UNIVERSIDADE FEDERAL DO PARANÁ

MAÍRA MACIEL TOMAZZOLI

PHYTOCHEMICAL EVALUATION, ANTIOXIDANT ACTIVITY AND PROPAGATION METHODS OF *Baccharis dracunculifolia* D.C.: THE MAIN BOTANICAL SOURCE OF BRAZILIAN GREEN PROPOLIS

CURITIBA

2020

MAÍRA MACIEL TOMAZZOLI

PHYTOCHEMICAL EVALUATION, ANTIOXIDANT ACTIVITY AND PROPAGATION METHODS OF *Baccharis dracunculifolia* D.C: THE MAIN BOTANICAL SOURCE OF BRAZILIAN GREEN PROPOLIS

Tese apresentada ao Curso de Pós-Graduação em Agronomia, Área de Concentração em Produção Vegetal, Departamento de Fitotecnia e Fitossanitarismo, Setor de Ciências Agrárias, Universidade Federal do Paraná, como como parte das exigências para obtenção do título de Doutor em Ciências.

Orientador: Prof. Dr. Cicero Deschamps
Co-orientador: Dr. Walderlei do Amaral

CURITIBA

2020

Tomazzoli, Maíra Maciel

Phytochemical evaluation, antioxidant activity and propagation methods of *Baccharis dracunculifolia* D.C.: the main botanical source of brazilian green propolis. / Maíra Maciel Tomazzoli. - Curitiba, 2020.

Tese (Doutorado) - Universidade Federal do Paraná. Setor de Ciências Agrárias, Programa de Pós-Graduação em Agronomia - Produção Vegetal.

Orientador: Cicero Deschamps.

Coorientador: Walderlei do Amaral.

1. Alecrim. 2. Óleos essenciais. 3. Extratos vegetais. 4. Própolis. 5. Reprodução vegetal. I. Deschamps, Cícero. II. Amaral, Wanderlei do. III. Título. IV. Universidade Federal do Paraná.

Sistema de Bibliotecas/UFPR

Guilherme Luiz Cintra Neves - CRB9/1572

MINISTÉRIO DA EDUCAÇÃO
SETOR DE CIENCIAS AGRARIAS
UNIVERSIDADE FEDERAL DO PARANÁ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO AGRONOMIA
(PRODUÇÃO VEGETAL) - 40001016031P6

TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em AGRONOMIA (PRODUÇÃO VEGETAL) da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **MAIRA MACIEL TOMAZZOLI** intitulada: **PHYTOCHEMICAL EVALUATION, ANTIOXIDANT ACTIVITY AND PROPAGATION METHODS OF *Baccharis dracunculifolia* DC.: THE MAIN BOTANICAL SOURCE OF BRAZILIAN GREEN PROPOLIS**, sob orientação do Prof. Dr. CICERO DESCHAMPS, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de doutor está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 09 de Março de 2020.

CICERO DESCHAMPS
Presidente da Banca Examinadora (UNIVERSIDADE FEDERAL DO PARANÁ)

WANDERLEI DO AMARAL
Avaliador Externo (UNIVERSIDADE FEDERAL DO PARANÁ)

LUIZ ANTONIO BIASI
Avaliador Interno (UNIVERSIDADE FEDERAL DO PARANÁ)

FABRICIO AUGUSTO HANSEL
Avaliador Externo (EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA)

Aos meus amados pais

Maristela Maciel Silva

Edison Ramos Tomazzoli

ACKNOWLEDGMENTS

A Deus, sob suas misteriosas formas na minha vida;

Aos meus amado pais Maristela Maciel Silva e Edison Ramos Tomazzoli, a quem dedico este trabalho, e ao meu irmão Thiago Maciel Tomazzoli. Obrigada pelo apoio durante todas as fases da minha vida, por todo o amor, carinho e por sempre acreditarem nos meus sonhos. Sem vocês este trabalho não seria possível;

Ao meu amado Leandro Specht, pelo companheirismo por todos esses anos, por sempre se fazer presente, pelo incentivo constante e pelos valorosos conselhos profissionais e de vida. Agradeço especialmente pela compreensão nos momentos de ausência. Sem você este trabalho não seria o mesmo;

À toda familía Specht: Lúcia, Douglas, Patrícia, Gustavo, Daiana, Joseandro e Valentina. Obrigada pelo incentivo e inspiração de vida;

Ao meu orientador Cicero Deschamps, por acreditar nesse trabalho desde o início, pela confiança a mim depositada, pela orientação, pelos ensinamentos e conselhos e pela primordial educação;

Ao meu co-orientador Wanderlei do Amaral, por todo o apoio e suporte nas coletas a campo, definições metodológicas e conversas científicas;

Aos professores Peter Brooks e Stephen Trueman da 'University of the Sunshine Coast' da Austrália, por terem me proporcionado essa experiência incrível de fazer pesquisa em uma Instituição estrangeira, obrigada pela generosidade, paciência, ensinamentos e profissionalismo;

À Jéssica de Cássia Tomasi, por dividir as angústias e conquistas dessa jornada, por todo o auxílio nos experimentos, pelos ensinamentos e por ser um exemplo de profissional a ser seguido, tenho certeza que és uma inspiração para muitas pessoas;

À Cintia de Moraes Fagundes e Juliana Amatussi, presentes que a UFPR me deu, obrigada por serem minha luz em todos os momentos desse doutorado e pela ajuda nos longos experimentos acadêmicos e de vida;

À Renata Grunennvaldt pela primordial ajuda em diversas fases desse doutorado, em especial para a execução do doutorado sanduíche na Austrália, sem você essa experiência incrível não teria sido possível;

Aos amigos da UFPR: Juliane, Erik, Leandro, Mariana, Tales, Laudiane e Manoela, por toda ajuda nos experimentos, risadas e mateadas compartilhadas;

Aos amigos da Agronomia/UFSC: Carina, Caroline, Francine, Maria Luiza, Ana Luiza, Ana Heck e Bruno, não importa o rumo que as nossas vidas tomaram, sempre levo um pouco de vocês comigo;

Aos amigos: Thais, Karine, Carla, Elisa, Teresa, Kamilla, Jimi e Alice, por todos apoio, amizade e respeito;

As amigas da 'University of the Sunshine Coast': Anushika Lakshrini De Silva e Tsvakai Gama, agradeço a amizade, companheirismo, ensinamentos durante essa caminhada australiana;

Aos colegas da 'University of the Sunshine Coast': Trong Tran e Simon Williams, pelos auxílios laboratoriais e discussões valorosas;

À Kareen Dwyer, Noel Davidson e Matilda Davidson, por serem minha família australiana, obrigada por me receber em sua casa, pelo incentivo e ensinamentos diários;

Ao Laboratório de Produtos Naturais e Ecologia Química da UFPR, em especial a professora Dra. Beatriz H. L. N. S Maia, pela realização das análises dos óleos essenciais;

Ao técnico de laboratório e colega de doutorado Róger Raupp Cipriano, obrigada por todo auxílio nas análises laboratoriais, pelas discussões científicas e pelo seu profissionalismo;

À professora Maristela Panobianco por todo apoio e auxílio durante a condução dos experimento de sementes e pelas discussões científicas;

Ao professor Walmes Marques Zeviani por todo auxílio nas análises estatísticas, pela prestatividade e profissionalismo;

A todos os professores do Programa de Pós-graduação em Agronomia - Produção Vegetal (PGAPV) pelos ensinamentos, em especial aos professores Luiz Antonio Biasi e Katia Christina Zuffellato Ribas por serem fontes de inspiração como profissionais para mim;

Aos técnicos de laboratório Rose, Carlos Maduro e Maria Emília, pelo apoio na condução dos experimentos;

À secretária do PGAPV Lucimara Antunes, pelo seu profissionalismo, prestatividade e eficiência;

À Universidade Federal do Paraná e ao Programa de Pós-graduação em Agronomia (Produção Vegetal) por viabilizar a realização deste doutorado;

À ‘University of the Sunshine Coast’ por proporcionar a realização de uma parte do meu doutorado em uma Instituição estrangeira;

À CAPES pelo suporte financeiro para realização deste doutorado no Brasil e do doutorado sanduíche na Austrália;

“A chave da nossa felicidade depende da capacidade de estarmos satisfeitos”

(DALAI LAMA)

RESUMO

A espécie *Baccharis dracunculifolia* DC., conhecida popularmente como “alecrim-do-campo”, é um arbusto dióico nativo do Brasil com grande importância econômica devido aos seus metabólitos secundários. A planta é conhecida por ser a principal fonte botânica de um importante produto comercial conhecido como "própolis verde", que possui várias propriedades benéficas para a saúde humana, entre elas propriedades antitumorais. O metabolismo secundário vegetal é influenciado por fatores genéticos e ambientais, e o entendimento de como esses fatores influenciam a diversidade química em populações naturais é o primeiro passo para satisfazer com sucesso as demandas industriais, sendo possível, posteriormente, selecionar acessos altamente produtivos para cultivos e programas de domesticação. Neste sentido, o objetivo principal deste trabalho foi avaliar a variabilidade química dos metabólitos secundários das folhas de *B. dracunculifolia* e suas atividades antioxidantes em populações de ocorrência natural no sul do Brasil, além de avaliar as técnicas de propagação da espécie. Os extratos vegetais foram analisados por HPLC-DAD e LC-MS, a análise da composição do óleo essencial (OE) foi realizada por GC-FID e GC-MS e as atividades antioxidantes foram analisadas pelos métodos DPPH e ABTS. Além disso, foram avaliadas técnicas de propagação (1) por reprodução sexual: curva de maturação para determinar o ponto de maturidade fisiológica e definir melhores condições (temperatura) para a germinação; (2) por reprodução assexuada: a influência do sexo da planta matriz no enraizamento de estacas de caule de acordo com diferentes concentrações de ácido indolbutírico. As populações naturais estudadas produziram diferentes teores de óleo essencial e distintas quantidades de compostos terpênicos, e isso pode estar relacionado principalmente ao fator genético e secundariamente aos fatores bióticos e abióticos a que as plantas foram expostas, o que também teve reflexo nas atividades antioxidantes apresentadas. Apesar da diferença quantitativa nos compostos, a composição química do óleo essencial não foi alterada. As populações naturais estudadas podem ser divididas em três grupos principais, de acordo com os níveis de seus compostos terpênicos. Ao longo do ano existem variações no teor, na quantidade dos compostos e na atividade antioxidante do óleo essencial, que podem ser divididos em duas épocas: I) verão e outono, fase de floração, onde o OE produz maior porcentagem de *(E)*-nerolidol; II) inverno e primavera, fase vegetativa, OE produz maior porcentagem de β-pineno. O metabolismo vegetal de *B. dracunculifolia* é afetado tanto pelo gênero da planta e quanto pelo local de crescimento. As plantas masculinas produzem níveis mais altos de compostos fenólicos, assim como maior teor de óleo essencial e maior capacidade antioxidante. O artepillin C, composto mais importante para a comercialização da própolis verde, apresenta maior conteúdo nas folhas de plantas masculinas do que nas femininas. A maturidade fisiológica das sementes ocorre aos 40 dias após a antese e as temperaturas de 25°C e 20-30°C são as melhores para a germinação da espécie. A aplicação do IBA é essencial para o enraizamento de estacas, mas mesmo assim o enraizamento é baixo e não foi influenciado pelo gênero da planta matriz. Este é o primeiro relato de propagação vegetativa de *B. dracunculifolia*, entretanto estudos adicionais são necessários para otimização da metodologia de propagação da espécie.

Palavras-chave: Alecrim-do-campo. Óleo essencial. Extratos. Própolis verde. Reprodução vegetal.

ABSTRACT

Baccharis dracunculifolia DC., which widely known as “alecrim-do-campo” is a dioecious shrub native to Brazil. This plant has great economical importance due to its secondary metabolites. This species is popular for being the botanical source of the important commercial product "green propolis”, which has several beneficial properties for human health, such as anti-tumor activity. Secondary plant metabolism is influenced by genetic and environmental factors. Thus, understanding how these factors influence chemical diversity in wild populations is the first step in successfully satisfying industrial demands, by being able to select highly productive accessions for crops and improvement of domestication. In this sense, the main objective of this study was to evaluate the chemical variability of secondary metabolites of *B. dracunculifolia*, and their antioxidant activities of wild populations in southern Brazil (Parana state) and also to assess its propagation techniques. The plant extracts were analyzed by HPLC-DAD and LC-MS, the essential oil composition was determined by GC-FID and GC-MS and their antioxidant activities were analyzed by DPPH and ABTS methods. In addition, propagation techniques was evaluated (1) by sexual reproduction: maturation curve to determine the physiological maturity point and define better condition (temperature) for germination; (2) by asexual reproduction: the influence of the plant sex on rooting of stem cuttings with different concentrations of indolbutyric acid (IBA). The wild populations reported to produce different essential oil contents and different amounts of terpenic compounds. This may be related mainly to the genetic factor and secondarily to the biotic and abiotic factors that the plants were exposed. It also had an impact on the antioxidant activities presented. The essential oil shows quantitative changes in its compounds, however, the chemical composition remained unchanged. In relation to the results, this population can be divided into three main groups, according to the levels of their terpenic compounds. Throughout the year, there were variations in the content, quantity of the compounds and antioxidant activity of the essential oils, which can be divided into two seasons: I) summer and autumn, flowering phase, EO produces a higher percentage of *(E)*-nerolidol; II) winter and spring, vegetative phase, EO produces a higher percentage of β-pinene. The secondary metabolism of *B. dracunculifolia* is affected both by the plant's gender and by the growth site. Male plants produced higher levels of phenolic compounds and essential oil contents and also showed greater antioxidant capacity compared with female plants. Artepillin C, the most important compound for the commercialization of green propolis, was present in higher content in the leaves of the male plant than that of female plant. Moreover, the physiological maturity of seeds occur at 40 days after anthesis which being the best time for seed collection in the field. The temperatures of 25°C and 20-30°C are the best for germination tests of the species. The application of IBA is essential for rooting of stem cuttings, but even so the rooting is low and was not influenced by the gender of the matrix plant. This is the first study report on vegetative propagation of *B. dracunculifolia*, however further work is required to optimize the propagation methodology of the plant.

Keywords: Alecrim-do-campo. Essential oil. Extracts. Green propolis. Plant reproduction.

LIST OF FIGURES

FIGURE 1.1 - (A) GENERAL APPEARANCE OF *B. dracunculifolia;* (B) DETAILS OF BRANCHES IN VEGETATIVE STAGE; (C) MALE PLANT FLOWERS; (D) FEMALE PLANT FLOWERS; (E) FEMALE FLOWERS, FRUITS AND SEED..... 22
(B)

FIGURE 1.2 - MAIN SYNTHESIS PATHWAYS OFPLANT SECONDARY METABOLISM..... 24

FIGURE 1.3 - BIOSYNTHESIS OF TERPENES COMPOUNDS BY METHYLERYTHRITOL PHOSPHATE AND MEVALONATE PATHWAYS..... 25

FIGURE 1.4 - BIOSYNTHESIS OF PHENOLIC COMPOUNDS..... 26

FIGURE 2.1 - ESSENTIAL OIL YIELD (%) IN FRESH LEAVES OF TEN POPULATIONS (P) OF *B. dracunculifolia* FROM THE STATE OF PARANA, BRAZIL. MEANS FOLLOWED BY THE SAME LETTER DO NOT DIFFER BY SCOTT-KNOTT TEST AT 5% OF PROBABILITY..... 38

FIGURE 2.2 - PRINCIPAL COMPONENT ANALYSIS (PCA) OF THE ESSENTIAL OIL COMPOSITION IN POPULATIONS (P) OF B. dracunculifolia COLLECTED IN THE STATE OF PARANA, BRAZIL (MARCH, 2016)..... 44

FIGURE 2.3 - CLUSTER ANALYSIS (UPGMA METHOD - 75% COPHENETIC CORRELATION) OF THE ESSENTIAL OIL COMPOSITION IN POPULATIONS (P) OF *B. dracunculifolia* COLLECTED IN THE STATE OF PARANA, BRAZIL (MARCH, 2016)..... 44

FIGURE 2.4 - MEANS AND STANDARD ERRORS OF THE ANTIOXIDANT ACTIVITIES (DPPH METHOD) OF ESSENTIAL OILS OF TEN *B. dracunculifolia* populations (P) FROM THE STATE OF PARANA, BRAZIL (MARCH, 2016). MEANS FOLLOWED BY THE SAME LETTER DO NOT DIFFER BY SCOTT-KNOTT TEST AT 5% OF PROBABILITY..... 46

FIGURE 3.1 - ESSENTIAL OIL YIELD (%) IN FRESH LEAVES OF *B. dracunculifolia* COLECTED IN AUTUMN/2016, WINTER/2016, SPRING/2016 AND SUMMER/2017. COEFFICIENT OF VARIATION = 18.40%. MEANS FOLLOWED BY THE SAME LETTER DO NOT DIFFER BY SCOTT-KNOTT TEST AT 5% OF PROBABILITY..... 63

FIGURE 3.2 - PRECIPITATION (MM) AND AVERAGE TEMPERATURE (T°C) IN THE COLLECTION SITE, PARANA STATE, BRAZIL. THE RED ARROWS INDICATE THE PLANT HARVEST TIME. PHENOLOGICAL STAGES OF THE PLANT DURING HARVEST: FLOWERING (F) AND VEGETATIVE (V)..... 65

FIGURE 3.3 - RADIATION (W/m²) AND RELATIVE HUMIDITY (%) IN THE COLLECTION SITE, PARANA STATE, BRAZIL. THE RED ARROWS INDICATE THE PLANT HARVEST TIME. PHENOLOGICAL STAGES OF THE PLANT DURING HARVEST: FLOWERING (F) AND VEGETATIVE (V)..... 65

FIGURE 3.4 - PRINCIPAL COMPONENT ANALYSIS (PCA) OF THE ESSENTIAL OIL COMPOSITION OF *B. dracunculifolia* LEAVES COLLECTED

DURING AUTUMN/2016, WINTER/2016, SPRING/2016 AND SUMMER/2017..... 69

FIGURE 3.5 - CLUSTER ANALYSIS (UPGMA METHOD - 96% COPHENETIC CORRELATION) OF THE ESSENTIAL OIL COMPOSITION OF B. dracunculifolia LEAVES COLLECTED DURING AUTUMN/2016, WINTER/2016, SPRING/2016 AND SUMMER/2017..... 69

FIGURE 3.6 - MEANS AND STANDARD ERRORS OF THE ANTIOXIDANT ACTIVITIES (DPPH METHOD) OF ESSENTIAL OILS IN FRESH LEAVES OF *B. dracunculifolia* COLLECTED DURING AUTUMN/2016, WINTER/2016, SPRING/2016 AND SUMMER/2017. COEFFICIENT OF VARIATION = %. MEANS FOLLOWED BY THE SAME LETTER DO NOT DIFFER BY SCOTT-KNOTT TEST AT 5% OF PROBABILITY..... 72

FIGURE 4.1 - CHROMATOGRAPHIC PROFILES (HPLC-DAD, λ = 316 nm) OF MALE AND FEMALE LEAVES OF *B. dracunculifolia* FROM POPULATION OF PALMEIRA (PPA) AND POPULATION OF PIRAQUARA (PPI)..... 94

FIGURE 4.2 - CLIMATE CONDITIONS OF PRECIPITATION (mm) AND AVERAGE TEMPERATURE (T°C) IN POPULATION OF PALMEIRA (PPA) AND PIRAQUARA (PPI)..... 95

FIGURE 4.3 - CLIMATE CONDITIONS OF RADIATION (W/m²) AND WIND SPEED (m/s) IN POPULATION OF PALMEIRA (PPA) AND POPULATION OF PIRAQUARA (PPI)..... 96

FIGURE 4.4 - MEANS AND STANDARD DEVIATION OF ANTIOXIDANT ACTIVITY BY ABTS ($\mu m.g^{-1}$) OF MALE AND FEMALE LEAVES OF *B. dracunculifolia* FROM POPULATION OF PALMEIRA (PPA) AND POPULATION OF PIRAQUARA (PPI). MEANS FOLLOWED BY THE SAME LOWER CASE LETTERS FOR POPULATIONS AND THE SAME LOWER CASE LETTERS FOR GENDER ARE NOT STATISCALLY DIFFERENT AT 5% LEVEL OF SIGNIFICANCE ACCORDING TO TUKEY TEST..... 99

FIGURE 4.5 - ESSENTIAL OIL CONTENT (%) OF FEMALE AND MALE PLANTS OF B. dracunculifolia FROM POPULATION OF PALMEIRA (PPA) AND POPULATION OF PIRAQUARA (PPI). COEFFICIENT OF VARIATION = 16.51%. MEANS FOLLOWED BY THE SAME LOWER CASE LETTERS FOR POPULATIONS AND THE SAME LOWER CASE LETTERS FOR GENDER ARE NOT STATISCALLY DIFFERENT AT 5% LEVEL OF SIGNIFICANCE ACCORDING TO TUKEY TEST..... 101

FIGURE 4.6 - MEANS AND STANDARD DEVIATION FROM IC_{50} OF ANTIOXIDANT ACTIVIT ($\mu g.ml^{-1}$) BY DPPH OF MALE AND FEMALE LEAVES OF *B. dracunculifolia* FROM POPULATION OF PALMEIRA (PPA) AND POPULATION OF PIRAQUARA (PPI). COEFFICIENT OF VARIATION= 9.24%. MEANS FOLLOWED BY THE SAME LOWER CASE LETTERS FOR POPULATIONS AND THE SAME LOWER CASE LETTERS FOR GENDER ARE NOT STATISCALLY DIFFERENT AT 5% LEVEL OF SIGNIFICANCE ACCORDING TO TUKEY TEST. NS= NOT SIGNIFICANT..... 107

FIGURE 5.1 - CHARACTERISTIC OF *B. dracunculifolia* BRANCHES AND SEEDS DURING PHYSIOLOGICAL MATURITY: (A) FLOWER BUDS; (B) ANTHESIS AND (C) 19 DAYS; (D) 26 DAYS; (E) 29 DAYS; (F) 33

DAYS; (G) 36 DAYS AND (H) 40 DAYS AFTER ANTHESIS..... 128

FIGURE 5.2 - DRY MASS ACCUMULATION (%) IN *B. dracunculifolia* SEEDS THROUGHOUT MATURATION PROCESS. (**) SIGNIFICANCE P ≤0.01 ACCORDING TO THE F TEST..... 129

FIGURE 5.3 - DETAILS OF *B. dracunculifolia* BRANCHES AND FRUITS AT THE POINT OF PHYSIOLOGICAL MATURITY..... 130

FIGURE 5.4 - PERCENTEGE OF GERMINATION (NORMAL SEEDLINGS) PER DAY OF B. dracunculifolia SEEDS AT 20, 25, 30 AND 20-30°C..... 132

FIGURE 5.5 - *Baccharis dracunculifolia* STEM CUTTINGS AFTER 120 DAYS FROM PLANTING: A) CALLUSES FORMATION; B) ALIVE CUTTINGS (NEITHER CALLUSES NOR ROOT FORMATION); DEAD CUTTINGS (NECROSED)..... 135

FIGURE 5.6 - REGRESSION ANALYSIS FOR THE VARIABLES OF ROOTING (A), CALLUSES (B), AVERAGE NUMBER OF ROOTS PER CUTTINGS (C), AVERAGE LENGHT OF ROOTS (D) IN *B. DRACUNCULIFOLIA* STEM CUTTINGS TREATED WITH INCREASING CONCENTRATIONS OF IBA ($mg.L^{-1}$)..... 136

FIGURE 5.7- EFFECT OF DIFFERENT IBA TREATMENTS ($mg.L^{-1}$) ON *B. dracunculifolia* STEM ROOT FORMATION..... 137

LIST OF TABLES

TABLE 2.1 - LOCATION OF *B. dracunculifolia* POPULATIONS IN THE STATE OF PARANA, BRAZIL, AND CLIMATIC CONDITIONS OF THE COLLECTION SITES (AVERAGE OF MARCH/2016)..... 35

TABLE 2.2 - MEAN AND STANDARD ERROR OF THE COMPOUNDS PRESENT IN THE ESSENTIAL OILS % (GC/FID) OF TEN POPULATIONS (P) OF *B. dracunculifolia* COLLECTED IN THE STATE OF PARANA, BRAZIL (MARCH, 2016)..... 41

TABLE 2.3 - PRINCIPAL COMPONENTS, VARIANCE (EIGENVALUE) AND CUMULATIVE VARIANCE (%) OBTAINED FROM THE CHEMICAL COMPOSITION (≥ 3.0%) AND THE ANTIOXIDANT/DPPH ANALYSIS OF THE ESSENTIAL OILS OF *B. dracunculifolia* FROM TEN POPULATIONS (P) COLLECTED IN THE STATE OF PARANA, BRAZIL..... 45

TABLE 2.4 - PEARSON CORRELATION COEFFICIENTS BETWEEN *B. dracunculifolia* ESSENTIAL OIL CONTITUENTS, ANTIOXIDANT ACTIVITY (DPPH) AND CLIMATIC FACTORS: ALTITUDE, RADIATION (RAD), TEMPERATURE (TEMP) AND PRECIPITATION (PREC)..... 47

TABLE 3.1 - COMPOSITION OF ESSENTIAL OIL % BY GC/FID (MEAN AND STANDARD ERROR) AND OF LEAVES OF B. dracunculifolia PLANTS COLLECTED DURING AUTUMN/2016, WINTER/2016, SPRING/2016 AND SUMMER/2017..... 66

TABLE 3.2 - PRINCIPAL COMPONENTS, VARIANCE (EIGENVALUE) AND CUMULATIVE VARIANCE (%) OBTAINED FROM THE CHEMICAL COMPOSITION (≥ 3.0%) ANALYSIS OF THE ESSENTIAL OILS OF LEAVES OF B. dracunculifolia..... 68

TABLE 3.3 - PEARSON CORRELATION COEFFICIENTS BETWEEN *B. dracunculifolia* ESSENTIAL OIL CONSTITUENTS AND ANTIOXIDANT ACTIVITY (DPPH)..... 73

TABLE 4.1 - COMPOSITION OF *B. dracunculifolia* LEAVES BY LC-MS/MS..... 90

TABLE 4.2 - MEANS AND STANDARD DEVIATION OF COMPOSITION CONTENT $mg.g^{-1}$ OF MALE AND FEMALE LEAVES OF *B. dracunculifolia* PLANTS FROM POPULATION OF PALMEIRA (PPA) AND POPULATION OF PIRAQUARA (PPI) CHARACTERIZED BY HPLC-DAD..... 93

TABLE 4.3 - MEANS AND STANDARD DEVIATION OF COMPOSITION CONTENT $mg.g^{-1}$ OF LEAVES OF *B. dracunculifolia* PLANTS FROM POPULATION OF PALMEIRA (PPA) AND POPULATION OF PIRAQUARA (PPI) CHARACTERIZED BY HPLC-DAD..... 93

TABLE 4.4 - MEANS AND STANDARD DEVIATION OF COMPOSITION CONTENT $mg.g^{-1}$ OF LEAVES OF *B. dracunculifolia* PLANTS FROM POPULATION OF PALMEIRA (PPA) AND POPULATION OF PIRAQUARA (PPI) CHARACTERIZED BY HPLC-DAD..... 93

TABLE 4.5 - MEANS AND STANDARD DEVIATION FROM IC50 OF ANTIOXIDANT ACTIVIT ($\mu g.ml^{-1}$) BY DPPH OF MALE AND FEMALE LEAVES OF *B. dracunculifolia* FROM POPULATION OF PALMEIRA (PPA) AND POPULATION OF PIRAQUARA (PPI)..... 99

TABLE 4.6 - COMPOSITION OF ESSENTIAL OIL% BY GC/MS (MEAN AND STANDARD DEVIATION) OF FRESH LEAVES OF FEMALE AND MALE PLANTS OF *B. dracunculifolia* FROM POPULATION OF PALMEIRA (PPA) AND POPULATION OF PIRAQUARA (PPI)..... 104

TABLE 4.7 - MEANS AND STANDARD DEVIATION OF ESSENTIAL OIL COMPOSITION % OF FRESH LEAVES OF *B. dracunculifolia* PLANTS FROM POPULATION OF PALMEIRA (PPA) AND POPULATION OF PIRAQUARA (PPI) CHARACTERIZED BY GC/FID..... 105

TABLE 4.8 - MEANS AND STANDARD DEVIATION OF ESSENTIAL OIL COMPOSITION % OF FRESH LEAVES OF *B. dracunculifolia* PLANTS FROM TWO GENDERS CHARACTERIZED BY GC/FID.... 106

TABLE 5.1 - MEAN AND STANDARD ERROR OF GERMINATION (%) AND GERMINATION SPEED INDEX (GSI) OF *B. dracunculifolia* SEEDS, BY DIFFERENT INCUBATION TEMPERATURES AND USING BLOTTER PAPER AS SUBSTRATE..... 131

TABLE 5.2 - SUMMARIES OF DEVIANCE ANALYSIS CHART OF ROOTING, SPROUTING, LEAVE RETENTION, CALLUSES, MORTALITY, SURVIVAL, NUMBER OF ROOTS AND AVERAGE LENGTH OF ROOTS OF *B. dracunculifolia* STEM CUTTINGS..... 134

LIST OF ABBREVIATIONS

%	- Percentage
°C	- Degree celsius
µg	- Microgram
µl	- Microliter
AA	- Antioxidant activity
ALIVE	- Live cuttings
ANOVA	- Analysis of variance
CALL	- Cuttings with callus
DAD	- Diode array detector
DC	- De Candole
DAA	- Days after anthesis
DEAD	- Dead cuttings
DPPH	- 2,2-Diphenyl-1-picrylhydrazyl
FDA	- United States by the Food and Drug Administration
FID	- Flame ionization detector
g	- Gram
GC	- gas chromatography
GHS	- Enzymes glutathione
GLM	- Generalized linear models
H_2O_2	- Hydrogen peroxide
CA	- Cluster analysis
HPLC	- Performance Liquid Chromatography
IAA	- Indole-acetic acid
IBA	- Indole-butyric acid
IC_{50}	- Inhibitory Concentration 50%
ISO	- Isoprene units
LEAV	- Cuttings with leaves
LENG	- Average length of roots per cutting
M^{-1}	- Molecular mass
MBM	- Municipal Botanical Museum of Curitiba
MEP	- 2-C-methyl-D-erythritol 4-phosphate
MEV	- Mevalonate

mg	- Miligram
min	- Minute
mL	- Milliliter
mm	- Millimeter
MS	- Mass spectrometry detector
n	- Number
NaCl	- Sodium chloride
nm	- Nanometer
NUMB	- Number of roots per cutting
O_2–	- Superoxide anion
OH	- Hydroxyl radicals
P	- Population
PAL	- Phenylalanine ammonia lyase
PCA	- Principal component analysis
PPA	- Population of Palmeira
PPI	- Population of Piraquara
PREC	- Precipitation
r^2	- Coefficient of determination
RAD	- Radiation
RI	- Retention index
ROOT	- Rooted cuttings
ROS	- Reactive oxygen species
Rt	- Retention time
SIMEPAR	- Parana Meteorological System
SOD	- Superoxide dismutase
SPRO	- Cuttings with sprout
TEMP	- Temperature
UPGMA	- Non-weighted arithmetic mean
UV	- Ultraviolet
UV-Vis	- Ultraviolet Visible
v/v	- Volume/volume
λ	- Lamda
µm	- Micrometer

SUMMARY

1 GENERAL INTRODUCTION....22
2 CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF ESSENTIAL OILS FROM POPULATIONS OF *Baccharis dracunculifolia* DC. IN SOUTHERN BRAZIL....31
ABSTRACT....31
2.1 INTRODUCTION....32
2.2 MATERIAL AND METHODS....34
2.2.1 Collection of plant material and climatic conditions....34
2.2.2 Essential oils isolation and chemical analysis....35
2.2.3 Antioxidant activity assay....36
2.2.4 Experimental design and statistical analysis....36
2.3 RESULTS AND DISCUSSION....37
2.3.1 Essential oil yield (%)....37
2.3.2 Essential oil chemical composition....39
2.3.3 Antioxidant activity....46
2.4 CONCLUSIONS....49
REFERENCES....49
3 SEASONAL EVALUATION OF VOLATILE COMPOUNDS AND ANTIOXIDANT ACTIVITY OF *Baccharis dracunculifolia* DC....57
ABSTRACT....57
3.1 INTRODUCTION....58
3.2 MATERIAL AND METHODS....60
3.2.1 Collection of plant material and essential oil extraction....60
3.2.2 Determination and quantification of chemical composition....61
3.2.3 Determination of antioxidant activity....61
3.2.4 Statistical analysis....62
3.3 RESULTS AND DISCUSSIONS....62
3.3.1 Essential oil yield (%)....62
3.3.2 Chemical composition....66
3.3.3 Antioxidant activity....72
3.4 CONCLUSIONS....74
REFERENCES....75

4 GENDER DIFFERENCE IN *Baccharis dracunculifolia* DC. PLANTS: PHYTOCHEMICAL EVALUATION AND ANTIOXIDANT ACTIVITY....82
ABSTRACT....82
4.1 INTRODUCTION....83
4.2 MATERIAL AND METHODS....85
4.2.1 Material collection and proceedings....85
4.2.2 Preparation and extraction of constituents for HPLC analysis....85
4.2.3 HPLC-DAD analysis....86
4.2.4 HPLC-MS analysis....86
4.2.5 Extraction and quantification of the essential oil....87
4.2.6 Determination of antioxidant activity....88
4.2.6.1 DPPH radical scavenging assay....88
4.2.6.2 ABTS radical scavenging assay....88
4.2.7 Statistical analysis....89
4.3 RESULTS AND DISCUSSIONS....89
4.3.1 Analysis of the extracts....89
4.3.1.1 Chemical characterization of the extracts....89
4.3.1.2 Chemical content of the extracts....91
4.3.1.3 Antioxidant activities of the extracts....98
4.3.2 Analysis of the essential oils....101
4.3.2.1 Essential oil content (%)....101
4.3.2.2 Chemical composition of essential oils....103
4.3.2.3 Antioxidant activity of essential oils....107
4.4 CONCLUSIONS....108
REFERENCES....109
5 SEXUAL AND ASSEXUADED PROPAGATION OF *Baccharis dracunculifolia* DC....121
ABSTRACT....121
5.1 INTRODUCTION....122
5.2 MATERIAL AND METHODS....123
5.2.1 Seed propagation....123
5.2.1.1 Monitoring of seed maturation....123
5.2.1.2 Dry mass analysis....124
5.2.1.3 Seed germination test....125

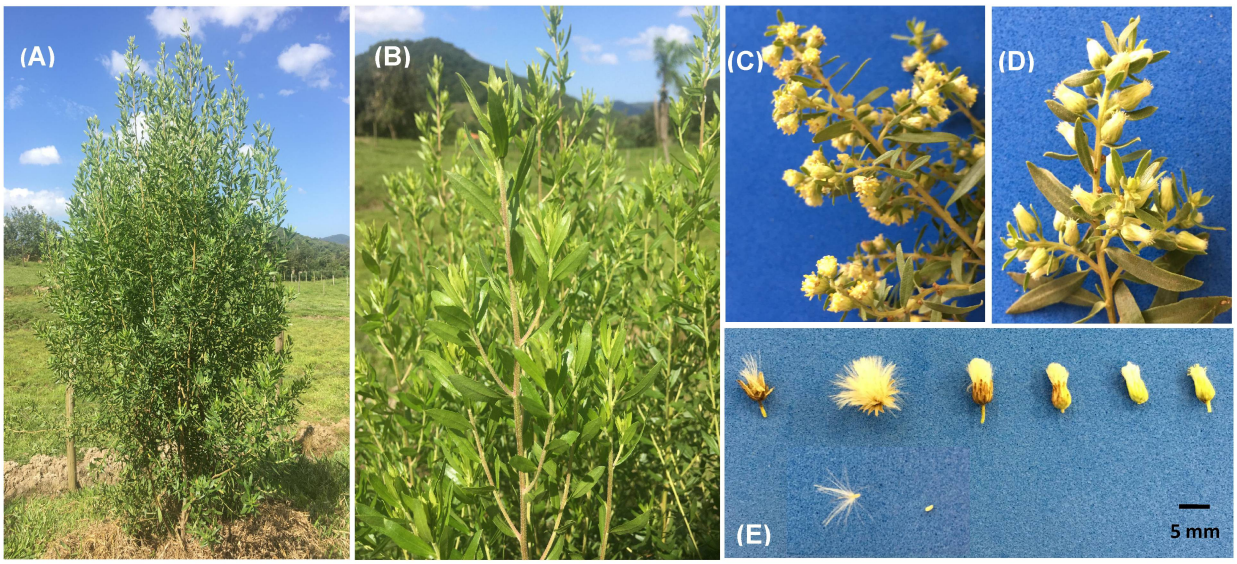
5.2.1.4 Data analysis....125
5.2.2 Vegetative propagation - Stem cuttings....126
5.2.2.1 Plant material collection site....126
5.2.2.2 Preparation and evaluation of stem cuttings....126
5.2.2.3 Data analysis....127
5.3 RESULTS AND DISCUSSIONS....127
5.3.1 Seed propagation....127
5.3.1.1 Monitoring of seed maturation....127
5.3.1.2 Seed germination test....130
5.3.2 Stem cuttings propagation....134
5.4 CONCLUSIONS....139
REFERENCES....140
GENERAL CONCLUSIONS....145
FINAL CONSIDERATIONS....147
GENERAL REFERENCES....149

1 GENERAL INTRODUCTION

The genus *Baccharis* belongs to the botanical family Ateraceae, to the tribe Astereae and to the subtribe Baccharidinae. This genus includes more than 500 plant species, widely distributed from the United States to Argentina. The great majority of *Baccharis* species (90%) occurs naturally in South America and 120 species are found in Brazil. Due to the large concentration of species in Brazil and the Andes, studies indicate that this area is probably the center of origin of the taxon (BUDEL et al., 2005).

Baccharis dracunculifolia De Candole (1836), popularly known as “alecrim-do-campo”, "vassourinha" and "alecrim-vassoura", is a dioecious shrub with a height of 2-3 meters, pilous branches, lanceolate leaves (1-2.5 cm long and 4 mm wide) with whole margins or 1-3 dentate margin, flowers with multiflorum chapter and achene (1.5 mm long) type fruit (BARROSO; BUENO, 2002; BUDEL et al., 2005). The general appearance of *Baccharis dracunculifolia* is shown in Figure 1.1. Its leaves have tectoral and glandular trichomes that, besides acting as a barrier to the predatory insects attacks, help in the species interaction with bees and for collection of their resinous material (SFORCIN et al., 2012).

FIGURE 1.1 - (A) GENERAL APPEARANCE OF *B. dracunculifolia;* (B) DETAILS OF BRANCHES IN VEGETATIVE STAGE; (C) MALE PLANT FLOWERS; (D) FEMALE PLANT FLOWERS; (E) FEMALE FLOWERS, FRUITS AND SEED.



SOURCE: The author (2019).

Baccharis dracunculifolia is well known for an important commercial product called 'green propolis' produced by honeybees (*Apis mellifera* L.) from vegetative buds of this plant. For green propolis production, honeybees cut out the vegetative buds of *B. dracunculifolia* and add to salivary enzymes, beeswax and pollen, with the purpose to seal openings in the hive, repairing combs and deter the entrance of intruders (BANKOVA et al., 2000; PARK et al., 2004; SALATINO et al., 2005; TEIXEIRA et al., 2005).

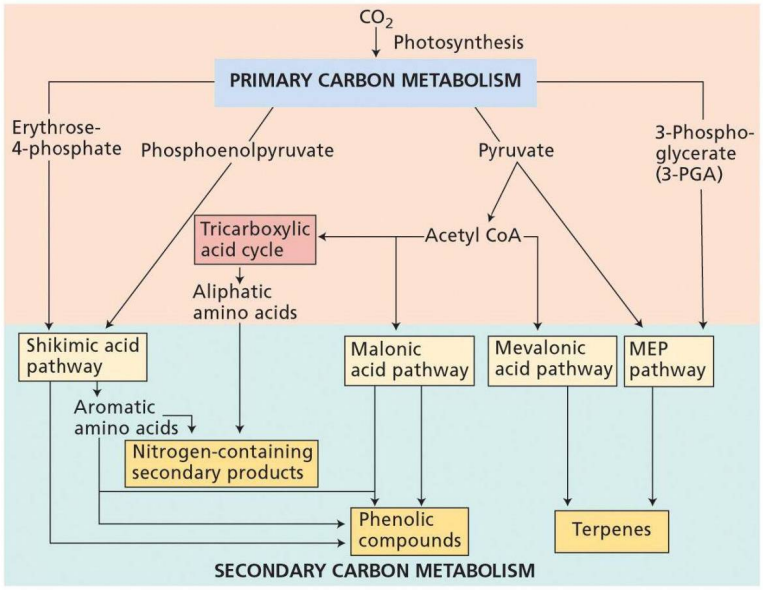
The plant has great economic importance due to its secondary metabolites, which have a series of applications for the medicinal, cosmetic and ecological fields. Related to medicinal importance, the compound commercially called Artepillin C (3,5-diprenyl-4-hydroxycinnamic acid) and present in high levels both in the plant and in green propolis, has a series of beneficial effects to human health, including the treatment of tumors. Such feature greatly increasing the studies and the demand for the product on the market (BANSKOTA et al., 1990; PARK et al., 2004; TEIXEIRA et al., 2005; AHN et al., 2007; MESSERLI et al., 2009). In addition, its essential oil has uses in the medical and cosmetic fields, since they have a series of biological activities and are also used as an ingredient in fine fragrances, cosmetics, shampoos, toilet soaps, cleaners and detergents (LAPCZYNSKI et al., 2008; PARREIRA et al., 2010; FLORÃO et al., 2012; CHAABAN et al., 2017). From an ecological point of view, the secondary metabolites present in the plant assist in the interaction of the species with the bees for the collection of resinous material for the production of propolis and also for the pollination of the species (MAFFEI, 2010; BASTOS et al., 2011). Besides these characteristics, among the plants of the genus *Baccharis* found in Brazil, *B. dracunculifolia* is the species with the most characteristic of anthropic pioneer and has a high rate of natural regeneration which emphasizes its ecological importance in the recovery of degraded areas (BARROSO; BUENO, 2002; FERREIRA et al., 2009).

Despite the plant's application in the production of various derived products of commercial value, until now there is no commercial cultivation of the species, where the production of these products originating from vegetal extractivism. In addition, in view of the potential to recover degraded areas of the species, commercial seedling production can bring additional economic benefits to producers.

The secondary metabolism of this species is quite complex, the several compounds present in the plant belong to different classes and can originate from

different metabolic pathways (TAIZ; ZEIGER, 2013), as shown in Figure 1.2, Figure 1.3 and Figure 1.4 .

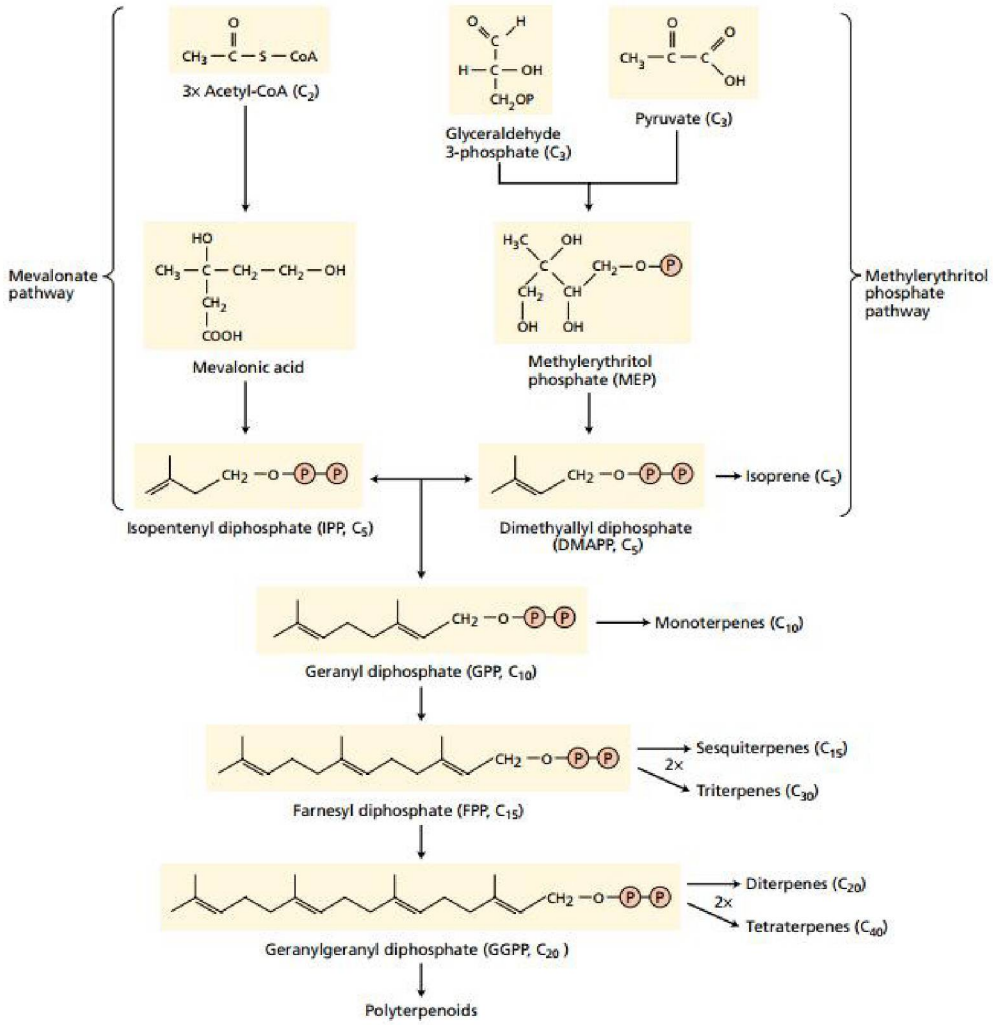
FIGURE 1.2 - MAIN SYNTHESIS PATHWAYS OFPLANT SECONDARY METABOLISM.



SOURCE: TAIZ; ZEIGER (2013).

The essential oils produced occur by 2-C-methyl-D-erythritol 4-phosphate (MEP) in the plastids and by mevalonate (MVA) in the cytoplasm, as shown in Figure 1.3. The terpene syntheses are the main enzymes responsible for the production of the various terpene compounds (VRANOVÁ et al., 2013; PAZOUKI et al., 2016). Terpenes are formed by adding isoprene units (C_5H_8) and are classified according to the number of these isoprene units (ISO), where: hemiterpene (1 ISO), monoterpenes (2 ISO), sesquiterpenes (3 ISO), diterpenes (4 ISO), triterpenes (6 ISO) and tetraterpenes (8 ISO) (TAIZ; ZEIGER, 2013). The plant extracts, mostly belong to the class of phenolics, that in plants occur via the shikimic acid pathway (FIGURE 1.4) and the phenylalanine ammonia lyase (PAL) is the enzyme that regulates the synthesis of these compounds (TAIZ; ZEIGER et al., 2013).

FIGURE 1.3 - BIOSYNTHESIS OF TERPENES COMPOUNDS BY METHYLERYTHRITOL PHOSPHATE AND MEVALONATE PATHWAYS.



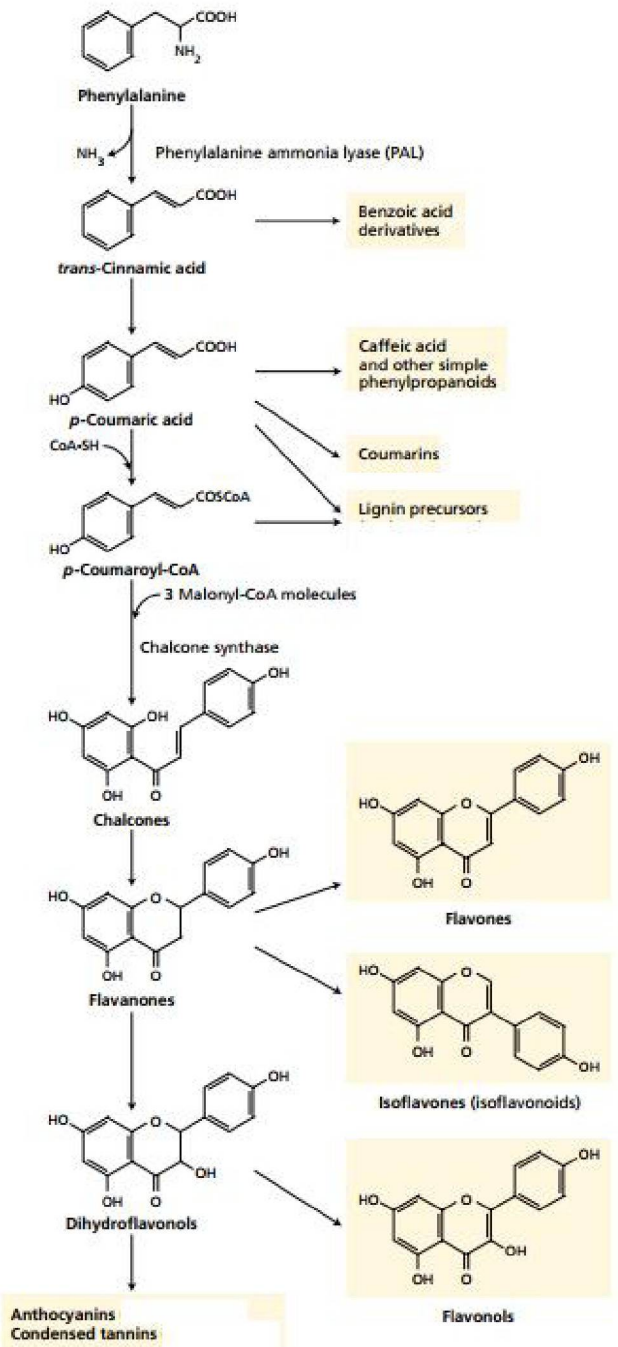
SOURCE: TAIZ; ZEIGER (2013).

The main compounds that characterize the essential oil are *(E)*-nerolidol, spathulenol, α-pinene, β-pinene, β-Caryophyllene, germacrene D, bicyclogermacrene, mircene, limonene and δ-cadinene (LOAYZA et al., 1995; DE SOUSA et al., 2009; PARREIRA et al., 2010; SANTOS et al., 2012; SALAZAR et al., 2018), and have been decribed several biological properties such as antimicrobial (FERRONATTO et al., 2007), antibacterial (BARBOSA et al., 2015), antiprotozoal (PARREIRA et al., 2010), insecticide (CHAABAN et al., 2017), anti-inflammatory (FLORÃO et al., 2012) and acaridae (LAGE et al., 2015) activities.

Among the main compounds found in the extract of the plant are the prenylated phenylpropanoids acid derivatives like artepillin C, baccharin, drupanin and *p*-coumaric acid, and caffeoylquinic acids such as 3,4 and 3,5-Di-caffeoylquinic

acid (BANSKOTA et al., 1990; PARK et al., 2004; MISHIMA et al., 2005; TEIXEIRA et al., 2005; TOMAZZOLI et al., 2020). The biosynthesis pathway for some phenolic compounds is described in Figure 1.4. Related to biological property, previous studies identified activities antiulcer (LEMOS et al., 2007), antimicrobial (FILHO et al., 2008), antimutagenic (MUNARI et al., 2008), anti-inflammatory (PAULINO et al., 2008) and radical scavenging (NAKANISHI et al., 2003).

FIGURE 1.4 - BIOSYNTHESIS OF PHENOLIC COMPOUNDS.



SOURCE: TAIZ; ZEIGER (2013).

Such applications, coupled with new properties discovered as research progresses, tend to increase the uses of essential oil, plant extract and propolis from *B. dracunculifolia* plant as raw material for the industries and, for this purpose, a constant offer of standardized plant material of these products will become increasingly important. The overall chemical composition and the content of specific compounds are the main factors affecting the type of industry in which the final product will be applied and, therefore, their final market value.

With regard to essential oils, their chemical diversity is strongly influenced by genetic and environmental factors such as photoperiod, radiation, seasonality, nutrients availability, soil salinity, humidity and temperature (FIGUEIREDO et al., 2008; BANO et al., 2016). In addition, the seasonality has an important influence on the yield and chemical composition of essential oils, where large changes in the plant chemical profile may occur. According to the phenological stage of the plant, metabolic pathways may be directed to plant growth during the vegetative stage in relation to flowering and fruiting plants and may also be related to the activity of pollinating insects in flowering seasons (FIGUEIREDO et al., 2008; MAFFEI, 2010; SALEM et al., 2018).

Another factor that can influence secondary plant metabolism is the plant's gender, since many studies developed with dioecious species, such as *B. dracunculifoli*a, have shown morphological and physiological differences between male and female individuals (ESPIRITO-SANTO et al., 2003; BESTEN et al., 2013; LI et al., 2016). This kind of species can present different allocations of products during the vegetative and reproductive phases, studies point out that reproductive investments are greater in female individuals than in male individuals and these diferences may also affect secondary plant metabolism, since primary and secondary metabolic pathway share common precursors and intermediates (HERMS; MATTSON, 1992; ANTOS; ALLEN, 1999; SUZUKI, 2005; CAPEDA-CORNEJO; DIRZO, 2010). Studies focusing on chemical and essential oil content, together with the composition of the extracts and antioxidant activity comparison between the female and male individuals of *B. dracunculifolia* were not developed until then and this can greatly contribute to understanding the interaction between bee and plant in the production of green propolis, to achieve a standardized raw material of green propolis, plant extract and essential oil.

Therefore, the understanding of chemical diversity in wild populations and, subsequently, the selection of highly productive accessions for domestication, cultivation and breeding are some of the initial steps to successfully satisfy the industrial demands (DAJIC-STEVANOVIC et al., 2000). Despite previous studies pointing out numerous potentials for the use of *B. dracunculifolia*, there are few reports about its propagation. So far, only studies with the propagation by seeds were carried out with *B. dracunculifolia*, despite the high genetic variability linked to this kind of reproduction. Previous studies developed with its seeds found that the best conditions for seed germination are at temperatures between 15 to 20ºC in the presence of light (GOMES; FERNANDES, 2002). *B. dracunculifolia* seeds can be stored during 3, 6, and 9 months at 6°C and during 45 days at -20°C and moisture content reduced to 3.3%, without loss of viability (SANTOS et al., 2016). However, there is no information about optimal point of harvest of *B. dracunculifolia* seeds in the field, and this data is extremely important to identify the physiological maturity of the seeds, and consequently increasing the success of producing quality seedlings.

There are also no studies on the vegetative propagation of this species, but for other species of the genus *Baccharis* such as *Baccharis trimera*, *Baccharis articulata* and *Baccharis stenocephala*, the vegetative propagation by stem cuttings has been shown to be effective (BONA et al., 2005). In adittion, previous studies of vegetative propagation in dioecious species, identified the influence of plant sex on stem cuttings rooting (CARVALHO et al., 2007; WENDLING et al., 2016), and this also needs to be investigated for *B. dracunculifolia*. Although the vegetative propagation be considered more costly (per unit propagule) than propagation by seeds, for many species, including medicinal species as *B. dracunculifolia*, the possible superiority of some clones can justifies the higher cash value associated with this kind of propagation (HARTMANN et al., 2014), being more likely to maintain uniformity of production of secondary metabolites, since the genetic factor among individuals is the same.

In this sense, the main objective of this study was to evaluate the chemical variability of secondary metabolites of *B. dracunculifolia*, their antioxidant activities and evaluate the propagation techniques of this species by sexual (seeds) and asexual reproduction (stem cuttings).

This study was divided into four chapters. Chapter I: Aimed to evaluate the content, the chemical composition and antioxidant activity of essential oils from ten

populations of *B. dracunculifolia* in Parana state, Brazil. Chapter II: The population with the highest content of essential oil was selected and its content, chemical composition and antioxidant activity were evaluated over the seasons for one year. Chapter III: Compare the chemical profile of secondary metabolites, i.e. essential oil and extracts, and their antioxidant activities of male and female plants of *B. dracunculifolia* from two different populations of Parana state, Brazil. Chapter IV: The last one evaluated the propagation techniques of the species (1) by sexual reproduction: maturation curve to determine the physiological maturity point for seed collection and define better condition (temperature) for germination; (2) by asexual reproduction: the influence of the plant sex on stem cuttings rooting according to different concentrations of IBA.

2 COMPOSIÇÃO QUÍMICA E ATIVIDADE ANTIOXIDANTE DE ÓLEOS ESSENCIAIS DE POPULAÇÕES DE *Baccharis dracunculifolia* DC. DO SUL DO BRASIL

RESUMO

Baccharis dracunculifolia DC. é uma espécie vegetal nativa do Brasil com ampla diversidade química e inúmeras aplicações industriais, e.g. farmacêuticas e cosméticas. Esta pesquisa avaliou o teor, a atividade antioxidante e a similaridade química de óleos essenciais de 10 populações de *B. dracunculifolia* no estado do Paraná, sul do Brasil. A extração dos compostos voláteis foi realizada por hidrodestilação, a composição química foi determinada por GC/FID e GC/MS e a atividade antioxidante pelo método DPPH. A produção de óleo essencial das populações naturais de *B. dracunculifolia* variou de 0,14 a 0,87%. Os óleos foram compostos predominantemente por sesquiterpenos oxigenados (34,16-51,01%), hidrocarbonetos monoterpenos (18,02-46,17%) e hidrocarbonetos sesquiterpenos (9,60-17,70%). Os principais compostos encontrados em todas as populações foram β-pineno (7,65-29,8%) e *(E)*-nerolidol (9,11-21,68%). Soluções de óleo essencial (20%) de diferentes populações apresentaram capacidade antioxidante variando de 27,78 a 91,67%. Foi encontrada uma correlação negativa entre a atividade antioxidante e o spathulenol (r=-0,696). As análises multivariadas separaram as populações em três grupos: (1) baixas concentrações de α-pineno (2,02-2,06%), (2) altas concentrações de α-pineno (4,17-4,61%) e β-pineno (22,54 - 29,80%) e (3) concentrações intermediárias de α-pineno (2,38-3,31%), β-pineno (12,77-19,03%) e espatulenol (6,02-9,06%).

Palavras-chave: β-pineno, *(E)*-nerolidol, Alecrim-do-campo, DPPH.

2 CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF ESSENTIAL OILS FROM POPULATIONS OF *Baccharis dracunculifolia* DC. IN SOUTHERN BRAZIL

ABSTRACT

Baccharis dracunculifolia DC. is a Brazilian native plant, presenting wide chemical diversity and numerous industrial applications, e.g. pharmaceutical and cosmetic. This research assessed the yield, antioxidant activity and the chemical similarity of essential oils from 10 populations of *B. dracunculifolia* in the state of Parana, southern Brazil. The extraction of the volatile compounds was carried out by hydrodistillation, the chemical composition was determined by GC/FID and GC/MS and the antioxidant activity by the DPPH method. There essential oil yield of wild *B. dracunculifolia* populations ranged from 0.14 to 0.87%. The oils were predominantly composed of oxygenated sesquiterpenes (34.16-51.01%), monoterpene hydrocarbons (18.02-46.17%) and sesquiterpenes hydrocarbons (9.60-17.70%). The major compounds found in all populations were β-pinene (7.65-29.8%) and *(E)*-nerolidol (9.11-21.68%). Essential oil solutions (20%) from different populations presented antioxidant capacity ranging from 27.78 to 91.67%. A negative correlation was found between the antioxidant activity and spathulenol (r=-0.696). Multivariate analyses separated the populations into three groups: (1) low concentrations of α-pinene (2.02-2.06%), (2) high concentrations of α-pinene (4.17-4.61%) and β-pinene (22.54-29.80%), and (3) intermediate concentrations of α-pinene (2.38-3.31%), β-pinene (12.77-19.03%) and spathulenol (6.02-9.06%).

Keywords: β-pinene, *(E)*-nerolidol, Alecrim-do-campo, DPPH.

2.1 INTRODUCTION

Baccharis dracunculifolia DC. (Asteraceae) is a dioecious, perennial, 2-3 meters high woody shrub, usually propagated by seeds. The species is native to Brazil, with a wide distribution in the South, Southeast and Center-West regions of the country, especially in areas of Cerrado (BOLDT, 1989; BARROSO; BUENO, 2002; PARK et al., 2004). It also occurs naturally in other countries of South America such as Bolivia, Paraguay, Argentina, and Uruguay (BOLDT, 1989).

In addition to being widely known as the major botanical source of the so-called green propolis (PARK et al., 2004; SFORCIN et al., 2012), one of the main commercial uses of *B. dracunculifolia* relies on the aroma and medicinal properties of its essential oil. The volatile oils produced in the leaves of this species have been shown to have several biological properties such as antimicrobial (FERRONATTO et al., 2007), antibacterial (BARBOSA et al., 2015), antiprotozoal (PARREIRA et al., 2010), insecticide (CHAABAN et al., 2017), anti-inflammatory (FLORÃO et al., 2012) and acaridae (LAGE et al., 2015) activities. Additionally, a previous study found that hydoethanolic extracts and essential oil of *B. dracunculifolia* leaves shows cytotoxic activity against HEp-2 cells (Human laryngeal epidermoid carcinoma) *in vitro* (BÚFALO et al., 2010). *B. dracunculifolia* oil is also widely used as a raw material in perfumery, representing a high value product for the fragrance industries (VERDI et al., 2005).

The study of *B. dracunculifolia* populations from Southern Brazil, Uruguay and Bolivia has shown two distinct chemotypes, indicating large fluctuations in the quality of the essential oil due to genetic and environmental factors (FRIZZO et al., 2008). Despite the sesquiterpenes (*E*)-nerolidol (1.2 – 32.0%) and spathulenol (6.0 - 21.0%) being frequently reported as major components of the volatile oil of *B. dracunculifolia*, other valuable compounds are also commonly identified, such as α-pinene (1.01 - 5.8%), β-pinene (1.93 - 43.4%), myrcene (0-2.8%), limonene (1.15-13%), (E)-β-caryophyllene (0.4-6.5), germacrene D (0.2-18.4%), bicyclogermacrene (0.7-8.4%), γ-muurolene (0.2 – 5.51%), δ-cadinene (0.1-3.64%), viridiflorol (0.8-16.4%), caryophyllene oxide (5.7-6.35%), (E)-β-oxyhemoglobin (0.2 - 1.7%) epi-α-muurolol (0-2.9%) and α-muurolol (0.2-4.7%) (PARREIRA et al., 2010; SANTOS et al., 2012); SALAZAR et al., 2018).

The overall chemical composition and the presence/relative content of specific compounds are the main factors affecting the type of industry in which essential oils will be applied and, therefore, their final market value. Bioactive compounds extracted from plants can be used in several areas and important studies demonstrate their effectiveness as food natural preservatives (SELANI et al., 2011; VAITHIYANATHAN et al., 2011). Furthermore, studies indicate that, due to the antioxidant activity of *B. dracunculifolia*, the plant has great potential for the manufacture of various products in the areas of medicine, cosmetics and food (CASAGRANDE et al., 2018). One of the major compounds of *B. dracunculifolia* oil, (*E*)-nerolidol, for example, was recently approved in the United States by the Food and Drug Administration (FDA) as a flavoring agent in the food industry and is also known to inhibit the growth of *Leishmania amazonenses* (ARRUDA et al., 2005). Spathulenol, in turn, has demonstrated promising antibacterial (ULUBELEN et al., 1994) and moderate cytotoxic activities (PACCIARONI et al., 2000).

Such applications, coupled with new properties discovered as research progresses, tend to increase the use of *B. dracunculifolia* essential oil as a raw material for the pharmaceutical and flavor industries and, for this purpose, a constant offer of standardized plant material/essential oils will become increasingly important. Therefore, the understanding of chemical diversity in wild populations and, subsequently, the selection of highly productive accessions for domestication, cultivation and breeding are some of the initial steps to successfully satisfy the industrial demands (DAJIC-STEVANOVIC; PLJEVLJAKUSIC, 2015).

Chemical diversity, including the content and chemical composition of essential oils, in plant species is ultimately determined by environmental (photoperiod, radiation, seasonality, nutrients availability, soil salinity, humidity and temperature) and genetic factors (FIGUEIREDO et al., 2008; BANO et al., 2016). The genetic variability of plant populations, in turn, is mainly related to their size, geographic variations, reproductive habit and mechanism of seed dispersion (SCHONEWALD-COX et al., 1983). For allogamous species as *B. dracunculifolia*, most of the genetic variation is found within the population (URBANSKA, 1987).

In addition to genetic variations, geographic conditions can greatly affect the yield and chemical composition of essential oils. If the same genotype of a given aromatic plant is grown in different regions, there may be considerable differences in

the chemical profile, reflecting the way plants adapt to ecological circumstances of each site (FIGUEIREDO et al., 2008).

Studies addressing essential oil yield and chemical variability in *B. dracunculifolia* populations are relatively scarce and limited to a few regions, more specifically the southeastern, (São Paulo state), and southern regions of Brazil, (Rio Grande do Sul state). Additional studies, in other regions of southern Brazil such as the state of Parana, are important to understand how the secondary metabolism of the species behaves in different environmental conditions and also to identify potential accessions for domestication and breeding programs. In this regard, multivariate statistics tools can be used to detect patterns of similarity among the essential oils of different wild plant populations and may provide additional help for a better understanding of the main compounds that can be used as biomarkers of oil quality.

In this sense, the aim of this study was to evaluate the content and chemical composition of essential oils from different populations of *B. dracunculifolia*, their chemical similarity and antioxidant activity.

2.2 MATERIAL AND METHODS

2.2.1 Collection of plant material and climatic conditions

Samples were collected in March 2016 from ten wild *B. dracunculifolia* populations in the State of Parana, South of Brazil. All plant materials were collected in floral bud stage. Branches with leaves were collected from approximately twenty-five plants at each collection site. The climate of the region is classified as Cfb according to Alvares et al. (2013). The precise location and specific climatic data of each population (average of March 2016) are described in TABLE 2.1. The registration number of the species in the '*Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado* - SisGen' is AC74344.

TABLE 2.1 - LOCATION OF *B. dracunculifolia* POPULATIONS IN THE STATE OF PARANA, BRAZIL, AND CLIMATIC CONDITIONS OF THE COLLECTION SITES (AVERAGE OF MARCH/2016).

Popula-tions	County	Latitude (S)*	Longitude (W)*	Voucher code	Altitude (m)*	Precipitation (mm)**	Radiation (W/m²)**	Average temperature (°C)**
P1	São Luiz do Purunã[1]	25°28'40''	49°42'22''	MBM-308.071	1055	12.8	1143	19.85
P2	São Luiz do Purunã[1]	25°28'13''	49°43'42''	MBM-326.212	1037	12.8	1143	19.85
P3	São José dos Pinhais[2]	25°39'77''	49°08'80''	MBM-336.907	835	91	1065	20.24
P4	São José dos Pinhais[2]	25°43'95''	49°05'75''	MBM-336.908	999	91	1065	20.24
P5	São José dos Pinhais[2]	25°45'047'	49°01'91''	MBM-341.117	886	91	1065	20.24
P6	Piraquara[3]	25°32'19''	49°04'35''	MBM-343.530	908	92.2	1064	20.1
P7	Piraquara[3]	25°30'64''	49°02'06''	MBM-348.144	916	92.2	1064	20.1
P8	Piraquara[3]	25°30'309'	49°00'54''	MBM-350.603	940	92.2	1064	20.1
P9	Palmeira[4]	25°28'18''	49°38'42''	MBM-332.462	1194	92	1197	20.81
P10	Palmeira[4]	25°19'80''	49°48'35''	MBM-334.697	1027	92	1197	20.81

SOURCE: *The author (2017) and **Parana Meteorological System - SIMEPAR, (2016).
SUBTITLE: Voucher specimens were deposited in the Municipal Botanical Museum of Curitiba - MBM Herbarium, Parana state, Brazil. Due to the lack of SIMEPAR stations in these cities, the data presented are related to the nearest stations: [1] Lapa station; [2] Curitiba station; [3] Pinhais station; [4] Ponta Grossa station.

2.2.2 Essential oils isolation and chemical analysis

Samples of 100g of fresh leaves from each *B. dracunculifolia* population were submitted to hydrodistillation in Clevenger apparatus for 2 hours and 30 minutes. The oil yield was calculated in dry base, by volumetric moisture determination and the results were expressed as the percentage of leaves dry mass (%), considering the mean value of a triplicate for each population. The samples were stored in a freezer at -5°C until the chemical composition and antioxidant analyses were performed.

Essential oil chemical composition was assessed by gas chromatography (GC) coupled to flame ionization detector – FID (Agilent 7890A) and mass spectrometry detector – MS (Shimadzu-2010 Plus), both equipped with HP-5MS capillary column (30 mx 0. 25 mm x 0.25 µm). Hydrogen for GC/FID (flow 1.5 mL / min) and helium for GC/MS (flow 1.0 mL/min) were used as the carrier gases. For both equipment the injector temperature remained constant throughout the analysis at 250°C and the The initial temperature of the oven was 60°C, rising to 240°C at the rate of

3°C/minute. For GC–MS, the injection volume was 1uL of the sample, 1% (w/v) in hexane, the interface temperature was at 300°C and mass range of m/z 40–400. The mass detector was operated in the ionization mode (electron impact 70 eV) at 3.15 scan s^{-1} and the ion source was maintained at 230 °C.

A homologous series of alkanes (C7-C30) was used to identify the chemical constituents, through the linear retention index (VAN DEN DOOL, 1963). In addition to the retention index, the mass spectrum of each compound was compared with data from the literature (ADAMS, 2007). The quantification of the composition was performed by dividing the peak area of each compound by the total area of identified compounds (%) by GC-FID.

2.2.3 Antioxidant activity assay

The antioxidant activity of *B. dracunculifolia* essential oils was determined by the radical and oxidizing agent 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (BRAND-WILLIAMS et al., 1995). Initially, essential oil samples were diluted in methanol (PA) at a concentration of 20%. The control solution was prepared diluting 2.366 mg of DPPH in 100 mL of methanol. Subsequently, a 0.1 mL aliquot of the essential oil/methanol solution was added to 3.9 mL of DPPH solution. After 30 minutes of incubation in dark, the solution was subjected to UV-Vis spectrophotometry to determine absorbances (λ= 515 nm). The results were expressed as percent inhibition, according to the formula below:

$$AA\% = 100 - \{[(absorbance._{sample} - absorbance._{control}) \times 100] \div absorbance._{control}\}$$

2.2.4 Experimental design and statistical analysis

Data were analyzed in a completely randomized design, comparing the essential oil yield and the antioxidant activity of 10 populations, each one with 3 repetitions. The results were submitted to the Bartlett test to verify the homogeneity of variances. The F-test of analysis of variance (ANOVA) was applied and, when significant, the data were submitted to the Scott-Knott test at 5% probability ($p < 0.05$), using the statistical software ASSISTAT (SILVA; AZEVEDO, 2016). The mean values of antioxidant activity and chemical composition of the essential oils were analyzed by Pearson correlation analysis and multivariate statistics, using the principal

components (PCA) and cluster analysis (CA), using the statistical software R (v. 3.1. 1) (R CORE TEAM, 2018). In the cluster analysis, the Euclidean distance was used as mean of similarity and the non-weighted arithmetic mean (UPGMA) method was used for the hierarchical grouping process. The analyses were performed for the compounds with means higher than 3.0% in at least one of the populations.

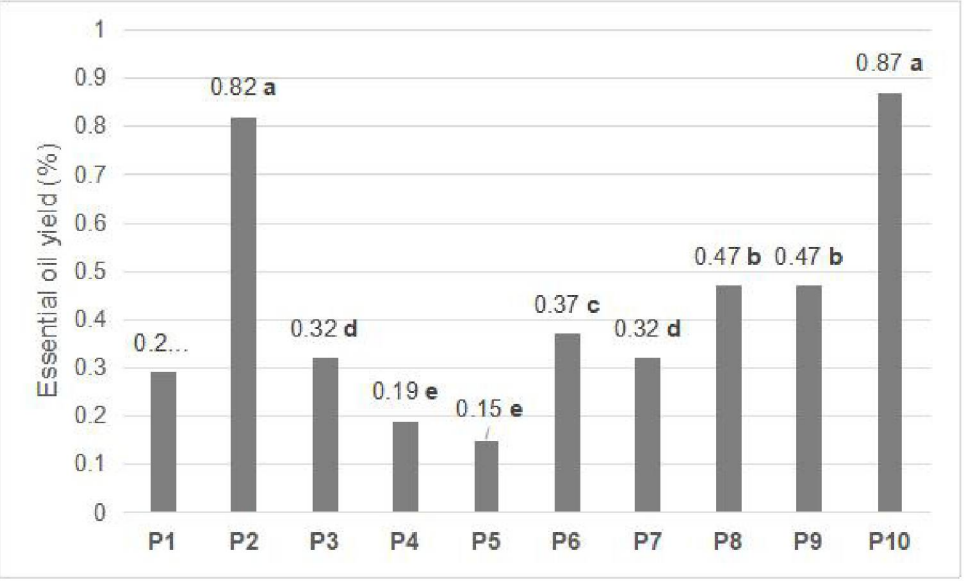
2.3 RESULTS AND DISCUSSION

2.3.1 Essential oil yield (%)

There were significant differences in the essential oil yield among *B. dracunculifolia* wild populations, ranging from 0.14 to 0.87%. The populations that presented the highest values were P2 (0.82%) and P10 (0.87%) (FIGURE 2.1). In a previous study carried out with cultivated accessions of *B. dracunculifolia* from different Brazilian states, essential oil yield ranged from 0.31 to 0.70% (SFORCIN et al., 2012). Similarly, *B. dracunculifolia* plants treated with different doses of organic composting at the time of planting had oil contents ranging from 0.26 to 0.40% (SANTOS et al., 2012). *B. dracunculifolia* leaves from different stages of maturity produced different levels of essential oil, with yields of 1.2% for young leaves and 1.7% for a mixture of young and developed leaves (MARTINEZ-CORREIA et al., 2012). Taken together, those studies illustrate the effect of genetic and environmental factors on oil content of this species.

The work developed by De Sousa et al. (2009), cultivated *B. dracunculifolia* plants from seeds of ten different populations of Brazil, the author identified different essential oils yield (%) from these populations, even though they were grown in the same place and under the same climatic conditions. Besides that, another study have observed that most of the genetic diversity of *B. dracunculifolia* is intrapopulation, with low interpopulation diversity (RIGOTTI et al., 2011), i.e. genetic variation within the population is greater than between populations. This can occur mainly due to its reproductive habit (allogamy) and mechanism of seed dispersal (anemochory) (SCHONEWALD-COX et al., 1983). In this sense, for this work, considering that different yields essential oil were produced from populations that grew under the influence of the same climatic conditions, these different yields are possibly related with the genetic effects of this species.

FIGURE 2.1 - ESSENTIAL OIL YIELD (%) IN FRESH LEAVES OF TEN POPULATIONS (P) OF *B. dracunculifolia* FROM THE STATE OF PARANA, BRAZIL. MEANS FOLLOWED BY THE SAME LETTER DO NOT DIFFER BY SCOTT-KNOTT TEST AT 5% OF PROBABILITY.



SOURCE: The author (2017).

However, in addition to genetic factors, environmental factors may also have influenced the production of essential oils in plants. Among the environmental conditions that can influence the content of essential oil is the solar radiation, where species that grow under high luminous intensities undergo a series of adaptations such as developing thicker leaves as a result of greater development of the palisade parenchyma and the vascular system. These structural modifications result in a greater capacity to capture solar radiation and, consequently, greater photosynthetic rates (RAVEN et al., 2005; SOUZA et al, 2008). Yet another modification plants use in order to overcome the excessive radiation is the development of trichomes. The essential oil of *B. dracunculifolia* is stored in pluricellular glandular trichomes, whose ecological function is thought to be increasing the light reflectance, avoiding excessive water loss and regulating the foliar temperature (RAVEN et al., 2005; PEGORINI et al., 2008).

In addition, different wavelengths of biologically active light can cause important effects in plant growth and responses to the environment and this may directly affect the synthesis of terpenes in the plant (LIU et al., 2017). Some terpenes have well-established functions, such as isoprene, which protect leaves from UV-B heating (TIIVA et al., 2007). The synthesis of terpenes in plants can occur

by two pathways: the MEP in the plastids and the mevalonate in the cytoplasm and terpene synthesi are the main enzymes responsible for the production of the various terpene compounds (VRANOVÁ et al., 2013; PAZOUKI et al., 2016). In the plastids, the terpene synthase usually is reponsible for the formation of monoterpenes using geranyl diphosphate and in the cytoplasm this enzyme originates sesquiterpenes from farnesyl diphosphate (PAZOUKI et al., 2016). The family of genes encoding terpene synthase enzymes have different members with high sequence homology. The size of terpene synthase's family with the promiscuity of each member contribute to the complexity of terpenoids produced in plants (LIU et al., 2017).

Water availability also affects the production of essential oils and several plant species present increases in their content under conditions of low precipitation (LOPES et al., 2001). The lower precipitation in the collection site of P2 may have contributed to the higher essential oil content of that population. One of the main damages caused by prolonged water stress is the production of reactive oxygen species (ROS), which will ultimately interfere in the structure of biomolecules and jeopardize overall plant physiology (CHAI et al., 2005; JEYARAMRAJAA et al., 2005). Therefore, the production of essential oils may represent a biochemical defense, as several terpenes present significant antioxidant activities (ABDEL-LTEIF et al., 2016; CHAN et al., 2016).

However, possibly other environmental conditions besides those described in Table 2.1 combined with other factors, such as genetic factors, incidence of pests and diseases, different soil types and nutrient availability, may also have influenced this essential oil production. In response to adverse conditions plants may develop certain regulatory mechanisms through the induction of transcription factors. Thus, terpene production levels, for example, may vary according to the need for plant defense (VERMA et al., 2015). Such regulatory mechanisms, coupled with highly productive genotypes, might be explored by growers in order to induce higher oil productivity. Future studies should address such strategies to improve overall yield and quality of *B. dracunculifolia* essential oil.

2.3.2 Essential oil chemical composition

A total of 35 chemical constituents were identified in the essential oil from wild populations of *B. dracunculifolia*. The oils were predominantly composed of

oxygenated sesquiterpenes (34.16 - 51.01%), monoterpene hydrocarbons (18.02-46.17%) and sesquiterpenes hydrocarbons (9.60 - 17.70%). The major compounds found in all ten populations were β-pinene (7.65 - 29.80%) and (*E*)-nerolidol (9.11 - 21.68%) (TABLE 2.2).

The major compounds identified in the present study are similar to previous reports on essential oils of *B. dracunculifolia* collected/grown in the southern and southeastern regions of Brazil. For plants grown in the state of São Paulo, (*E*)-nerolidol (33.51%) and spathulenol (16.24%) were identified as the major compounds (QUEIROGA et al., 2008). (*E*)-nerolidol was also the major compound (22.3%) in samples collected during winter in the state of Minas Gerais, followed by germacrene-D, limonene, and β-pinene (LAGE et al., 2015). For plants collected at a different region in the state of São Paulo, (*E*)-nerolidol was also the major compound (25.84%) followed by spathulenol (13.14%), germacrene-D (7.62%), limonene (6.93%) and β-pinene (4.24%) (BARBOSA et al., 2015). For plants collected in the State of Santa Catarina, β-pinene (9.94%) D-limonene (9.59%), β-nerolidol (7.93%) and caryophyllene (7.69%) were identified as major compounds (CHAABAN et al., 2017).

TABLE 2.2 - MEAN AND STANDARD ERROR OF THE COMPOUNDS PRESENT IN THE ESSENTIAL OILS % (GC/FID) OF TEN POPULATIONS (P) OF *B. dracunculifolia* COLLECTED IN THE STATE OF PARANA, BRAZIL (MARCH, 2016).

(to be continued)

Compounds	IR^{lit}	IR^{cal}	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
1- α-tujene	924	925	-	0.22 ± 0.04	-	0.71 ± 0.00	-	0.21 ± 0.01	0.22 ± 0.03	-	-	-
2- α-pinene	**932**	**932**	**2.06 ± 0.86 a**	**4.61 ± 0.64 a**	**3.06 ± 1.43 a**	**3.16 ± 0.55 a**	**2.02 ± 1.12 a**	**2.38 ± 1.12 a**	**2.76 ± 0.59 a**	**4.17 ± 0.16 a**	**3.12 ± 1.09 a**	**3.31 ± 0.80 a**
3- sabinene	969	972	-	-	-	0.72 ± 0.00	0.18 ± 0.05	-	-	0.37 ± 0.00	-	0.21 ± 0.00
4- β-pinene	**974**	**976**	**17.85 ± 1.15 c**	**29.80 ± 4.20a**	**16.34±1.27 c**	**15.14 ±1.21 c**	**7.65 ± 1.52 d**	**19.03±1.18 c**	**19.03 ± 1.15 c**	**22.54 ±1.15 b**	**16.40± 2.45 c**	**17.85 ±1.15 c**
5- myrcene	988	990	0.89 ± 0.39	1.93 ± 0.21	2.21 ± 0.02	1.14 ± 0.84	0.87 ± 0.17	1.81 ± 0.01	2.13 ± 0.04	1.86 ± 0.12	1.29 ± 0.46	1.62 ± 0.27
6- limonene	**1024**	**1026**	**2.60 ± 1.09 d**	**8.46 ± 1.19 c**	**11.66±0.58b**	**9.61 ± 1.41 c**	**6.90 ± 1.87 c**	**11.24±1.79 b**	**16.72 ± 0.04 a**	**15.13± 1.14 a**	**5.52 ± 1.00 d**	**12.58 ± 2.83 b**
7- (E)-β-ocimene	1044	1045	1.17 ± 0.47	1.69 ± 0.21	1.03 ± 0.14	0.69 ± 0.46	0.40 ± 0.06	0.89 ± 0.04	1.53 ± 0.03	1.14 ± 0.06	0.75 ± 0.21	0.9 ± 0.13
8- terpinen-4-ol	1174	1174	0.27 ± 0.01	0.30 ± 0.03	0.38 ± 0.01	0.32 ± 0.14	0.14 ± 0.01	0.30 ± 0.00	0.22 ± 0.04	0.26 ± 0.03	0.26 ± 0.04	0.27 ± 0.04
9- α-terpineol	1186	1188	0.49 ± 0.14	0.47 ± 0.06	0.45 ± 0.02	0.44 ± 0.05	0.24 ± 0.04	0.47 ± 0.08	0.28 ± 0.08	0.67 ± 0.05	0.45 ± 0.04	0.63 ± 0.09
10- δ-elemene	1335	1333	0.17 ± 0.04	0.13 ± 0.00	-	0.16 ± 0.00	0.15 ± 0.03	0.23 ± 0.00	0.70 ± 0.09	-	0.12 ± 0.00	-
11- β-elemene	1389	1388	0.37 ± 0.07	0.25 ± 0.04	0.26 ± 0.02	0.33 ± 0.07	0.36 ± 0.05	0.30 ± 0.12	-	-	0.34 ± 0.03	0.15 ± 0.01
12- (E)-β-caryophyllene	1417	1413	2.75 ± 0.51	0.99 ± 0.11	1.57 ± 0.11	1.77 ± 0.55	2.10 ± 0.33	1.24 ± 0.09	2.89 ± 0.2	1.31 ± 0.14	2.30 ± 0.23	2.06 ± 0.03
13- aromadendrene	1439	1432	0.36 ± 0.08	0.19 ± 0.02	0.27 ± 0.03	0.29 ± 0.01	0.35 ± 0.01	0.29 ± 0.00	0.29 ± 0.12	0.28 ± 0.03	0.45 ± 0.04	0.47 ± 0.02
14- cabreuva oxide B	1462	1456	0.57 ± 0.09	0.32 ± 0.02	0.56 ± 0.11	0.64 ± 0.02	0.84 ± 0.13	0.60 ± 0.16	0.38 ± 0.17	0.54 ± 0.04	0.67 ± 0.05	0.83 ± 0.05
15- dauca-5,8-diene	1471	1468	0.20 ± 0.02	0.12 ± 0.01	-	0.20 ± 0.00	0.15 ± 0.00	0.19 ± 0.07	0.16 ± 0.04	0.14 ± 0.01	0.15 ± 0.01	0.14 ± 0.01
16- germacrene D	**1484**	**1474**	**3.46 ± 0.64 a**	**1.84 ± 0.26 a**	**2.29 ± 0.68 a**	**2.65 ± 0.66 a**	**3.39 ± 0.23 a**	**3.02 ± 1.11 a**	**1.52 ± 0.45 a**	**2.39 ± 0.06 a**	**3.50 ± 0.24 a**	**1.87 ± 0.07 a**
17- bicyclogermacrene	**1500**	**1490**	**6.36 ± 1.25 a**	**3.82 ± 0.45 a**	**3.71 ± 1.07 a**	**4.39 ± 1.07 a**	**5.59 ± 0.97 a**	**4.84 ± 1.71 a**	**3.42 ± 0.81 a**	**3.75 ± 041 a**	**3.83 ± 0.46 a**	**3.54 ± 010 a**
18- α-muurolene	1500	1495	0.42 ± 0.07	0.20 ± 0.06	-	0.33 ± 0.09	0.38 ± 0.03	0.37 ± 0.14	0.28 ± 0.06	0.21 ± 0.02	0.33 ± 0.03	0.21 ± 0.03
19- γ-cadinene	1513	1508	0.57 ± 0.09	0.36 ± 0.08	-	0.42 ± 0.13	0.52 ± 0.01	0.50 ± 0.17	0.51 ± 0.08	0.33 ± 0.02	0.45 ± 0.06	0.32 ± 0.04
20- δ-cadinene	1522	1519	2.47 ± 0.39	1.38 ± 0.19	1.62 ± 0.09	1.88 ± 0.05	2.07 ± 0.13	1.60 ± 0.02	1.43 ± 0.10	1.51 ± 0.10	1.77 ± 0.15	1.37 ± 0.15
21- (*E*)-nerolidol	**1561**	**1564**	**14.93± 1.78 b**	**13.20± 1.71 b**	**19.35±4.49 a**	**18.30± 3.64 a**	**20.71± 1.28 b**	**13.38±2.53 b**	**13.98 ± 2.47 b**	**9.11 ± 0.16 b**	**22.60± 3.47 a**	**21.68 ± 3.35 a**
22- spathulenol	**1577**	**1570**	**7.02 ± 0,57 a**	**6.70 ± 0.63 a**	**6.68 ± 1.25 a**	**8.23 ± 1.68 a**	**8.49 ± 0.13 a**	**7.52 ± 1.21 a**	**9.60 ± 1.86 a**	**7.69 ± 0.51 a**	**6.02 ± 0.97 a**	**6.91 ± 0.42 a**
23- β-Copaen-4α-ol	**1590**	**1576**	**4.71 ± 0.72 a**	**2.79 ± 0.28 a**	**3.46 ± 0.91 a**	**3.88 ± 0.93 a**	**4.13 ± 0.11 a**	**3.42 ± 0.76 a**	**3.78 ± 0.96 a**	**3.33 ± 0.08 a**	**3.22 ± 0.53 a**	**4.22 ± 0.20 a**
24- viridiflorol	1592	1583	1.13 ± 0.27	1.65 ± 0.29	2.70 ± 0.31	1.96 ± 0.10	2.10 ± 0.51	1.84 ± 0.15	2.23 ± 0.18	1.78 ± 0.13	2.24 ± 0.29	1.02 ± 0.03
25- cubeban-11-ol	1595	1586	0.28 ± 0.07	0.14 ± 0.02	-	0.31 ± 0.00	0.25 ± 0.02	0.22 ± 0.08	-	0.16 ± 0.02	0.18 ± 0.04	0.21 ± 0.01
26- ledol	1602	1594	2.18 ± 0.37	1.72 ± 0.17	1.55 ± 0.22	2.19 ± 0.20	2.55 ± 0.1	1.44 ± 0.06	1.59 ± 0.23	2.07 ± 0.08	2.11 ± 0.29	2.01 ± 0.10
27- β-oplopenone	1607	1604	0.28 ± 0.04	0.21 ± 0.03	0.27 ± 0.07	0.31 ± 0.11	0.33 ± 0.03	0.29 ± 0.07	0.26 ± 0.08	0.28 ± 0.02	0.27 ± 0.05	0.25 ± 0.02
28- junenol	1618	1615	0.25 ± 0.07	0.16 ± 0.01	0.27 ± 0.07	0.32 ± 0.00	0.23 ± 0.02	0.19 ± 0.06	0.33 ± 0.00	0.15 ± 0.01	0.20 ± 0.04	0.26 ± 0.01
29- 1-epi-cubenol	1627	1621	0.95 ± 0.14	0.53 ± 0.05	0.27 ± 0.07	0.71 ± 0.02	0.82 ± 0.08	0.46 ± 0.10	0.51 ± 0.12	0.47 ± 0.03	0.76 ± 0.08	0.42 ± 0.03
30- epi-α-muurolol	1640	1635	3.13 ± 0.56	1.91 ± 0.27	1.67 ± 0.05	2.33 ± 0.71	2.87 ± 0.12	1.66 ± 0.07	1.26 ± 0.14	2.00 ± 0.12	2.17 ± 0.22	1.89 ± 0.23
31- α-muurolol	1644	1640	0.94 ± 0.17	0.59 ± 0.09	0.66 ± 0.20	2.33 ± 0.71	1.11 ± 0.17	0.59 ± 0.16	0.54 ± 0.17	0.62 ± 0.04	0.75 ± 0.11	0.56 ± 0.05
32- α-cadinol	**1652**	**1647**	**3.85 ± 0.83 a**	**2.56 ± 0.59 a**	**2.79 ± 0.79 a**	**2.95 ± 0.76 a**	**3.93 ± 0.16 a**	**2.10 ± 0.09 a**	**2.07 ± 0.53 a**	**2.75 ± 0.19 a**	**2.66 ± 0.36 a**	**2.21 ± 0.26 a**
33-germacra-4(15),5,10(14)-trien-1α-ol	1685	1678	1.87 ± 0.38	1.37 ± 0.31	1.14 ± 0.18	1.91 ± 0.1	2.51 ± 0.35	1.32 ± 0.10	1.10 ± 0.30	1.99 ± 0.15	2.25 ± 0.46	1.32 ± 0.17
34- isobicyclogermacrenal	1733	1724	0.34 ± 0.07	0.37 ± 0.07	0.41 ± 0.12	0.48 ± 0.19	0.60 ± 0.05	0.43 ± 0.13	0.26 ± 0.01	0.50 ± 0.04	0.42 ± 0.10	0.36 ± 0.03
35- khusimol	1741	1733	0.27 ± 0.06	0.26 ± 0.07	0.28 ± 0.10	0.33 ± 0.16	0.38 ± 0.00	0.29 ± 0.10	0.32 ± 0.14	0.37 ± 0.04	0.26 ± 0.08	0.25 ± 0.04
Monoterpene hydrocarbons			24.57	46.71	34.30	31.17	18.02	35.56	37.85	45.21	27.07	31.39

(finish)

Oxygenated monoterpenes	0.76	0.77	0.83	0.76	0.38	0.77	0.50	0.93	0.71	0.90
Sesquiterpene hydrocarbons	17.70	9.60	10.28	13.06	15.90	13.18	14.40	10.25	13.91	10.96
Oxygenated sesquiterpenes	42.13	34.16	41.50	46.54	51.01	36.05	37.83	33.16	46.11	43.57
Identified compounds %	84.45	91.24	86.91	91.53	85.31	85.56	90.58	89.55	87.80	86.82

SOURCE: The author (2017)

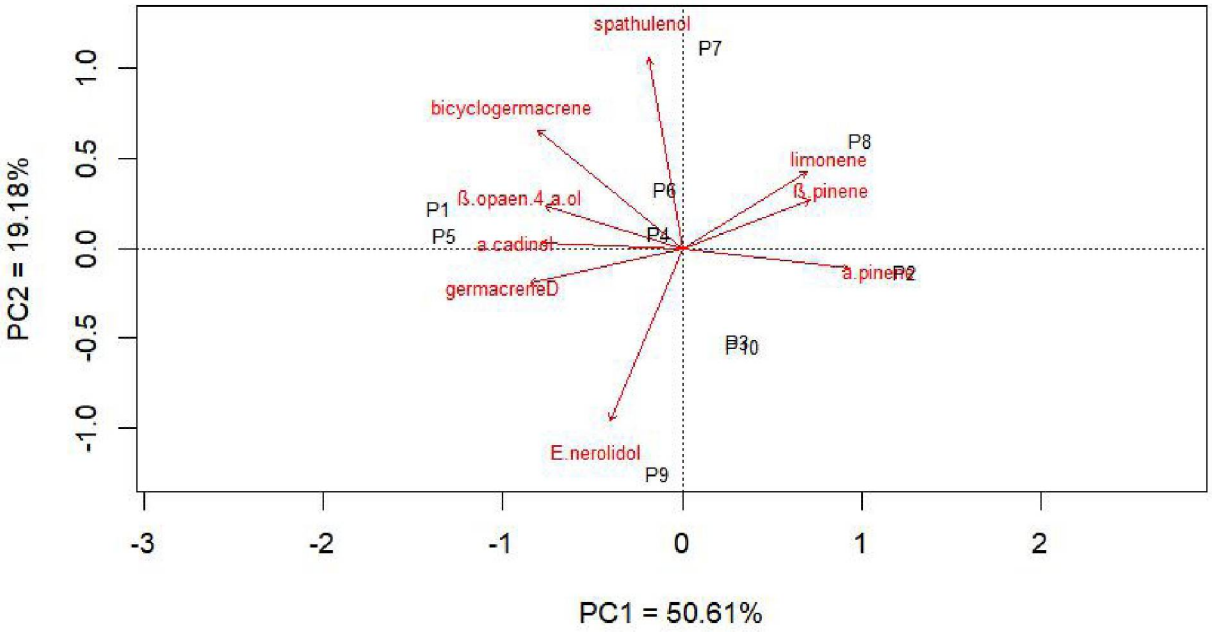
SUBTITLE: RI^{lit}: retention index of the literature Adams (2007). RI^{cal}: calculated retention index. – trace element <0.1. Means followed by the same letter in line do not differ by Scott-Knott test at 5% of probability.

In a previous study conducted with leaves in different stages of development, a similar chemical profile was found for all samples, with slight quantitative variations. The main compounds detected in the essential oil were: germacrene D, 12.99 - 8.79%; trans-caryophyllene, 6.81 - 7.71%; nerolidol, 5.73 - 6.50%; and spathulenol, 3.81 - 4.55% (MARTINEZ-CORREA et al., 2012). Analysis of the essential oil of *Baccharis milleflora* species showed similar compounds to *B. dracunculifolia*: trans-caryophyllene (7.65-13.41%), germacrene-D (6.83-11.18%), bicyclogermacrene (9.99-12.89%) were the major compounds, besides presenting antimicrobial activity against *Staphylococcus aureus* (PEREIRA et al., 2016). As previously mentioned, the knowledge of essential oils chemical composition is a fundamental aspect of their marketing and, therefore, is one of the most important selection criteria for exploitation of wild aromatic plant populations and/or for breeding and domestication programs.

In order to better understand the chemical variability of the studied populations of *B. dracunculifolia,* principal component analysis - PCA (FIGURE 2.2) and cluster analysis (FIGURE 2.3) were performed. These analyses considered only the chemical constituents with averages higher than 3.0% in at least one of the populations (TABLE 2.2). In the PCA analysis, the first three main components explained 86.10% of the sample variability. The compounds that were most significant to order the populations were spathulenol (0.6298), β-pinene (0.5306) and α-pinene (0.4332) (TABLE 2.3).

The populations were separated into three groups. The first group consisted of populations P1 and P5, characterized by low concentrations of α-pinene (2.02-2.06%). The second group included populations P2 and P8, with high levels of α-pinene (4.17-4.61%) and β-pinene (22.54-29.80%) ($p>0.05$). Finally, the third group was formed by the other populations, characterized by intermediate concentrations of α-pinene (2.38-3.31%), β-pinene (12.77-19.03%) and spathulenol (6.02 -9.06%). The differences in the essential oils in each group, however, are mainly quantitative and do not seem to indicate the presence of different chemotypes.

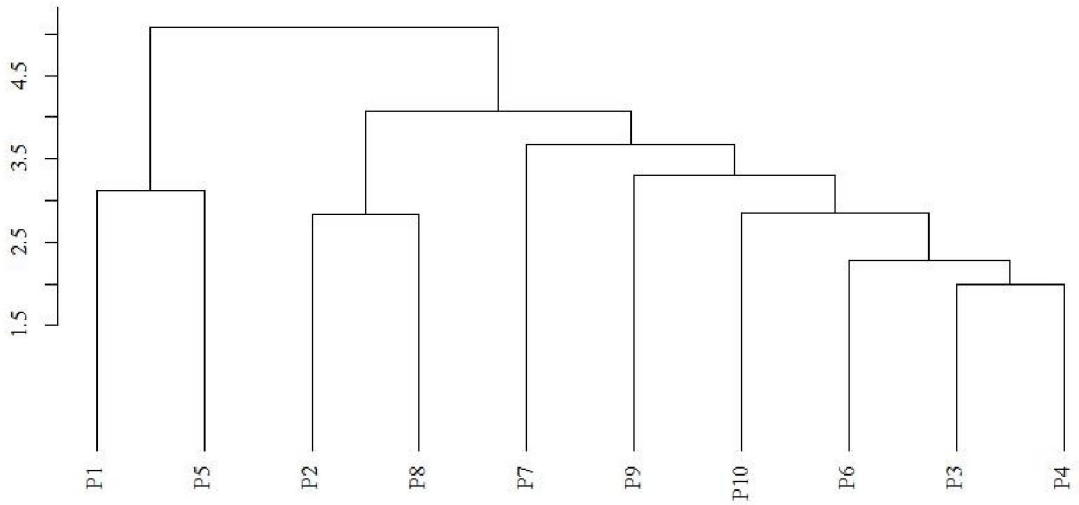
FIGURE 2.2 - PRINCIPAL COMPONENT ANALYSIS (PCA) OF THE ESSENTIAL OIL COMPOSITION IN POPULATIONS (P) OF *B. dracunculifolia* COLLECTED IN THE STATE OF PARANA, BRAZIL (MARCH, 2016).



SOURCE: The author (2017).

.

FIGURE 2.3 - CLUSTER ANALYSIS (UPGMA METHOD - 75% COPHENETIC CORRELATION) OF THE ESSENTIAL OIL COMPOSITION IN POPULATIONS (P) OF *B. dracunculifolia* COLLECTED IN THE STATE OF PARANA, BRAZIL (MARCH, 2016).



SOURCE: The author (2017).

TABLE 2.3 - PRINCIPAL COMPONENTS, VARIANCE (EIGENVALUE) AND CUMULATIVE VARIANCE (%) OBTAINED FROM THE CHEMICAL COMPOSITION (≥ 3.0%) AND THE ANTIOXIDANT/DPPH ANALYSIS OF THE ESSENTIAL OILS OF *B. dracunculifolia* FROM TEN POPULATIONS (P) COLLECTED IN THE STATE OF PARANA, BRAZIL.

Composition	Main component		
	PC1	PC2	PC3
α-pinene	**0.4332**	-0.0669	0.1265
β-pinene	0.3310	0.1603	**0.5306**
Limonene	0.3220	0.2527	-0.4388
Germacrene D	-0.3942	-0.1144	0.2433
bicyclogermacrene	-0.374	0.3921	0.1880
(E)-nerolidol	-0.1869	-0.5681	-0.3871
spathulenol	-0.0862	**0.6298**	-0.3202
β-opaen-4-α-ol	-0.3564	0.1385	-0.2562
α-cadinol	-0.3657	0.0180	0.3116
Variance (eigenvalue)	4.555	1.727	1.467
Cumulative variance (%)	50.61	69.80	86.10

SOURCE: The author (2017).

In a previous study, multivariate tools were also applied to understand the chemical diversity of volatile compounds in 9 populations of *B. dracunculifolia.* Different from the observed in the present study, however, the analysis revealed the existence of two chemotypes: one characterized by the predominance of alcohols derived from aromadendrene (spathulenol, globulol and viridiflorol) and the other with the predominance of cadinane-derived compounds (γ-cadinene, δ-cadinene, T-cadinol and α-cadinol) (FRIZZO et al., 2008).

For *Baccharis trimera* samples collected in different states of southern Brazil, the PCA analysis separated the samples into two distinct groups: volatile samples extracted from the cladodes and from shoots or inflorescences. Nevertheless, similarly to the results here presented, the authors suggested that the same chemotype of *B. trimera* occurs in the three southern states of Brazil and the strongest factor to discriminate the oils in that species seems to be the plant organ from where it is collected (BESTEN et al., 2013). For the same species, environmental conditions have been shown to exert significant influence on essential oil biosynthesis (SILVA et al., 2007).

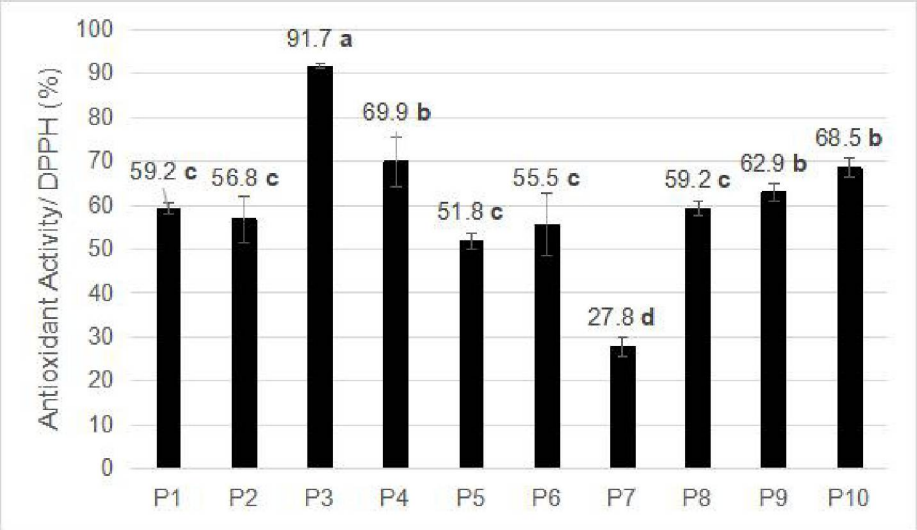
Previous studies have also shown that the chemical composition of *B. dracunculifolia* volatile oils can be greatly influenced by external factors, including the altitude and latitude of a particular site (FRIZZO et al., 2008). However, populations from different sites, under the influence of the different climatic conditions were separated into the same group according to their chemical composition, such as I) P1

and P5; II) P2 and P8 (FIGURE 2.3). This reinforces that other factors besides those evaluated in this study, e.g. genetic and climatic, may have influenced the terpenic composition of the plant.

2.3.3 Antioxidant activity

The essential oils from different *B. dracunculifolia* populations presented antioxidant activities ranged from 27.78 to 91.67%. The highest free radical-scavenging capacity was found in population P3 (FIGURE 2.4). Pearson's correlation analysis showed a significant negative correlation between the antioxidant activity and the compound spathulenol (r = -0.696) in the oils (TABLE 2.4).

FIGURE 2.4 - MEANS AND STANDARD ERRORS OF THE ANTIOXIDANT ACTIVITIES (DPPH METHOD) OF ESSENTIAL OILS OF TEN *B. dracunculifolia* populations (P) FROM THE STATE OF PARANA, BRAZIL (MARCH, 2016). MEANS FOLLOWED BY THE SAME LETTER DO NOT DIFFER BY SCOTT-KNOTT TEST AT 5% OF PROBABILITY.



SOURCE: The author (2017).

Antioxidant compounds are widely studied because of their ability to retard or inhibit reactions that cause ROS damage to living cells, therefore protecting biological systems and functionality of important biomolecules such as lipids, proteins and DNA (AHMAD et al., 2008). Studies on biological activities of *Baccharis* species have shown a wide array of possible applications, mainly related to the allelopathic, antimicrobial, cytotoxic, anti-inflammatory and antioxidant effects (VERDI et al., 2005).

TABLE 2.4 - PEARSON CORRELATION COEFFICIENTS BETWEEN *B. dracunculifolia* ESSENTIAL OIL CONTITUENTS, ANTIOXIDANT ACTIVITY (DPPH) AND CLIMATIC FACTORS: ALTITUDE, RADIATION (RAD), TEMPERATURE (TEMP) AND PRECIPITATION (PREC).

Composition	DPPH	p-value	Altitute	p-value	Rad	p-value	Temp °C	p-value	Prec	p-value
β-pinene	-0.24	0.68	0.04	0.64	0.04	0.62	-0.53	0.93	-0.54	0.1
α-pinene	0.01	0.73	0.17	0.77	0.17	0.62	-0.03	0.11	-0.15	0.66
limonene	0.03	0.92	-0.40	0.11	-0.40	0.24	0.10	0.77	0.57	0.08
spathulenol	**-0.69***	0.03	0.53	0.11	**-0.67***	0.03	-0.31	0.37	0.31	0.38
β-opaen-4-α-ol	-0.01	0.86	0.05	0.88	0.05	0.87	0.037	0.91	-0.05	0.87
bicyclogermacrene	-0.50	0.12	-0.14	0.69	0.26	0.46	-0.49	0.14	-0.30	0.38
α-cadinol	0.09	0.78	0.12	0.77	0.20	0.56	-0.35	0.31	-0.29	0.41
germacrene D	-0.11	0.71	0.25	0.48	0.01	0.95	0.047	0.89	0.01	0.99
(*E*)-nerolidol	0.47	0.267	0.27	0.43	0.45	0.19	**0.76***	0.01	0.30	0.38

SOURCE: The author (2019).
SUBTITLE: *significant at 5% of probability.

Antioxidant compounds are widely studied because of their ability to retard or inhibit reactions that cause ROS damage to living cells, therefore protecting biological systems and functionality of important biomolecules such as lipids, proteins and DNA (AHMAD et al., 2008). Studies on biological activities of *Baccharis* species have shown a wide array of possible applications, mainly related to the allelopathic, antimicrobial, cytotoxic, anti-inflammatory and antioxidant effects (VERDI et al., 2005).

Scavenging of reactive oxygen species, such as superoxide, hydrogen peroxide, hydroxyl radical, among others, is possible by the use of enzymatic (e.g. superoxide dismutase, catalase, ascorbate peroxidase, among others) and non-enzymatic (i.e. antioxidant compounds) mechanisms (AHMAD et al., 2008; TAIZ; ZEIGER, 2013). Neto et al. (2013) showed a significant increase in the activity of superoxide dismutase after (*E*)-nerolidol treatment in mice, inferring the antioxidant-stimulant effect of this sesquiterpene.

Significant antioxidant activities have been previously reported for the major components here identified in *B. dracunculifolia* essential oils (i.e: β-pinene, (*E*)-nerolidol and limonene) (DORMAN et al., 2000; CHAN et al., 2016; YU et al., 2017), suggesting the possibility of using such compounds and the oils from the different populations in pharmaceutical and cosmetic formulations. On other hand, low antioxidant activity was reported for the essential oils of *B. dracunculifolia* and other plant species by Miranda (2016). This may occur because some compounds do not have hydrogen atoms in the allylic and/or benzyl positions.

Previous studies conducted on *Baccharis* species which produced β-pinene as major compound or in high contents showed low free radical capture by DPPH (RUBERTO et al., 2000; SILVA et al., 2018). Therefore, such compound is probably not the main responsible for the antioxidant activity of the essential oils of various plant species.

Although it was not possible to identify a positive correlation of the antioxidant activity with the grouping of the populations or with any specific compound in the oils, the antioxidant potential found in some samples can be a result of synergistic effects of two or more compounds. Accordingly, studies with essential oil of *Calophyllum Cardiopetalum* leaves, for example, have not correlated isolated compounds with antioxidant activity, however, when β-caryophyllene was combined with phenolic compounds, an evident increase in antioxidant capacity by means of synergistic effects was observed (XAVIER et al., 2016). Therefore, the correlation between compounds that have potential biological activities is a complex feature, as the synergistic effect between metabolites can have major effects on these activities (BITTENCOURT et al., 2015).

Interestingly, spathulenol showed significant negative correlation with both antioxidant activity and radiation levels (TABLE 2.4). Such phenomena may suggest that other compounds or group of compounds, with stronger antioxidant activity, are accumulated in detriment of spathulenol to alleviate oxidative stress generated by radiation in *B. dracunculifolia*. Similarly, spathulenol contents in sage (*Salvia officinalis* L.) fruits are reported to decrease under NaCl stress (TAARIT et al., 2009), a condition that is also known to produce oxidative damages in plant cells. *(E)*-nerolidol, on the other hand, showed positive correlation with temperature, suggesting that it may play some role on alleviation of heat stress on *B. dracunculifolia.* Similarly, heat stressed chamomile plants were reported to produce higher amounts of nerolidol (GHASEMI et al., 2016). Another possibility is the fact that higher temperatures, up to 30°C, increase herbivory (LEMOINE et al., 2014), and nerolidol, as a well studied inducible herbivore-defense mechanism (CHAN et al., 2016), may be accumulated in response to such environmental conditions.

The results here presented highlight one of the many natural products biosynthesized by *B. dracunculifolia*. This versatile aromatic species can also be explored by beekeepers for the production of Brazilian green propolis and the extract from the leaves can yield significant amounts of the high added-value compound

Artepillin C (3,5-diprenyl-4-hydroxycinnamic acid) (PARK et al., 2004; SFORCIN et al., 2012). The knowledge of phytochemical diversity and biological activities are crucial steps for sustainable exploitation and must be followed by agronomic studies in order to assure the availability of raw material for current and future uses of this species as the understanding of its biological properties progresses.

2.4 CONCLUSIONS

In view of the chemical variability the populations are separated into three groups according to the concentration of its terpenic compounds: I) populations with low concentration of α-pinene, II) populations with high concentrations of α-pinene and β-pinene and III) populations with intermediate concentrations of α-pinene, β-pinene and spathulenol.

The populations have the same terpenic compounds, but with quantitative differences between them.

The differences in essential oil yield and quantitative composition may be related to genetic factors and other factors not explored in this study.

The major components of the studied populations are β-pinene and (*E*)-nerolidol.

The antioxidant capacity of oils from different populations presents a wide variation, related to its chemical composition, and with a negative correlation between this activity and spathulenol.

REFERENCES

ABDEL-LATEIF, K. S.; ELDEAB, H. A.; MAGHRABI, I. A. The plant natural products: their antioxidants, free radical scavengers, DNA protection and antimicrobial activities. **Journal of Bioprocessing and Biotechniques**, v. 6, p. 2-7, 2016.

ADAMS, R. P. **Identification of essential oil components by gas chromatography/mass spectrometry**. Carol Stream: Allured Publishing Corporation, 2007.

AHMAD, P.; SARWAT, M.; SHARMA, S. Reactive oxygen species, antioxidants and signaling in plants. **Journal of Plant Biology**, v. 51, p. 167-173, 2008.

ALVARES, C. A. et al. Koppen's climate classification map for Brazil. **Meteorologische Zeitschrift**, v. 22, n. 6, p. 711–728, 2013.

ARRUDA, D. C. et al. Antileishmanial activity of the terpene nerolidol. **Antimicrobial Agents and Chemotherapy**, v. 5, p. 1679-1687, 2005.

BANO, U. et al. Effect of plant growth regulators on essential oil yield in aromatic plants. **Journal of Chemical and Pharmaceutical Research**, v. 8, p. 733-739, 2016.

BARBOSA, L. N. et al. *In vitro* antibacterial and chemical properties of essential oils including native plants from Brazil against pathogenic and resistant bacteria. **Journal of Oleo Science**, v. 64, p. 289-298, 2015.

BARROSO, G. M.; BUENO, O. L. **Flora ilustrada catarinense plantas compostas-subtribo: Baccharidinae**. Itajaí: Herbário Barbosa Rodrigues (HBS), 2002.

BESTEN, M. A. et al. Chemical composition of volatiles from male and female specimens of *Baccharis trimera* collected in two distant regions of southern Brazil: a comparative study using chemometrics. **Química Nova**, v. 36, p. 1096-1100, 2013.

BITTENCOURT, M. L. F. et al. Metabolite profiling, antioxidant and antibacterial activities of Brazilian propolis: Use of correlation and multivariate analyses to identify potential bioactive compounds. **Food Research International**, v. 76, p. 449-457, 2015.

BOLDT, P. E. ***Baccharis*** **(Asteraceae). A Review of its taxonomy, phytochemistry, ecology, economic status, natural enemies and potential for its biological control in the United States**. Washington: USDA. Agricultural Research Service Grassland, 1989.

BRAND-WILLIAMS, W.; CUVELIER, M. E.; BERSET, C. Use of a free radical method to evaluate antioxidant activity. **LWT- Food Science and Technology**, v. 28, p. 25-30, 1995.

BÚFALO, M. C. et al. In vitro cytotoxic activity of *Baccharis dracunculifolia* and propolis against HEp-2 cells. **Natural Product Research**, v. 24, p. 1710–1718, 2010.

CASAGRANDE, M. et al. Influence of time, temperature and solvent on the extraction of bioactive compounds of *Baccharis dracunculifolia*: *In vitro* antioxidant activity, antimicrobial potential, and phenolic compound quantification. **Industrial Crops and**

Products, v. 125, p. 207–219, 2018.

CHAABAN, A. et al. Insecticide activity of *Baccharis dracunculifolia* essential oil against *Cochliomyia macellaria* (Diptera: Calliphoridae). **Natural Product Research**, v. 32, p. 2954-2958, 2017.

CHAI, T.T. et al. Water stress-induced oxidative damage and antioxidant responses in micropropagated banana plantlets. **Biologia Plantarum**, v. 49, p. 153-156, 2005.

CHAN, W. K. et al. Nerolidol: a sesquiterpene alcohol with multi-faceted pharmacological and biological activities. **Molecules**, v. 21, p. 1-40, 2016.

DAJIC-STEVANOVIC, Z.; PLJEVLJAKUSIC, D. **Challenges and decision making in cultivation of medicinal and aromatic plants**. Dordrecht: Springer, 2015.

DE SOUSA, J. P. et al. Seasonal variation of the *(E)*-nerolidol and other volatile compounds within ten different cultivated populations of *Baccharis dracunculifolia* D.C. (Asteraceae). **Journal of Essential Oil Research**, v. 21, p. 308-314, 2009.

DORMAN H. D. et al. *In vitro* evaluation of antioxidant activity of essential oils and their components. **Flavour and Fragrance Journal**, v. 15, p. 12-16, 2000.

FERRONATTO, R. et al. Antimicrobial activity of essential oils produced by *Baccharis dracunculifolia* D.C. and *Baccharis uncinella* D.C. (Asteraceae). **Revista Brasileira de Farmacognosia**, v. 17, p. 224-230, 2007.

FIGUEIREDO, A. C. et al. Factors affecting secondary metabolite production in plants: volatile components and essential oils. **Flavour and Fragrance Journal**, v. 23, p. 213-226, 2008.

FLORÃO, A. et al. Essential oils from *Baccharis* species (Asteraceae) have anti-inflammatory effects for human cells. **Journal of Essential Oil Research**, v. 24, p. 561-570, 2012.

FRIZZO, C. D. et al. Essential oil variability in *Baccharis uncinella* DC and *Baccharis dracunculifolia* DC growing wild in southern Brazil, Bolivia and Uruguay. **Flavour and Fragrance Journal**, v. 23, p. 99-106, 2008.

GHASEMI, M. et al. Increase of chamazulene and α-bisabolol contents of the

essential oil of german chamomile (*Matricaria chamomilla* L.) using salicylic acid treatments under normal and heat stress conditions. **Foods**, v. 5, p. 1-14, 2016.

JEYARAMRAJAA, P.R. et al. Water deficit induced oxidative damage in tea (*Camellia sinensis*) plants. **Journal of Plant Physiology**, v. 162, p. 413-419, 2005.

LAGE, T. C. A. et al. Chemical composition and acaricidal activity of the essential oil of *Baccharis dracunculifolia* De Candole (1836) and its constituents nerolidol and limonene on larvae and engorged females of *Rhipicephalus microplus* (Acari: Ixodidae). **Experimental Parasitology**, v. 148, p. 24-29, 2015.

LEMOINE, N. P.; BURKEPILE, D. E.; PARKER, J. D. Variable effects of temperature on insect herbivory. **PeerJ**, v. 2, p. e376, 2014.

LIU, H. et al. UV-B irradiation differentially regulates terpene synthases and terpene content of peach. **Plant, Cell & Environment**, v. 40, p. 2261–2275, 2017.

LOPES, R. C. et al. Different hydric influencing essential oil production from *Plygonum punctatum* Ell. **Revista Brasileira de Plantas Medicinais**, v. 3, p. 7-10, 2001.

MARTINEZ-CORREA, H. Á. et al. Extracts from the leaves of *Baccharis dracunculifolia* obtained by a combination of extraction processes with supercritical CO2, ethanol and water. **The Journal of Supercritical Fluids**, v. 63, p. 31-39, 2012.

MIRANDA, C. A. S. F. et al. Essential oils from leaves of various species: antioxidant and antibacterial properties on growth in pathogenic species. **Revista Ciência Agronômica**, v. 47, p. 213-220, 2016.

NETO, J. D. N. et al. Antioxidant effects of nerolidol in mice hippocampus after open field test. **Neurochemical Research**, v. 38, p. 1861-1870, 2013.

PACCIARONI, A. V. et al. Bioactive constituents of *Conyza albida*. **Planta Medica**, v. 66, p. 720-723, 2000.

PARK, Y. K. et al. Chemical constituents in *Baccharis dracunculifolia* as the main botanical origin of southeastern brazilian propolis. **Journal of Agricultural and Food Chemistry**, v. 52, p. 1100-1103, 2004.

PARREIRA, N. A. et al. Antiprotozoal, schistosomicidal, and antimicrobial activities of the essential oil from the leaves of *Baccharis dracunculifolia*. **Chemistry & Biodiversity**, v. 7, p. 993-1001, 2010.

PAZOUKI, L.; NIINEMETS, Ü. Multi-substrate terpene synthases: their occurrence and physiological significance. **Frontiers in Plant Science**, v. 7, p. 1-16, 2016.

PEGORINI, F.; MARANHÃO, L. T.; ROCHA, L. D. Organização estrutural das folhas de *Baccharis dracunculifolia* DC. Asteraceae. **Revista Brasileira de Farmácia**, v. 89, p. 272-275, 2008.

PEREIRA, C. B. et al. Chemical composition and biological activities of *Baccharis milleflora* essential oil. **Latin American Journal of Pharmacy**, v. 35, p. 2225-2233, 2016.

QUEIROGA, C. L. et al. Comparison of the chemical composition of the essential oil and the water soluble oil of *Baccharis dracunculifolia* DC. (Asteraceae). **Journal of Essential Oil Research**, v. 20, p. 111-114, 2008.

R CORE TEAM. R: **A language and environment for statistical computing.** R Foundation for Statistical Computing, Vienna, Austria, 2018.

RAVEN, P. H.; EVERT, R. F.; EICHHORN, S. E**. Biology of plants**. New York: W. H. Freeman and Company, 2005.

RIGOTTI, M. ***Baccharis dracunculifolia*** **D.C.: diversidade genética e química de populações.** 2011. 94 f. Tese (Doutorado em Agronomia - Horticultura) - Faculdade de Ciências Agronômicas, Universidade Estadual Paulista "Júlio de Mesquita Filho", Botucatu (SP), 2011. Disponível em: https://repositorio.unesp.br/bitstream/handle/11449/133997/000858227.pdf?sequence=1&isAllowed=y. Acesso em 04 mai. 2019.

RUBERTO, G.; BARATTA, M. T. Antioxidant activity of selected essential oil components in two lipid model systems. **Food Chemistry**, v. 69, p. 167-174, 2000.

SALAZAR, G. J. T. et al. Phytochemical characterization of the *Baccharis dracunculifolia* DC (Asteraceae) essential oil and antibacterial activity evaluation. **Industrial Crops and Products**, v. 122, p. 591-595, 2018.

SANTOS, R.F. et al. Composição química e produtividade dos principais

componentes do óleo essencial de *Baccharis dracunculifolia* DC. em função da adubação orgânica. **Revista Brasileira de Plantas Medicinais,** v. 14, p. 224-234, 2012.

SCHONEWALD-COX, M. C. et al. **Genetics and conservation: a reference for managing wild animal and plant populations**. Menlo Park: Benjamin Cummings Publishing Company, 1983.

SELANI, M. M. et al. Wine industry residues extracts as natural antioxidants in raw and cooked chicken meat during frozen storage. **Meat Science**, v. 88, p. 397–403, 2011.

SFORCIN, J. M. et al. *Baccharis dracunculifolia*, **uma das principais fontes vegetais da própolis brasileira**. São Paulo: Unesp, 2012.

SILVA, E. R. Antioxidant Evaluation of *Baccharis* patens and *Baccharis psiadioides* essential oils. **Journal of Essential Oil Research**, v. 21, p. 485-492, 2018.

SILVA, F. A. S.; AZEVEDO, C. A. V. The assistat software version 7.7 and its use in the analysis of experimental data. **African Journal of Agricultural Research**, v. 11, p. 3733–3740, 2016.

SILVA, F. G. et al. Seasonal variability in the essential oils of wild and cultivated *Baccharis trimera*. **Journal of the Brazilian Chemical Society**, v. 18, p. 990-997, 2007.

SOUZA, J. R. P. et al. Development of holy-thorn under different light intensities and pruning levels. **Horticultura Brasileira**, v. 26, p. 40-44, 2008.

TAARIT, M. B. et al. Plant growth, essential oil yield and composition of sage (*Salvia officinalis* L.) fruits cultivated under salt stress conditions. **Industrial Crops and Products**, v. 30, p. 333-337, 2009.

TAIZ, L.; ZEIGER, E. **Plant Physiology**, Sunderland: Sinauer Associates Inc, 2013.

TIIVA, P. T. et al. Isoprene emission from a subarctic peatland under enhanced UV-B radiation. **New Phytologist**, v. 176, p. 346-355, 2007.

ULUBELEN, A. et al. Terpenoids from *Salvia sclarea*. **Phytochemistry**, v. 36, p. 971-

974, 1994.

URBANSKA, K. M. **Differentiation Patterns in Higher Plants**. New York: Academic Press, 1987.

VAITHIYANATHAN, S. et al. Effect of dipping in pomegranate (*Punica granatum*) fruit juice phenolic solution on the shelf life of chicken meat under refrigerated storage (4 °C). **Meat Science**, v. 88 p. 409-414, 2011.

VAN DEN DOOL, H.; KRATZ, P. D. A generalisation of the retention index system including linear temperature programmed gas-liquid chromatography. **Journal of Chromatography**, v. 11, p. 463-471, 1963.

VERDI, L. G.; BRIGHENTE, I. M. C.; PIZZOLATTI, M. G. Gênero *Baccharis* (Asteraceae): aspectos químicos, econômicos e biológicos. **Química Nova**, v. 28, p. 85-94, 2005.

VERMA, N.; SHUKLA, S. Impact of various factors responsible for fluctuation in plant secondary metabolites. **Journal of Applied Research on Medicinal and Aromatic Plants**, v. 2, p. 105-113, 2015.

VRANOVÁ, E.; COMAN, D.; GRUISSEM, W. Network analysis of the MVA and MEP pathways for isoprenoid synthesis**. Annual Review of Plant Biology**, v. 64, p. 665-700, 2013.

XAVIER, M. N. et al. Chemical composition from essential oil of *Cardiopetalum calophyllum* Schltdl. (Annonaceae) and their antioxidant, antibacterial and antifungal activities. **Revista Virtual de Química**, v. 8, p. 1433-1448, 2016.

YU, L.; YAN, J.; SUN Z. D-limonene exhibits anti-inflammatory and antioxidant properties in an ulcerative colitis rat model via regulation of iNOS, COX-2, PGE2 and ERK signaling pathways. **Molecular Medicine Reports**, v. 15, p. 2339-2346, 2017.

3 AVALIAÇÃO SAZONAL DOS COMPOSTOS VOLÁTEIS E DA ATIVIDADE ANTIOXIDANTE DE *Baccharis dracunculifolia* DC.

RESUMO

Baccharis dracunculifolia DC. (Asteraceae) é uma espécie vegetal nativa brasileira amplamente distribuída na América do Sul. O óleo essencial produzido pela planta possui várias atividades biológicas e aplicações cosméticas. A produção de compostos terpenos pode ser afetada por diferentes fatores, tais como a sazonalidade, que pode influenciar diretamente o rendimento e a composição química dos óleos essenciais. Nesse sentido, o principal objetivo deste estudo foi avaliar o efeito da sazonalidade no rendimento, na composição química e na atividade antioxidante dos óleos essenciais de *B. dracunculifolia.* A extração dos compostos voláteis foi realizada por hidrodestilação, a composição química das espécies foi realizada por GC-FID e GC-MS e a atividade antioxidante avaliada pelo método DPPH. Diferenças significativas foram verificadas no teor de óleos essenciais durante as quatro estações do ano. O conteúdo variou de 0,39 a 0,87% e no outono houve maior produção de óleo essencial (0,87%). As principais classes de compostos identificados foram os sesquiterpenos oxigenados (34,08-51,59%), hidrocarbonetos monoterpenos (29,29-35,8%), hidrocarbonetos sesquiterpenos (10,96-21,92%) e monoterpenos oxigenados (0,59-0,90%). Os principais compostos foram: *(E)*-nerolidol (8,91-21,68%), β-pineno (8,94-16,15%) e limoneno (11,74-13,64%). A maior atividade antioxidante foi identificada durante o verão (88,89%), com uma correlação positiva alta com limoneno (r= 0,977). De acordo com análises multivariadas, as amostras foram separadas em dois grupos principais: I) inverno e primavera: altos níveis de α-pineno (2,38-4,48%), β-pineno (15,58-16,15%) e germacreno D (3,03-5,55%) ; II) verão e outono: altos níveis de *(E)*-nerolidol (17,81-21,68%). Esses resultados sugerem que o período mais apropriado para a coleta e processamento de *B. dracunculifolia* para obtenção de óleo essencial com maiores teores de *(E)*-nerolidol é durante o verão e outono (fase de floração) e β-pineno durante o inverno e a primavera (fase vegetativa).

Palavras-chave: Alecrim-do-campo. Estação. *(E)*-Nerolidol. β-pineno. DPPH.

3 SEASONAL EVALUATION OF VOLATILE COMPOUNDS AND ANTIOXIDANT ACTIVITY OF *Baccharis dracunculifolia* DC.

ABSTRACT

Baccharis dracunculifolia DC. is a Brazilian native plant species (Asteraceae) widely distributed in South America. The essential oil produced by the plant has several biological activities and cosmetic applications. The production of terpene compounds can be affected by different factors such as seasonality, that has an important influence on the yield and chemical composition of essential oils. In this sense, the main goal of this study was to evaluate the effect of seasonality on the yield, chemical composition and antioxidant activity of *B. dracunculifolia* essential oils. The extraction of the volatile compounds was carried out by hydrodistillation, the analyses of chemical composition of the species were performed by GC-FID and GC-MS, while antioxidant activity was evaluated by the DPPH method. Significant differences were observed in the content of essential oils among the four seasons. The contents ranged from 0.39 to 0.87% and there was a higher production of essential oil during autumn (0.87%). The chemical characterization were predominantly composed of oxygenated sesquiterpenes (34.08-51.59%), monoterpene hydrocarbons (29.29-35.8%), sesquiterpenes hydrocarbons (10.96-21.92%) and oxygenated monoterpene (0.59-0.90%). The major compounds were: *(E)*-nerolidol (8.91-21.68%), β-pinene (8.94-16.15%) and limonene (11.74-3.64%).The highest antioxidant activity was identified during summer (88.89%), with a high positive correlation with limonene (r= 0.977). According to multivariate analyzes the samples were separated into two main groups: I) winter and spring: high levels of α-pinene (2.38-4.48%), β-pinene (15.58-16.15%) and germacrene D (3.03 - 5.55%); II) summer and autumn: high levels of *(E)*-nerolidol (17.81- 21.68%). These results suggest that the most appropriate season for the collection and processing of *B. dracunculifolia* to obtain essential oil with higher levels of *(E)*-nerolidol is during summer and autumn (flowering phase) and β-pinene during winter and spring (vegetative phase).

Keywords: Alecrim-do-campo. Season. *(E)* -Nerolidol. β-pinene. DPPH.

3.1 INTRODUCTION

Baccharis dracunculifolia DC. popularly known as ‘alecrim-do-campo’, ‘vassourinha’ and ‘alecrim-vassoura’ is a Brazilian native plant species (Asteraceae) widely distributed in South America (BARROSO; BUENO, 2002). The species is common in a specific biome of Brazil known as 'cerrado area’, also found in abandoned areas, areas in succession, easily adapting to habitats that have low nutrient levels (BOLDT, 1989). The plant is a semi-woody shrub, with a height of 2-3 meters, perennial, dioecious and reproduces by seeds. Although *B. dracunculifolia* is well known for the important commercial product called green propolis produced by honeybees (PARK et al., 2004), the plant is also used in folk medicine for treatment of gastrointestinal diseases and as a natural anti-inflammatory (LEMOS et al., 2007; FLORÃO et al., 2012).

The species produces an essential oil which is rich in monoterpenes hydrocarbon, oxygenated monoterpenes, sesquiterpene hydrocarbons and oxygenated sesquiterpenes (MIRANDA et al., 2016). Some terpenoids present in plants play important functions, such as the interaction between plant and insect for pollination, repellency against herbivory, and are directly related to plant defense and stress response (DUDAREVA et al., 2004). *B. dracunculifolia* essential oils are stored in glandular multicellular trichomes, which are also responsible for increasing the light radiation reflectance, preventing excessive water loss and assisting in maintaining leaf temperature (RAVEN, 2007; PEGORINI et al., 2008).

The major compounds identified in the essential oil of the species are sesquiterpenes *(E)*-nerolidol and Spathulenol, however, monoterpenos α-pinene, β-pinene, β-Caryophyllene, germacrene D, bicyclogermacrene, mircene and limonene are also found with relatively high contents (DE SOUSA et al., 2009; PARREIRA et al., 2010; SANTOS et al., 2012 a; SALAZAR et al., 2018). *(E)-n*erolidol is one of the major compounds present in the volatile fraction of the plant and previous studies have described important anti-ulcer activity of the compound (KLOPELL et al., 2007). Besides its pharmacological and biological activities (antioxidant, antibacterial, anti-fungal, antitrypanosomal and insecticidal (CHAN et al., 2016), (E)-nerolidol is used as an ingredient in fine fragrances, cosmetics, shampoos, toilet soaps, cleaners and detergents (LAPCZYNSKI et al., 2008). Spathulenol is another important compound present in the essential oil and previous studies showed that this compound has a

strong cytotoxicity against Leukemia Cell (L 1210) growth (FUKUDA et al., 2006) and moderately cytotoxicity against human oral epidermal carcinoma cellular line - KB cells (PACCIARONI et al., 2000). In addition, α-Pinene and β-Pinene enantiomers are common in many plant species, including *B. dracunculifolia*, and have important antifungal and antibacterial activities (LEITE et al., 2007; DA SILVA et al., 2012).

The production and constitution of volatile compounds can be qualitative and quantitative and depends on different factors such as plant development stage, pollinators, plant material type, mechanical injuries, pollution, diseases, photoperiod, radiation, seasonality, nutrition, salinity , humidity, temperature, plant regulators and storage structures (FIGUEIREDO et al., 2008; BANO et al., 2016). Among the factors mentioned, seasonality have an important influence on the yield and chemical composition of essential oils, for the same species from different regions, large changes in the plant chemical profile may occur, according to the phenological stage of the plant, since metabolic pathways may be directed to plant growth during the vegetative stage in relation to flowering and fruiting plants (FIGUEIREDO et al., 2008; SALEM et al., 2018). Additionally, the higher contents of these compounds may also be related to the activity of pollinating insects in flowering seasons (MAFFEI, 2010).

Besides that, several climatic factors common to each time of the year, such as radiation, may have an influence on the production of volatile compounds in plants, as phytoprotective responses in response to ultraviolet (UV) light, however a study has indicated reduction of 60% in the concentration of monoterpene linalol in response to incident radiation (LIU et al., 2017). In previous work developed by De Sousa et al. (2009), the major compounds of *B. dracunculifolia* plants, e.g. *(E)*-nerolidol and spathulenol, changed considerably over the year, where the concentration of *(E)*-nerolidol was five times higher during autumn than during winter. The spathulenol concentration increased about three times in autumn compared to winter. *Baccharis trimera* (Less.) DC. plants from natural populations also showed seasonal variations in secondary metabolism, where germacrene D was higher during spring and bicyclogermacrene during winter, spring and summer (SILVA et al., 2007).

The knowledge of the seasonal chemical profile variation of medicinal aromatic plants with wild growth in Brazil is extremely important to standardize their application in different areas. Since the use of *B. dracunculifolia* is wide in several areas, including pharmaceutical, cosmetic, ecological (interaction with honeybees

Apis mellifera L.) and apiculture products industry (especially green propolis production), this knowledge is even more necessary. Studies related to seasonal variation of chemical profile and yield of *B. dracunculifolia* essential oils are specific to some regions of Brazil, and information aderessing to the southern region, such as Parana state is needed. Therefore, to study this chemical variability, multivariate statistics tools like Principal Component and Cluster analysis were applied to detect patterns of similarity of the essential oil of the plant throughout the year. In this sense, the main goal of this study was to evaluate the effect of seasonality in the yield, chemical composition and antioxidant activity of *B. dracunculifolia* essential oils.

3.2 MATERIAL AND METHODS

3.2.1 Collection of plant material and essential oil extraction

Branches with leaves of approximately twenty-five *B. dracunculifolia* individuals were collected from the field in a natural population, in southern Brazil, Palmeira city, Parana state (25°19'80''S - 49°48'35''W, altitude 1027 meters) located in the area of 'Reserva Particular do Patrimônio Natural (RPPN) Butuquara', with the licenses (09.18) of the Environmental Institute of Paraná (IAP) for collecting and transporting plant material. The voucher specimen was deposited in the Municipal Botanical Museum of Curitiba - MBM Herbarium, with the deposit number: MBM-384.962. The registration number of the species in the '*Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado* - SisGen' is AC74344. The climate of the region is classified as Cfb according to the Alvares et al. (2013). In total, four seasonal collections were performed over a one year period: autumn/2016 (03/23/16), winter/2016 (07/13/16), spring/ 2016 (11/14/16) and summer/2017 (02/21/17).

The extraction of the essential oil was performed by hydrodistillation in Clevenger equipment for 2 hours and 30 minutes, using 100 g of fresh leaf mass in 1L off distilled water, in three replications. Additionally, 20g of aerial part leaves were weighed for oven drying (65°C) until a constant mass was reached. The essential oil yield was calculated based on the dry weight, by determining the moisture and the result was expressed as a percentage (%). The extracted oil was stored in a freezer at -5°C until the time of analysis.

3.2.2 Determination and quantification of chemical composition

Analyzes for the determination of chemical composition and quantification of oil compounds were performed by gas chromatography (GC), supported by GC/FID (Agilent 7890A) and GC/MS (Shimadzu-2010 Plus), both equipped with HP-5MS capillary column (30 m x 0,25 mm x 0,25 µm). The carrier gas was hydrogen for GC/FID (flow 1.5 mL/min) and helium for GC/MS (flow 1.0 mL/min). The injector temperature remained constant at 250°C throughout the analysis. The essential oil was injected into the equipment (1 µL) at 1% concentration by diluting it in hexane (HPLC grade - PA). The initial oven temperature was 60ºC, rising to 240ºC at a rate of 3ºC/min. For GC–MS, the interface temperature was at 300°C and mass range of m/z 40–400. The mass detector was operated in the ionization mode (electron impact 70 eV) at 3.15 scan s^{-1} and the ion source was maintained at 230 °C. For the identification of chemical constituents, a homologous series of alkanes (C7 - C30) was used to calculate the linear retention index (VAN DEN DOOL, 1963). In addition, the mass spectrum of each compound was compared with literature data (ADAMS, 2007). The quantification of constituents was performed by dividing the peak area of each compound by the total area of the identified compounds (%). This analysis was performed in three replications.

3.2.3 Determination of antioxidant activity

The antioxidant activity was determined by using the 2,2-diphenyl-1picri-1-hydrazyl radical and oxidizing agent (DPPH) as previously described (BRAND-WILLIAMS et al., 1995). The method is based on reducing the DPPH radical by transferring an antioxidant compound. When the radical is neutralized, the solution changes color from violet to light yellow. Initially, the essential oil was diluted in MeOH (PA) and analyzed at a concentration of 20%. After this, the DPPH control solution was prepared, where 2.366 mg DPPH was diluted in 100 mL MeOH (m/v) and an aliquot of 100 µl of the essential oil was added to 3.9 mL of the DPPH control solution (23.66 $\mu g.ml^{-1}$). After 30 minutes at room temperature, the solution was transferred to a quartz cuvette (3mL) and subjected to UV-Vis spectrophotometry to determine absorbance (λ= 515 ηm), in three replications. The results were expressed

as percentage inhibition: AA(%)= ((Abs.control - Abs.sample)/Abs.control)100 where 'Abs.control' is the absorbance of DPPH radical in methanol and 'Abs. sample' is the absorbance of DPPH radical solution + essential oil.

3.2.4 Statistical analysis

The experimental design was completely randomized, comparing the essential oil yield and the antioxidant activity during the four seasonal collections, each with 3 repetitions. Initially, the data were evaluated for the homogeneity of their variances by Bartlett's test. After this, analysis of variance (ANOVA) was applied and when significant differences were detected, the data were submitted to the Tukey at 5% probability ($p < 0.05$), performed by the software ASSISTAT® (SILVA; AZEVEDO, 2016). In addition, the antioxidant activity data and chemical composition of the essential oil were submitted to Pearson correlation analysis and multivariate statistics by principal component calculation (PCA) and cluster analysis (CA), using the statistical software R (v. 3.1. 1) (R CORE TEAM, 2016). The cluster analysis used the Euclidean distance as mean of similarity and the non-weighted arithmetic mean (UPGMA) method was used for the hierarchical grouping process. For these data, the analyzes were performed for compounds with averages ≥ 3.0% in at least one of the samples.

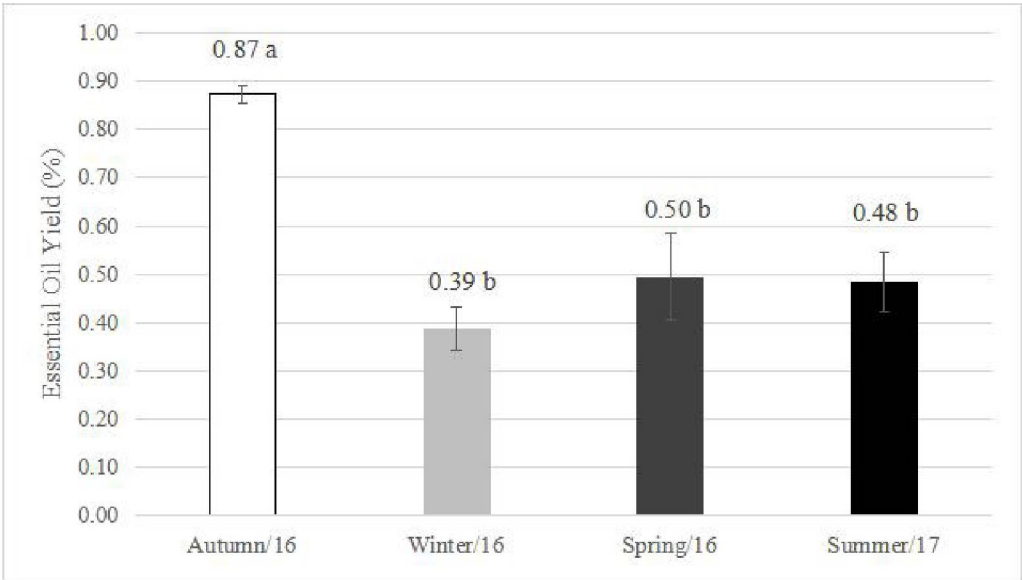
3.3 RESULTS AND DISCUSSIONS

3.3.1 Essential oil yield (%)

Significant differences were observed in *B. dracunculifolia* essential oil content among the four seasons (FIGURE 3.1). The contents ranged from 0.39 to 0.87%, however, during autumn there was a higher production of the essential oil (0.87%), when the plants were in the floral bud stage. Similar results were reported by De Sousa et al. (2009) for *B. dracunculifolia* from south-eastern Brazil - São Paulo state, where the highest levels of essential oil were obtained during the months of March and April (autumn season), 0.39 and 0.35%, respectively. The authors also observed that the oil content decreased from May (0.13%) to July (0.08%), which coincided with the flowering and fruiting period, similar to the present work. Previous work with

species of the Myrtaceae family identified a negative correlation between fruiting and essential oil accumulation (WANNES; MARZOUK, 2012). In addition, work developed with the species *Salvia officinalis* L. identified variation in essential oil accumulation related to plant development stage, where floral budding had higher oil content (0.9%) than vegetative stage (0.7%), flowering (0.5%), immature fruit (0.4%) and ripen fruit (0.2%) (MIRJALILI et al., 2006).

FIGURE 3.1 – ESSENTIAL OIL YIELD (%) IN FRESH LEAVES OF *B. dracunculifolia* COLECTED IN AUTUMN/2016, WINTER/2016, SPRING/2016 AND SUMMER/2017. COEFFICIENT OF VARIATION = 18.40%. MEANS FOLLOWED BY THE SAME LETTER DO NOT DIFFER BY SCOTT-KNOTT TEST AT 5% OF PROBABILITY.



SOURCE: The author (2018)

Numerous factors can affect essential oil production throughout the year, including the stage of plant development, pollinator activity cycle, disease, mechanical injury and climate variation (FIGUEIREDO et al., 2008). The developmental stage of the plant can be determine the production and composition of secondary metabolites, where some studies indicate the greater accumulation of essential oils in open flowers when compared to flower buds. In some cases, volatiles are produced on a larger scale in the flower bud phase (FIGUEIREDO et al., 2008).

In the present study, the increase in *B. dracunculifolia* oil content during the autumn season may correlate with the synthesis of gibberellins in the flowering phase of the species. As both terpenes and gibberellins shared the same pathway of

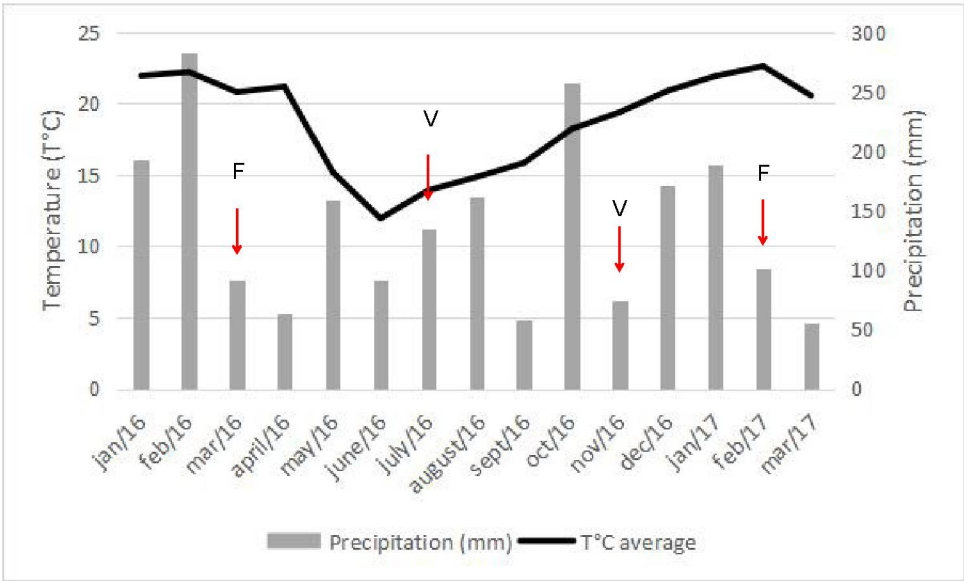
synthesis in the early stages, the increasing transcription of gibberellin biosynthesis genes at the flower bud emergence stage may provide more precursors for essential oil biosynthesis, which may explain the increase in oil content during the flowering phase (Yang et al., 2015). Similarly, for the species *Hyptis marrubioides* Epl. full flowering occurs during summer, being this period with the greatest essential oils accumulation (Botrel et al., 2010).

In addition, environmental conditions such as high temperatures and long days increase the production of essential oils, this is due to the increase in plant mass in response to photosynthetic carbon production to the monoterpene biosynthesis (SANTOS et al., 2012b). In this present work, higher production of essential oil during autumn may be related to weather conditions, such as low precipitation, high temperatures and high radiation levels especially during March/16 and April/16 (FIGURE 3.2, FIGURE 3.3). Many studies point out that low precipitation may contribute to the increase in essential oil content, oil yields are often higher in dry environments, correlating a possible increase in the terpene biosynthesis in response to drought stress (LOPES et al., 2001; FIGUEIREDO et al., 2008). For the native medicinal species of Brazil, *Polygonum punctatum* Ell., there was a 2.65-fold increase in the essential oil content of the plant without water regime (1.03%) when compared to treatment with water regime (0.39%) (LOPES et al., 2001). However, drought stress can limit photosynthesis, alter nutrient absorption, carbon flux, sugar, amino acids and inorganic ions, and consequently, the plant may become most susceptible to attack by pathogens or herbivores (FIGUEIREDO et al., 2008). In addition, high temperatures, common during the summer, can contribute to the volatilization of some constituents of the essential oil (HUSSAIN et al., 2008).

The effects of UV radiation on terpenoids vary with plant species (LLUSIA et al., 2012). Plants may develop structural adaptations such as thicker leaves and the development of multicellular trichomes to increase light reflectance in response to solar radiation, and this can increase essential oil production (RAVEN et al., 2005; SOUZA et al. 2008; PEGORINI et al., 2008). Some terpenoids such as isoprene have well-established specific functions such as the protection of leaves against heat (UV-B radiation) (TIIVA et al., 2007). A variety of terpene synthase (TPS) family enzymes are responsible for the formation of different terpenes in plants, and these enzymes are generally responsible for catalyzing the formation of monoterpenes in

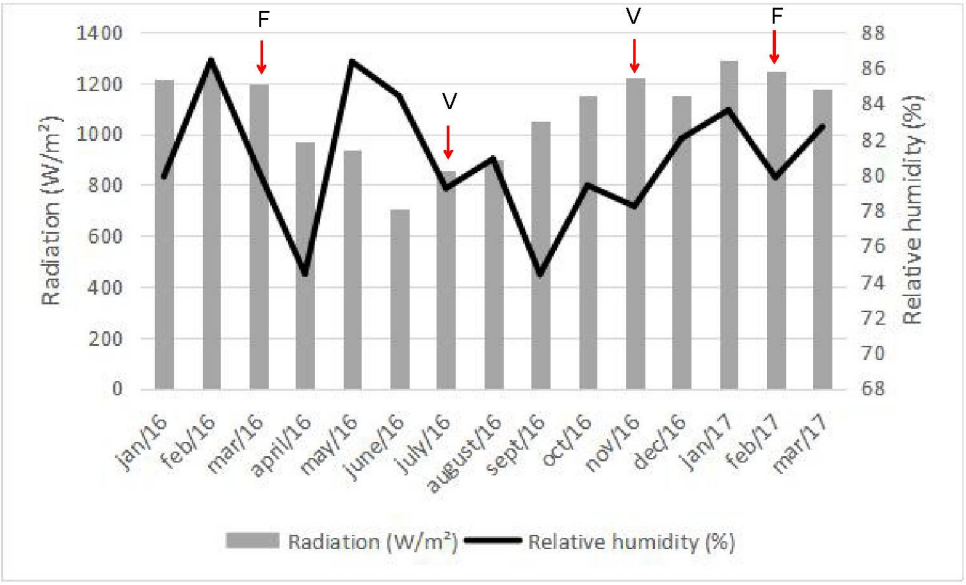
plastids using geranyl diphosphate as substrate and those in the cytoplasm form sequiterpenes from farnesyl diphosphate (PAZOUKI; NIINEMETS, 2016).

FIGURE 3.2 – PRECIPITATION (MM) AND AVERAGE TEMPERATURE (T°C) IN THE COLLECTION SITE, PARANA STATE, BRAZIL. THE RED ARROWS INDICATE THE PLANT HARVEST TIME. PHENOLOGICAL STAGES OF THE PLANT DURING HARVEST: FLOWERING (F) AND VEGETATIVE (V).



SOURCE: Parana Meteorological System - SIMEPAR (2016).

FIGURE 3.3 – RADIATION (W/m²) AND RELATIVE HUMIDITY (%) IN THE COLLECTION SITE, PARANA STATE, BRAZIL. THE RED ARROWS INDICATE THE PLANT HARVEST TIME. PHENOLOGICAL STAGES OF THE PLANT DURING HARVEST: FLOWERING (F) AND VEGETATIVE (V).



SOURCE: Parana Meteorological System - SIMEPAR (2016).

3.3.2 Chemical composition

Thirty-five (35) constituents were identified in the essential oil of *B. dracunculifolia* throughout the four seasons. The content and the chemical composition of the essential oil also change over the seasons. The samples collected seasonally showed the same compounds, but with quantitative variations (TABLE 3.1). The essential oil were predominantly composed of oxygenated sesquiterpenes (34.08-51.59%), monoterpene hydrocarbons (29.29-35.8%), sesquiterpenes hydrocarbons (10.96-21.92%) and oxygenated monoterpene (0.59-0.90%).

TABLE 3.1 - COMPOSITION OF ESSENTIAL OIL % BY GC/FID (MEAN AND STANDARD ERROR) AND OF LEAVES OF *B. dracunculifolia* PLANTS COLLECTED DURING AUTUMN/2016, WINTER/2016, SPRING/2016 AND SUMMER/2017.

(to be continued)

Compounds	RI^{lit}	RI^{cal}	Autumn/16	Winter/16	Spring/16	Summer/17
1- α-tujene	924	925	-	0.12 ± 0.00	0.18 ± 0.02	2.10 ± 0.20
2- α-pinene	**932**	**932**	**3.31 ± 0.80 a**	**2.38 ± 0.03 a**	**4.48 ± 0.41 a**	**2.96 ± 0.27 a**
3- sabinene	969	972	0.21 ± 0.00	ni	ni	ni
4- β-pinene	**974**	**976**	**12.77 ± 0.99 b**	**15.58 ± 0.39 a**	**16.15 ± 0.69 a**	**8.94 ± 0.67 c**
5- myrcene	988	990	1.62 ± 0.27	1.15 ± 0.03	2.01 ± 0.04	1.11 ± 0.49
6- limonene	**1024**	**1026**	**12.58 ± 2.83 a**	**13.06 ± 0.73 a**	**11.74 ± 0.40 a**	**13.64 ± 1.09 a**
7- (E)-β-ocimene	1044	1045	0.9 ± 0.13	1.49 ± 0.04	1.25 ± 0.07	0.54 ± 0.22
8- terpinen-4-ol	1174	1174	0.27 ± 0.04	0.24 ± 0.01	0.31 ± 0.02	0.23 ± 0.05
9- α-terpineol	1186	1188	0.63 ± 0.09	0.35 ± 0.03	0.42 ± 0.04	0.41 ± 0.10
10- δ-elemene	1335	1333	-	0.21 ± 0.01	0.20 ± 0.01	0.13 ± 0.02
11- β-elemene	1389	1388	0.15 ± 0.01	0.40 ± 0.03	0.25 ± 0.00	0.25 ± 0.04
12- (E)-β-caryophyllene	**1417**	**1413**	**2.06 ± 0.03 b**	**3.74 ± 0.36 a**	**3.56 ± 0.07 a**	**1.90 ± 0.26 b**
13- aromadendrene	1439	1432	0.47 ± 0.02	0.44 ± 0.03	0.47 ± 0.01	0.63 ± 0.07
14- cabreuva oxide B	1462	1456	0.83 ± 0.05	0.43 ± 0.03	0.49 ± 0.00	0.77 ± 0.04
15- dauca-5,8-diene	1471	1468	0.14 ± 0.01	0.16 ± 0.01	0.12 ± 0.01	0.21 ± 0.02
16- germacrene D	**1484**	**1474**	**1.87 ± 0.07 c**	**5.55 ± 0.38 a**	**3.03 ± 0.08 b**	**2.31 ± 0.24 c**
17- bicyclogermacrene	**1500**	**1490**	**3.54 ± 0.10 b**	**8.10 ± 0.43 a**	**7.82 ± 0.24 a**	**4.96 ± 0.87 b**
18- α-muurolene	1500	1495	0.21 ± 0.03	0.39 ± 0.02	0.30 ± 0.02	0.30 ± 0.03
19- γ-cadinene	1513	1508	0.32 ± 0.04	0.50 ± 0.05	0.30 ± 0.02	0.42 ± 0.05
20- δ-cadinene	1522	1519	1.37 ± 0.15	2.00 ± 0.07	1.52 ± 0.03	2.00 ± 0.22
21- (*E*)-nerolidol	**1561**	**1564**	**21.68 ± 3.35 a**	**12.96 ± 0.69 b**	**8.91 ± 0.16 b**	**17.81±2.84 a**
22- spathulenol	**1577**	**1570**	**6.91 ± 0.42 b**	**5.13 ± 0.35 c**	**7.35 ±0.08 b**	**9.43 ± 0.94 a**
23- β-Copaen-4α-ol	**1590**	**1576**	**4.22 ± 0.20 b**	**3.63 ± 0.07 b**	**4.71 ±0.11 a**	**5.62 ± 0.57 a**
24- viridiflorol	1592	1583	1.02 ± 0.03	2.08 ± 0.04	2.70 ± 0.04	3.11 ± 0.41
25- cubeban-11-ol	1595	1586	0.21 ± 0.01	0.23 ± 0.02	0.28 ± 0.02	0.37 ± 0.04
26- ledol	1602	1594	2.01 ± 0.10	1.80 ± 0.07	2.20 ± 0.06	2.89 ± 0.33
27- β-oplopenone	1607	1604	0.25 ± 0.02	0.19 ± 0.01	0.27 ± 0.01	0.39 ± 0.05
28- junenol	1618	1615	0.26 ± 0.01	0.24 ± 0.01	0.30 ± 0.01	0.33 ± 0.16
29- 1-epi-cubenol	1627	1621	0.42 ± 0.03	0.49 ± 0.01	0.49 ± 0.02	0.74 ± 0.08
30- epi-α-muurolol	1640	1635	1.89 ± 0.23	2.34 ± 0.11	2.01 ± 0.05	2.94 ± 0.45
31- α-muurolol	1644	1640	0.56 ± 0.05	0.63 ± 0.04	0.64 ± 0.03	0.91 ± 0.13
32- α-cadinol	**1652**	**1647**	**2.21 ± 0.26 a**	**2.71 ± 0.17 a**	**2.41 ± 0.07 a**	**3.54 ± 0.58 a**
33-germacra-4(15),5,10(14)-trien-1α-ol	1685	1678	1.32 ± 0.17	1.32 ± 0.15	1.22 ± 0.05	2.36 ± 0.41

						(finish)
34- isobicyclogermacrenal	1733	1724	0.36 ± 0.03	0.26 ± 0.01	0.37 ± 0.01	0.57 ± 0.07
35- khusimol	1741	1733	0.25 ± 0.04	0.13 ± 0.01	0.22 ± 0.00	0.40 ± 0.06
Monoterpene hydrocarbons	-	-	31.39	33.78	35.81	29.29
Oxygenated monoterpenes	-	-	0.90	0.59	0.73	0.64
Sesquiterpene hydrocarbons	-	-	10.96	21.92	18.06	13.88
Oxygenated sesquiterpenes	-	-	43.57	34.14	34.08	51.59
Identified compounds %		-	86.82	90.43	88.32	95.40

SOURCE: The author (2018).
SUBTILE: RI^{lit}: retention index of the literature Adams (2007). RI^{cal}: calculated retention index. – trace element <0.1%. Means followed by the same letter in line do not differ by Scott-Knott test at 5% of probability.

The major compounds were: *(E)*-nerolidol (8.91-21.68%), β-pinene (8.94-16.15%) and Limonene (11.74-13.64%). During winter and spring, β-pinene presented the highest concentrations, 15.58 and 16.15%, respectively. In autumn and summer the highest contents were *E*-nerolidol, 21.68 and 17.82%, respectively. Regarding the class of chemical compounds, monoterpenes hydrocarbons were predominant during the spring (35.81%), sesquiterpenes during the winter (21.92%) while oxygenated sesquiterpenes presented higher concentration in summer (51.59%).

The work developed by De Sousa et al. (2009) found different quantitative results for the essential oil of *B. dracunculifolia* from plants grown in the state of São Paulo - Brazil, where monoterpenes hydrocarbons (2.39-2.40%) and sesquiterpenes (13.00-13.18%) were predominant during the autumn and oxygenated sesquiterpenes during the spring (8.34-8.91%). Additionaly, the author found higher contents of *(E)*-nerolidol (32%), with little variation between the seasons (28.73-35.83%), while our work found a lower average of *(E)*-nerolidol (15%), and a wide variation between seasons (8.91-21.68%). The chemical variation of wild plant oil is quite wide, and studies are needed to identify these variations and provide support for potential species domestication and breeding programs.

The seasonal study of the chemical composition of *B. dracunculifolia* essential oils was performed by multivariate analysis. In the PCA analysis, the first two main components explained 93.94% of the sample variability. The compounds with the highest significance to the ordering of the samples were: α-pinene (0.6109), limonene (-0.4949), *(E)*-nerolidol (-0.4792) and α-cadinol (0.4815) (TABLE 3.2). Multivariate

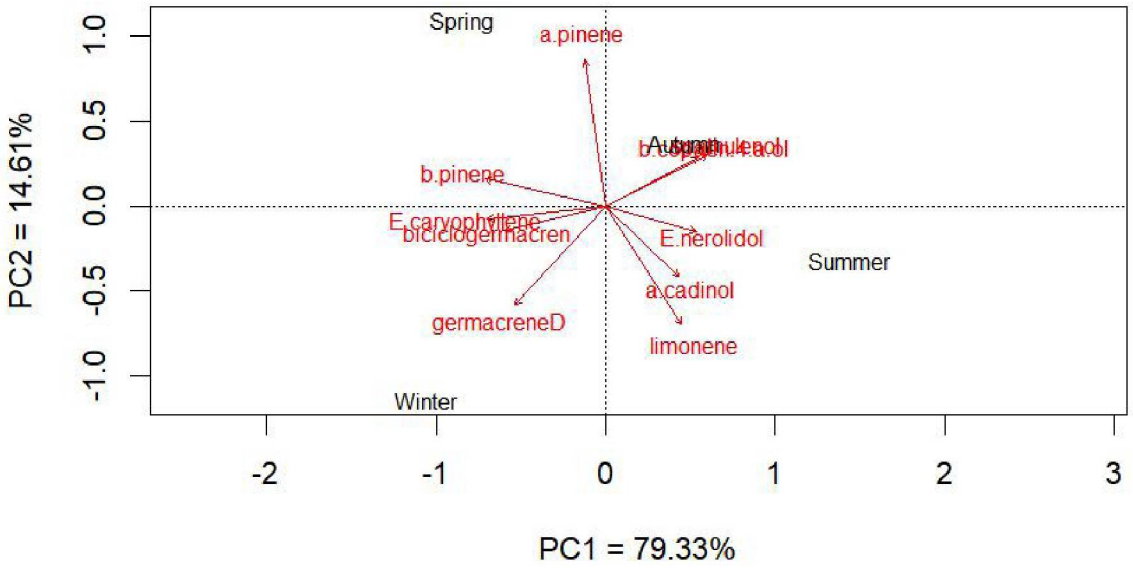
analyzes, such as principal component analysis - PCA (FIGURE 3.4) and cluster analysis (FIGURE 3.5) were applied to try to understand the chemical variability of essential oils between *B. dracunculifolia* collection times. According to the analysis, the samples were separated into two main groups. The first cluster consisted of winter and spring samples, characterized by higher levels of α-pinene (2.38-4.48%), β-pinene (15.58-16.15%) ($p<0.05$) and germacrene D (3.03-5.55%) ($p<0.05$). The second was composed of summer and autumn samples with higher levels of *(E)*-nerolidol (17.81-21.68%) ($p<0.05$). It was observed in this study that the phenological stage of flowering (including pre-flowering, flower bud and full bloom) occurred during the summer and autumn seasons (southern hemisphere). In this sense, the groups formed in this analysis are divided into the vegetative phase (Cluster I - winter and spring) and the flowering fase (Cluster II - summer and autumn).

TABLE 3.2 - PRINCIPAL COMPONENTS, VARIANCE (EIGENVALUE) AND CUMULATIVE VARIANCE (%) OBTAINED FROM THE CHEMICAL COMPOSITION (≥ 3.0%) ANALYSIS OF THE ESSENTIAL OILS OF LEAVES OF *B. dracunculifolia*.

Composition	Main component	
	PC1	PC2
α-pinene	-0.0696	**0.6109**
β-pinene	**-0.4126**	0.1124
limonene	0.2585	**-0.4949**
(*E*)-caryophyllene	-0.4047	-0.0546
Germacrene D	-0.3082	**-0.4151**
biciclogermacrene	-0.3445	-0.1006
(E)-nerolidol	0.3101	-0.1065
spathulenol	0.3485	0.2147
β-opaen-4-α-ol	0.3169	0.2006
α-cadinol	0.2498	-0.2930
Variance (eigenvalue)	5.633	2.496
Cumulative variance (%)	79.33	93.94

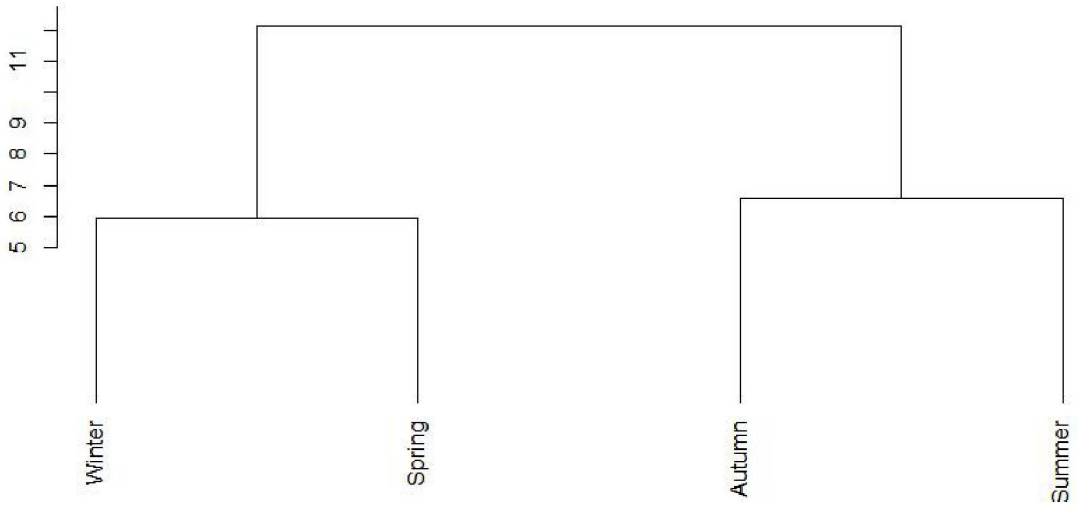
SOURCE: The author (2018).

FIGURE 3.4 - PRINCIPAL COMPONENT ANALYSIS (PCA) OF THE ESSENTIAL OIL COMPOSITION OF *B. dracunculifolia* LEAVES COLLECTED DURING AUTUMN/2016, WINTER/2016, SPRING/2016 AND SUMMER/2017.



SOURCE: The author (2018)

FIGURE 3.5 - CLUSTER ANALYSIS (UPGMA METHOD - 96% COPHENETIC CORRELATION) OF THE ESSENTIAL OIL COMPOSITION OF *B. dracunculifolia* LEAVES COLLECTED DURING AUTUMN/2016, WINTER/2016, SPRING/2016 AND SUMMER/2017.



SOURCE: The author (2018)

Cluster analysis applied to seasonally collected *Baccharis trimera* volatile compounds also identified groups according to the highest yields, where the cluster I was characterised by the samples harvested in March-May (flowering phase), with a high content of globulol (8.9%), spathulenol (8.7%), α-guaieno (3.4%), viridiflorol (3.4%), guaiol (3.1%), and δ-cadinene (6.6%); cluster II by samples harvested between June-February (vegetative phase) with Germacrene D (26.0%) and *(E)*-caryophyllene (19.0%) as principal constituents; and cluster III by samples harvested in the June-February (vegetative phase) with high amount of ledol (12.0%) (SILVA et al., 2007).

According some studies, the variation in essential oil composition related to season change can be associated with the flowering phase (PERRY et al., 1999; SANTOS-GOMES et al., 2001). The phenological phase of the plant can be crucial in the essential oils composition, where some compounds present levels close to 10% in the early stages of development, reaching 50-70% in full bloom. In other cases, volatiles are widely accumulated before the organ is fully expanded (FIGUEIREDO et al., 2008). A drastic variation in the content of some compounds may be related to the metabolic changes preceding the flowering period (SANTOS-GOMES et al., 2001). However, for this study, the pre-flowering period coincided with the summer season, which showed an increase in sesquiterpene contents, especially *(E)*-nerolidol and decrease of monoterpenes such as β-pinene. Similarly, the study developed by de Sousa et al. (2009) evaluated that the highest levels of *(E)*-nerolidol were detected in March, period between summer and autumn.

The major compounds present in *B. dracunculifolia* essential oil are β-pinene (monoterpene) and the *(E)*-nerolidol (sesquiterpene). In general, monoterpenes are related to leaf products, while sesquiterpenes are typical flower fragrances, although both are produced in considerable quantities in the glandular leaf trichomes (DUDAREVA et al., 2006; MAFFEI, 2010). Terpenoid synthesis can occur both via the 2-C-methyl-D-erythritol 4-phosphate (MEP) metabolic pathway in plastids, where monoterpenes are formed, as well as that of mevalonate (MVA) in cytoplasm, where sesquiterpenes are formed (MAFFEI, 2010; VRANOVÁ et al., 2013).

Regarding the major compounds present in *B. dracunculifolia* essential oil, *(E)*-nerolidol and β-pinene, these results suggest that the most appropriate period for the collection and processing of *B. dracunculifolia* to obtain essential oil with higher levels of *(E)*-nerolidol is during autumn and summer (flowering phase) and β-pinene

during winter and spring (vegetative phase). In view of the different metabolic pathways for synthesis of these compounds, their levels may be affected in inverse correlation depending on the phenological phase of the plant.

(E)-nerolidol is an important sesquiterpene with several applications. In addition to anti-ulcer activity (KLOPELL et al., 2007), *(E)*-Nerolidol also presents inhibitory properties over growth of *Leishmania amazonenses* (ARRUDA et al, 2005), antibacterial activity (ULBELEN et al., 1994) and its use has been approved in the United States by the Food and Drug Administration (FDA) as a flavoring agent in the food industry (ARRUDA et al, 2005). The compound is also widely used as a ingredient in fine fragrances, cosmetics, shampoos, toilet soaps, cleaners and detergents (LAPCZYNSKI et al., 2008).

In addition, abiotic factors can also directly influence the composition of essential oils (DUDAREVA et al., 2006; FIGUEIREDO et al., 2008; MAFFEI, 2010). Previous studies have shown that volatile terpenes, including monoterpenes and sesquiterpenes, are released from plant tissues of plants subjected to high radiation and temperature. Thus, these compounds would act as a tolerant term or in protecting the cell against oxidative stress (LORETO et al., 2001).

During the months of low temperature and lower solar radiation there is a decrease in volatile compounds production (FIGUEIREDO et al., 2008). In *Pinus elliotii*, the emission of monoterpenes, especially β-pinene, mircene, limonene and β-phellandreno, increased exponentially with a temperature increase between 20-46°C (TINGEY et al., 1980). However, excessive elevation can lead to volatilization and consequent loss of essential oils (PIRBALOUTI et al., 2013). In other cases, variation in oil production may be related to herbivory and pathogenic fungi attack, especially during rainy months (FIGUEIREDO et al., 2008).

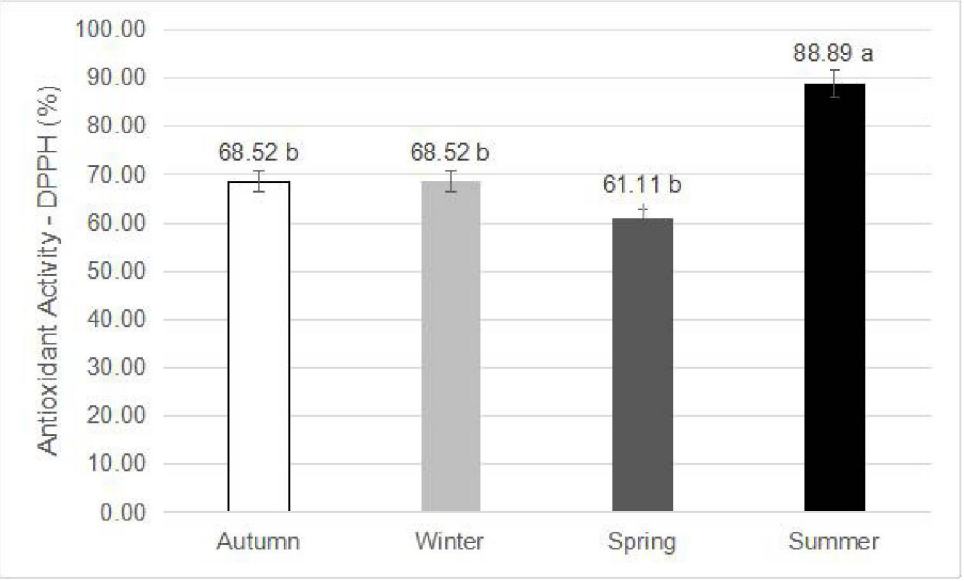
For some species as *B. dracunculifolia*, the essential oil composition may change throughout the year. Thus, the harvest time determination can have great influence from the agronomic and economic point of view (FIGUEIREDO et al., 2008). In this seasonality study, the contents of some major compounds were increased depending on time of year, emphasizing the importance of seasonal chemical characterization of the species. In view of the high levels of *(E)*-nerolidol produced by this plant during summer and autumn it is suggested that this is the best period of harvesting the leaves to obtain higher content of this compound. These results

corroborate the work of De Sousa (2009), with cultivation of the plant in the southeastern Brazil, state of São Paulo.

3.3.3 Antioxidant activity

Antioxidant activity was monitored throughout the four seasons of the year. The radical scavenging activity varied from 61.11 to 88.89% and the summer season had the highest antioxidant activity, 88.89% (FIGURE 3.6). Pearson correlation analysis detected a significant positive correlation between antioxidant/DPPH activity and limonene (r = 0.977), verifying the increase in antioxidant capacity as the limonene levels increased (TABLE 3.3).

FIGURE 3.6 - MEANS AND STANDARD ERRORS OF THE ANTIOXIDANT ACTIVITIES (DPPH METHOD) OF ESSENTIAL OILS IN FRESH LEAVES OF *B. dracunculifolia* COLLECTED DURING AUTUMN/2016, WINTER/2016, SPRING/2016 AND SUMMER/2017. COEFFICIENT OF VARIATION = %. MEANS FOLLOWED BY THE SAME LETTER DO NOT DIFFER BY SCOTT-KNOTT TEST AT 5% OF PROBABILITY.



SOURCE: The author (2018).

TABLE 3.3 - PEARSON CORRELATION COEFFICIENTS BETWEEN *B. dracunculifolia* ESSENTIAL OIL CONSTITUENTS AND ANTIOXIDANT ACTIVITY (DPPH).

Compounds	Antioxidant activity (DPPH)	p-value
α-pinene	-0.718	0.282
β-pinene	-0.79	0.209
limonene	0.977*	0.023
germacrene D	0.037	0.962
bicyclogermacrene	-0.253	0.747
(*E*)-nerolidol	0.396	0.603
spathulenol	0.438	0.56
β-opaen-4-α-ol	0.444	0.55
α-cadinol	0.908	0.09

SOURCE: The author (2018).
SUBTITLE: significant at 5% of probability.

Previous studies have reported the protective action of limonene against oxidative stress of cells, conferring its ability to increase activities of catalase, peroxidase and superoxide dismutase enzymes. Limonene is a monoterpene found naturally in foods, currently the compound has been used as a flavoring by the food industry, being used in the composition of various products (ROBERTO et al., 2009). In addition, sesquiterpenes in essential oils are known as important antioxidant compounds and *(E)*-nerolidol is an important compound from this group, exhibiting a DPPH scavenging activity (VINHOLES et al, 2014) and neuroprotective effects against neuronal damage induced by oxidative stress (*in vivo*) (NETO et al., 2013).

Reactive oxygen species (ROS), such as superoxide anion (O_2–), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH), are formed during the aerobic metabolism by the incomplete reduction of oxygen. The enzymes glutathione (GSH) reductase, GSH peroxidase, superoxide dismutase (SOD) and catalase usually help the cells with antioxidant defense, but sometimes free radical production is so intense that generates oxidative stress, causing cell damage (RAHMAN, 2007; LUSHCHAK, 2014; CHAN et al. 2016). In addition, ROS are normally produced during photosynthetic and respiratory transport processes. Under unfavorable conditions, such as extreme light and temperature levels, pollutants, and pathogen attack, plants can greatly increase the production of ROS inside their cells. Antioxidants act to

reduce free radicals by eliminating the highly reactive unpaired electron (Taiz; Zeiger, 2013).

The antioxidant activity of essential oils may also be related to the synergistic effect between one or more compounds, as already shown in previous studies (XAVIER et al., 2016). In addition, the effects of abiotics such as low precipitation, solar radiation and high temperature may also have influenced the production of antioxidant compounds. The low precipitation and high temperature detected in February/2017 may have influenced the higher production of reactive oxygen species (ROS), once those prolonged stress can cause physiological damage to the plant and the production of terpenoids with high antioxidant activity may have been produced as a defense (CHAI et al., 2005; JEYARAMRAJA et al., 2005). Structural modification in leaves that grow under high luminous rates may happened, such as developing thicker leaves. These adaptation result in greater capacity to capture solar radiation generating higher photosynthetic rates, which may have increased ROS production during the photosynthetic process (RAVEN et al., 2005; SOUZA et al., 2008).

Due to the economic importance of *B. dracunculifolia* species, e.g. green propolis and essential oil production, the knowledge and the understanding of the time of the year the plant produces the most compounds of interest is essential for the cultivation of quality medicinal aromatic plants.

3.4 CONCLUSIONS

The collection time of *B. dracunculifolia* leaves influence the essential oil accumulation in the leaves of the species, as autumn presented the highest yield. The highest antioxidant capacity is observed during summer. Variations in the levels of some compounds are observed over the seasons, where the contents of some major compounds is increased, emphasizing the importance of the seasonal chemical characterization of the species. Multivariate analyzes grouped the seasons in two groups: I) summer and autumn with high levels of (*E*)-nerolidol, II) winter and spring high levels of β-pinene, α-pinene and germacrene D. Biotic and abiotic factors from each specific season may have influenced the quantitative variation of the volatile compounds produced. These results suggest that the most appropriate period for the collection and processing of *B. dracunculifolia* to obtain essential oil

with higher levels of *(E)*-nerolidol is during autumn and summer (flowering phase) and β-pinene during winter and spring (vegetative phase).

REFERENCES

ADAMS, R. P. **Identification of essential oil components by gas chromatography/mass spectrometry**. Carol Stream, Illinois: Allured Publishing Corporation, 2007.

AIDI WANNES, W.; MARZOUK, B. Maturational effect on essential oil yield and composition of *Myrtus communis* var. baetica fruit. **Journal of Essential Oil Bearing Plants**, v.15, p. 847–853, 2012.

ALVARES, C. A. et al. Koppen's climate classification map for Brazil. **Meteorologische Zeitschrift**, v. 22, n. 6, p. 711–728, 2013.

ARRUDA, D.C. et al. Antileishmanial activity of the terpene nerolidol. **Antimicrobial Agents and Chemotherapy**, v. 5, p. 1679-1687, 2005.

BANO, U. et al. Effect of plant growth regulators on essential oil yield in aromatic plants. **Journal of Chemical and Pharmaceutical Research**, v. 8, p. 733-739, 2016.

BARROSO, G. M.; BUENO, O. L. Flora ilustrada catarinense plantas compostas-subtribo: Baccharidinae. Itajaí: **Herbário Barbosa Rodrigues (HBS)**, 2002.

BOLDT, P. E. *Baccharis* (Asteraceae). **A Review of its taxonomy, phytochemistry, ecology, economic status, natural enemies and potential for its biological control in the United States**. Washington: USDA. Agricultural Research Service Grassland, 1989.

BOTREL, P. et al. Teor e composição química do óleo essencial de *Hyptis marrubioides* Epl., Lamiaceae em função da sazonalidade. **Acta Scientiarum. Agronomy**, v. 32, p. 533-538, 2010.

BRAND-WILLIAMS, W.; CUVELIER, M. E.; BERSET, C. Use of a free radical method to evaluate antioxidant activity. **LWT - Food Science and Technology**, v. 28, p. 25-30, 1995.

CHAI, T. T. et al. Water stress-induced oxidative damage and antioxidant responses in micropropagated banana plantlets. **Biologia Plantarum**, v. 49, p. 153-156, 2005.

CHAN, W.K. et al. Nerolidol: A Sesquiterpene alcohol with multi-Faceted pharmacological and biological activities. **Molecules**, v. 21, n. 529, p. 1-40, 2016.
DA SILVA, A.C.R. et al. Biological activities of α-pinene and β-pinene enantiomers. **Molecules**, v. 17, p. 6305-6316, 2012.

DE SOUSA, J. P. et al. Seasonal variation of the *(E)*-Nerolidol and other volatile compounds within ten different cultivated populations of *Baccharis dracunculifolia* D.C. (Asteraceae). **Journal of Essential Oil Research**, v. 21, p. 308-314, 2009.

DUDAREVA, M.; PICHERSKY, E.; GERSHENZON, J. Biochemistry of plant volatiles. **Plant Physiology**, v. 135, p. 1893–1902, 2004.

FIGUEIREDO, A. C. et al. Factors affecting secondary metabolite production in plants: volatile components and essential oils. **Flavour and Fragrance Journal**, v. 23, p. 213–226, 2008.

FLORÃO, A. et al. Essential oils from Baccharis species (Asteraceae) have anti-inflammatory effects for human cells. Journal of Essential Oil Research, v.24, p. 561-570, 2012.

FRIZZO, C. D. et al. Essential oil variability in *Baccharis uncinella* DC and *Baccharis dracunculifolia* DC growing wild in southern Brazil, Bolivia and Uruguay. **Flavour and Fragrance Journal**, v. 23, p. 99-106, 2008.

FUKUDA. M, et al. Studies on the constituents of the leaves of *Baccharis dracunculifolia* (Asteraceae) and their cytotoxic activity. **Chemical & Pharmaceutical Bulletin**, v. 54, p. 1465–1468, 2006.

HUSSAIN, A. I. et. al. Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. **Food Chemistry**, v. 108, p. 986-995, 2008.

JEYARAMRAJA, P. R. et al. Water deficit induced oxidative damage in tea (*Camellia sinensis*) plants. **Journal of Plant Physiology**, v. 162, p. 413-419, 2005.

KLOPELL, F. C. et al. Nerolidol, an antiulcer constituent from essential oil of *Baccharis dracunculifolia* DC (Asteraceae). **Zeitschrift für Naturforschung**, v. 62c,

p. 537–542, 2007.

LAPCZYNSKI, A. et al. Fragrance material review on nerolidol (isomer unspecified). **Food and Chemical Toxicology**, v. 46, p. S247–S250, 2008.

LEITE, A.M. et al. Inhibitory effect of β-pinene, α-pinene and eugenol on the growth of potential infectious endocarditis causing Gram-positive bacteria. **Brazilian Journal of Pharmaceutical Sciences,** v. 43, n. 1, p. 121-126, 2007.

LEMOS, M. et al. *Baccharis dracunculifolia* the main botanical source of brazilian green propolis, displays antiulcer activity. **Journal of Pharmacy and Pharmacology**, v. 59, p. 603–608, 2007.

LIU, H. et al. UV-B irradiation differentially regulates terpene synthases and terpene content of peach. **Plant, Cell & Environment**, v. 40, p. 2261–2275, 2017.

LLUSIA, J. et al. Effects of UV radiation and water limitation on the volatile terpene emission rates, photosynthesis rates, and stomatal conductance in four Mediterranean species. **Acta Physiologiae Plantarum**, v. 34, p. 757–769, 2012.

LORETO, F.; VELIKOVA, V. Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. **Plant Physiology**, v. 127, p. 1781-1787, 2001.

LUSHCHAK, V. I. Free radicals, reactive oxygen species, oxidative stress and its classification. **Chemico-Biological Interactions**, v. 224, p. 164-175, 2014.

MAFFEI, M.E. Sites of synthesis, biochemistry and functional role of plant volatiles. **South African Journal of Botany**, v. 76, p. 612–631, 2010.

MIRANDA, C. A. S. F. et al. Essential oils from leaves of various species: antioxidant and antibacterial properties on growth in pathogenic species. **Revista Ciência Agronômica**, v. 47, p. 213-220, 2016.

MIRJALILI, M. H. et al. Essential oil variation of *Salvia officinalis* aerial parts during its phenological cycle. **Chemistry of Natural Compounds**, v. 42, p. 19–23, 2006.

NOGUEIRA NETO, J. et al. Antioxidant effects of nerolidol in mice hippocampus after open field test. **Neurochemical Research**, v. 38, 1861–1870, 2013.

PACCIARONI, A. V. et al. Bioactive Constituents of *Conyza albida*. **Planta Medica**, v. 66, p. 720-723, 2000.

PARK, Y. K. et al. Chemical Constituents in *Baccharis dracunculifolia* as the main botanical origin of southeastern Brazilian propolis. **Journal of Agricultural and Food Chemistry**, v. 52, p. 1100-1103, 2004.

PARREIRA, N. A. et al. Antiprotozoal, schistosomicidal, and antimicrobial activities of the essential oil from the leaves of *Baccharis dracunculifolia*. **Chemistry & Biodiversity**, v. 7, p. 993-1001, 2010.

PAZOUKI, L.; NIINEMETS, Ü. Multi-substrate terpene synthases: their occurrence and physiological significance. **Frontiers in Plant Science**, v. 7, p. 1019, 2016.

PEGORINI, F.; MARANHÃO, L. T.; ROCHA, L. D. Organização estrutural das folhas de *Baccharis dracunculifolia* DC., Asteraceae. **Revista Brasileira de Farmácia**, v. 89, p. 272-275, 2008.

PERRY, N. B. et al. Essential oils from dalmatian sage (*Salvia officinalis* L.): variations among individuals, plant parts, seasons, and sites. **Journal of Agricultural and Food Chemistry**, v. 47, p. 2048-2054, 1999.

PIRBALOUTI, A.G.; MAHDAD, E.; CRAKER, L. Effects of drying methods on qualitative and quantitative properties of essential oil of two basil landraces. **Food Chemistry**, v. 141, p. 2440-2449, 2013.

R CORE TEAM. **A language and environment for statistical computing. R Foundation for Statistical Computing**, Vienna, Austria, 2018.

RAHMAN, K. Studies on free radicals, antioxidants, and co-factors. **Clinical Interventions in Aging**, v. 2, p. 219-236, 2007.

RAVEN, P. H.; EVERT, R. F.; EICHHORN, S. E. **Biology of plants**. New York: W.H. Freeman, 2005.

ROBERTO, D. et al. Antioxidant activity of limonene on normal murine lymphocytes: relation to H2O2 modulation and cell proliferation. **Basic & Clinical Pharmacology & Toxicology**, v. 106, p. 38–44, 2009.

SALAZAR, G. J. T. et al. Phytochemical characterization of the *Baccharis dracunculifolia* DC (Asteraceae) essential oil and antibacterial activity evaluation. **Industrial Crops and Products**, v. 122, p. 591-595, 2018.

SALEM, N. et al. Variation in chemical composition of *Eucalyptus globulus* essential oil under phenological stages and evidence synergism with antimicrobial standards**.** **Industrial Crops & Products**, v. 124, p. 115–125, 2018.

SANTOS a, R. F. et al. Composição química e produtividade dos principais componentes do óleo essencial de *Baccharis dracunculifolia* DC. em função da adubação orgânica. **Revista Brasileira de Plantas Medicinais**, v. 14, p. 224-234, 2012.

SANTOS b, V. M. C. S. et al. Seasonal variation of vegetative growth, essential oil yield and composition of menthol mint genotypes at southern Brazil. **Bioscience Journal**, v. 28, p. 790–798, 2012.

SANTOS-GOMES, P.C.; FERNANDES-FERREIRA, M. Organ- and season-dependent variation in the essential oil composition of *Salvia officinalis* L. cultivated at two different sites. **Journal of Agricultural and Food Chemistry**, v. 49, p. 2908−2916 ,2001.

SILVA, F. A. S.; AZEVEDO, C. A. V. The assistat software version 7.7 and its use in the analysis of experimental data. **African Journal of Agricultural Research**, v. 11, p. 3733–3740, 2016.

SILVA, F. et al. Seasonal variability in the essential oils of wild and cultivated *Baccharis trimera*. **Journal of the Brazilian Chemical Society**, v. 18, n. 5, p. 990-997, 2007.

SOUZA, J. R. P. et al. Development of holy-thorn under different light intensities and pruning levels. **Horticultura Brasileira**, v. 26, p. 40-44, 2008.

TAIZ, L.; ZEIGER, E. **Fisiologia Vegetal**. Porto Alegre: Artmed , 2013.

TIIVA, P. T, et al. Isoprene emission from a subarctic peatland under enhanced UV-B radiation. **New Phytologist**, v. 176, p. 346–355, 2007.

TINGEY, D. T. et al. Influence of light and temperature on monoterpene emission rates from slash pine. **Plant Physiology**, v. 65, p. 797-801, 1980.

ULBELEN, A. et al. Terpenoides from *Salvia sclarea*. **Phytochemistry**, v. 36, p. 971-974, 1994.

VAN DEN DOOL, H. KRATZ, P. D. A generalisation of the retention index system including linear temperature programmed gas-liquid chromatography. **Journal of Chromatography**, v. 11, p. 463-471, 1963.

VINHOLES, J. et al. Assessment of the antioxidant and antiproliferative effects of sesquiterpenic compounds in in vitro Caco-2 cell models. **Food Chemistry**, v. 156, p. 204-211, 2014.

VRANOVÁ, E.; COMAN, D.; GRUISSEM, W. Network analysis of the MVA and MEP pathways for isoprenoid synthesis. **Annual Review of Plant Biology**, v. 64, p. 665–700, 2013.

XAVIER, M. N. et al. Chemical composition from essential oil of *Cardiopetalum calophyllum* Schltdl. (Annonaceae) and their antioxidant, antibacterial and antifungal activities. **Revista Virtual de Química**, v. 8, p. 1433-1448, 2016.

YANG, Y. et al. Environmental cues induce changes of steviol glycosides contents and transcription of corresponding biosynthetic genes in *Stevia rebaudiana*. Plant Physiology and Biochemistry, 86, 174-180 (2015).

4 DIFERENÇA DE GÊNERO EM PLANTAS DE *Baccharis dracunculifolia* DC.: AVALIAÇÃO FITOQUÍMICA E ATIVIDADE ANTIOXIDANTE

RESUMO

Baccharis dracunculifolia DC. é um arbusto dióico nativo do Brasil, a espécie é conhecida por ser a fonte botânica de um importante produto comercial produzido pelas abelhas (*Apis mellifera* L.) chamado de "própolis verde", produto com alto teor de artepillin C, composto que apresenta vários propriedades benéficas para a saúde humana, como atividades anti-tumorais. Muitos estudos desenvolvidos com espécies dióicas demonstraram diferenças morfológicas, fisiológicas e bioquímicas entre plantas masculinas e femininas. Nesse sentido, o objetivo principal deste estudo foi avaliar a fitoquímica de extratos, óleos essenciais e suas atividades antioxidantes em plantas masculinas e femininas de *B. dracunculifolia* originárias de diferentes populações do sul do Brasil. Para isso, ramos de plantas masculinas (n= 25) e femininas (n= 25) foram coletadas de duas populações do estado do Parana: Palmeira (PPA) e Piraquara (PPI). Os extratos das folhas (etanol: água 1:1 v/v) foram preparados e analisados por HPLC-DAD e LC-MS, enquanto que a composição química do óleo essencial foi analisada por GC-FID e GC-MS. As análises antioxidantes foram realizadas pelos métodos DPPH e ABTS. O delineamento experimental foi inteiramente casualizado em esquema fatorial 2x2 (populações e gêneros). Os dados foram submetidos à análise de variância e médias comparadas pelo teste de Tukey ($p< 0,05$). A análise cromatográfica dos extratos encontrou um total de 31 compostos, dos quais 17 foram identificados, sendo os principais compostos: ácido 3,5-di-caffeoilquinico (21,93-10,87 $mg.g^{-1}$), ácido 4,5-di-caffeoilquinico (15,25-3,72 $mg.g^{-1}$) e artepillin C (18,95-3,29 $mg.g^{-1}$). A caracterização química dos óleos essenciais foi composta predominantemente por sesquiterpenos oxigenados, hidrocarbonetos de monoterpenos e hidrocarbonetos de sesquiterpenos, sendo os principais compostos: *(E)*-nerolidol (13,03 - 19,68%), β-pineno (9,81 - 20,97%) e limoneno (6,17 - 18,21). %). As plantas masculinas produziram níveis mais altos de todos os compostos não-voláteis, maior teor de óleo essencial (0,728%) e maior capacidade antioxidante tanto para os extratos (DPPH - IC50 = 1121,42 µg.mL-1 e ABTS - 1569 µM.g-1) quanto para os óleos essenciais (DPPH - IC50 = 261,8 µg.mL-1). O artepillin C, composto mais importante para a comercialização da própolis verde, é produzido em maior quantidade nas folhas masculinas das duas populações estudadas, quando comparadas às plantas femininas. O metabolismo secundário de *B. dracuculifolia* é afetado pelo gênero da planta e pelos locais de crescimento.

Palavras-chave: Alecrim-do-campo. Própolis verde. Artepillin C. Óleo essencial. DPPH.

4 GENDER DIFFERENCE IN *Baccharis dracunculifolia* DC. PLANTS: PHYTOCHEMICAL EVALUATION AND ANTIOXIDANT ACTIVITY

ABSTRACT

Baccharis dracunculifolia DC. is a dioecious shrub native from Brazil and the species is well known for being the botanical source of an important commercial product produced by honeybees (*Apis mellifera* L.) called "green propolis". Green propolis possesses a high content of artepillin C, which has several beneficial properties for human health, such as anti-tumor treatments. Many studies developed with dioecious species have shown morphological, physiological, biochemical differences between male and female plants. The main goal of this study was to evaluate the phytochemical composition of extracts, essential oil and the antioxidant activity of male and female plants of *B. dracunculifolia* from different populations of southern Brazil. Branches of male (n=25) and female (n=25) plants of *B. dracunculifolia* were collected from two populations of Parana state: Palmeira (PPA) e Piraquara (PPI). Aqueous ethanol extracts (1:1 v/v) from the leaves were prepared and analyzed by HPLC-DAD and LC-MS, while the essential oil composition were performed by GC-FID and GC-MS. Antioxidant analyzes were performed by DPPH and ABTS methods. The experimental design was completely randomized in a 2x2 factorial scheme (populations and gender). The data were subjected to analysis of variance and means compared by Tukey test ($p< 0.05$). Chromatographic analysis of extracts found a total of 31 compounds, of these 17 were identified and the major compounds were 3,5-Di-caffeoylquinic acid (21.93-10.87 $mg.g^{-1}$), 4,5-Di-caffeoylquinic acid (15.25-3.72 $mg.g^{-1}$) and artepillin C (18.95-3.29 $mg.g^{-1}$). The chemical characterization of essential oils were predominantly composed of oxygenated sesquiterpenes, monoterpene hydrocarbons and sesquiterpenes hydrocarbons and the major compounds were *(E)*-nerolidol (13.03 - 19.68%), β-pinene (9.81 - 20.97%), limonene (6.17 - 18.21%). Male plants produce higher levels of all non-volatile compounds, higher amounts of essential oil content (0.728%) and higher antioxidant capacity for extracts (DPPH - IC_{50}= 1121.42 $\mu g.mL^{-1}$ and ABTS - 1569 $\mu M.g^{-1}$) and essential oil (DPPH - IC_{50}= 261.8 $\mu g.mL^{-1}$). Artepillin C, the most important compound for the commercialization of green propolis, is produced in higher amounts in the male leaves of the two populations studied, when compared to the female plants. The secondary metabolism of *B. dracunculifolia* is affected by the gender of the plant and by the growth sites.

Key words: Alecrim-do-campo. Green propolis. Artepillin C. Essential oil. DPPH.

4.1 INTRODUCTION

Baccharis dracunculifolia DC. is a dioecious shrub native from Brazil that has numerous medicinal properties (BOLDT, 1989; BARROSO; BUENO, 2002). The species is well known for being the botanical source of an important commercial product called “green propolis” (PARK et al., 2004). Green propolis is produced from vegetative buds of *B. dracunculifolia,* where honeybees cut out and add to salivary enzymes, beeswax and pollen (BANKOVA et al., 2000; TEIXEIRA et al., 2005).

The essential oil is characterized by the presence of *(E)*-nerolidol, spathulenol, α-pinene, β-pinene, β-Caryophyllene, germacrene D, bicyclogermacrene, mircene, limonene and δ-cadinene (LOAYZA et al., 1995; DE SOUSA et al., 2009; PARREIRA et al., 2010; SANTOS et al., 2012 a SALAZAR et al., 2018). Among the main compounds found in the extract of the plant and propolis are the prenylated phenylpropanoids like artepillin C (BANSKOTA et al., 1998; PARK et al., 2004; TEIXEIRA et al., 2005) and caffeoyl quinic acids (MISHIMA et al., 2005). The Japanese market has shown growing interest in Brazilian propolis (TORETI et al., 2013), where the propolis has been extensively used in foods and beverages with the aim of maintaining or improving human health (AGA et al., 1994). In addition, several studies have proven the anti-carcinogenic action of the compound artepillin C and various biological properties, greatly increasing the studies and the demand for the product on the market (AHN et al., 2007; MESSERLI et al., 2009).

Previous studies have identified numerous biological activities from extracts of *B. dracunculifolia* such as trypanocidal (FILHO et al., 2004), antiulcer (LEMOS et al., 2007), antimicrobial (FILHO et al., 2008), antimutagenic (MUNARI et al., 2008), anti-inflammatory (PAULINO et al., 2008) and radical scavenging (NAKANISHI et al., 2003). These biological activities, including antimicrobial and antioxidant activities, are mostly due to the high levels of prenylated cinnamic acid derivatives, including artepillin C (FERESIN et al. 2003). Besides that, the antibacterial activity might be increased by the increasing of the number of prenyl in the chemical structure (AGA et al., 1994). The mechanism of action of phenolic compounds against bacteria is due to their accumulation at the surface of bacteria and this accumulation depends of its interactions with the cell wall of bacteria (NEGI, 2012; BOUARAB-CHIBANE, 2019). The essential oils also presented several biological activities such as antimicrobial (FERRONATTO et al., 2007), antibacterial (BARBOSA et al, 2015), anti-inflammatory

(FLORÃO et al., 2012), insecticide (CHAABAN et al, 2017) and cito cytotoxic activities against HEp-2 cells (BÚFALO et al, 2010). In addition, the oil is used as an ingredient to produce fine fragrances (LAPCZYNSKI et al., 2008).

Many studies developed with dioecious species, such as *B. dracunculifolia,* have shown morphological and physiological differences between male and female individuals (ESPIRITO-SANTO et al., 2003; BESTEN et al., 2013; LI et al., 2016). Plants with dimorphism can present different allocations of products during the vegetative and reproductive phases, studies point out that reproductive investments are greater in female individuals than in male individuals (ANTOS; ALLEN, 1999; CAPEDA-CORNEJO; SUZUKI, 2005; DIRZO, 2010). In dioecious species, it can occur that part of the resources that could be used for the development of new tissues such as leaves, roots and stems, can be reallocated to the production of flowers, fruits and seeds (DUDLEY, 2006).

The physiological differences between female and male individuals may also affect secondary plant metabolism, once primary and secondary metabolic pathway share common precursors and intermediates (HERMS; MATTSON, 1992). Many dioecious species have more amounts of defense compounds like phenolic in female plants when compare with male, and with consequence are significantly better defended, with lower herbivore damage (CORNELISSEN; STILING, 2005; CEPEDA-CORNEJO; DIRZO, 2010).

Plant secondary metabolism can be influenced by several factors including environmental and genetic factors. Geographic conditions may influence the chemical profile of the plant, related to its ecological adaptation to the growth site. Environmental factors such as radiation, temperature, precipitation, photoperiod can affect the chemical constituents of plants (FIGUEIREDO et al., 2008; BANO et al., 2016).

The phytochemical and antioxidant activity comparison between the female and male individuals of *B. dracunculifolia* from different growth sites was not developed until then. This information can greatly contribute to understanding the interaction between bee and plant in the fabrication of green propolis, to assist assist in the production of high quality seedlings, both for the production of bee pasture for the production of green propolis and for the production of essential oil. The objective of this study was therefore to evaluate the phytochemical composition of extracts,

essential oil and their antioxidant activity of male and female plants of *B. dracunculifolia* from different populations of southern Brazil.

4.2 MATERIAL AND METHODS

4.2.1 Material collection and proceedings

Branches of male and female individuals of *B. dracunculifolia* were collected in April 2018 from two populations in southern Brazil during the flowering period: 1) Population of Palmeira (PPA) (25°19'80''S - 49°48'35''W, altitude 1027 meters) located in the area of 'Reserva Particular do Patrimônio Natural (RPPN) Butuquara', with the license number 09.18 from the Environmental Institute of Paraná- IAP for collecting and transporting plant material; 2) Population of Piraquara (PPI): 25°30'63"S - 49°02'58"W, altitude 891 meters.

The braches were collected from 25 female and 25 male individuals in the field and the sex of the plants was confirmed through botanical identification. Voucher specimens were deposited in the Municipal Botanical Museum of Curitiba - MBM Herbarium, with the deposit numbers: MBM-384.962 (Male – PPA), MBM-370.693 (Female – PPA); MBM-371.866 (Male – PPI); MBM-379.293 (Female – PPI). The registration number of the species in the '*Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado* - SisGen' is AC74344.

The leaves (female and male) from each population were separated from the branches and mixed each other in the laboratory. A part of the plant material was used for the production of the extract for HPLC analysis, and another part was used for the isolation of the essential oil. For the production of extract for analysis in HPLC, the leaves were immediately frozen in liquid nitrogen (-180°C) and stored in ultra-low freezer (-80°C) until further analysis. For the isolation of the essential oil, the fresh leaves (female and male) of each population were immediately separated for the extraction of the essential oil.

4.2.2 Preparation and extraction of constituents for HPLC analysis

The leaves were separately lyophilized (Liotop L101®) for 48 hours at a temperature of 35°C and vacuum pressure of 200 MBAR. After, the samples plants

were ground in a grinder and the extracts were prepared with water: ethanol (1:1 v/v) solution. For this, 10 $mg.ml^{-1}$ of the leaves were extracted in a water bath (Ratek TH5®) for 1h at 60 ºC. After this period, the solution was filtered through a 2 micron filter. For the chromatograph analysis 1000µl of the extracts were transferred into vial.

4.2.3 HPLC-DAD analysis

Aliquots (10 µl) of the hydroalcoholic extracts of leaves of *B. dracunculifolia* were injected in Agilent Technologies 1290 Infinity II Liquid Chromatography (HPLC), equipped with C18 reversed phase column (Synergy Fusion-RP 80A, 50mm x 4,6mm Ø, 2.5µm), C18 pre-column and diode array detector – DAD (Agilent Technologies 1260 Infinity II). The samples were eluted using the flow rate of 0.5mL/min and a linear gradient of methanol: water: formic acid (15: 84.9: 0.1, v/v/v, solvent A), and methanol: formic acid (99.9: 0.1, v/v, solvent B). The gradient profile started with 0-10 min (0% B), 10-60 min (72.2% B), 60-65 min (72.2% B), 65-67 min (0% B), 67-75 min (0% B). The chromatograms profiles were recorded at 200, 220, 260, 280, 290, 300 and 316 nm.

For the semi-quantification of the compounds was calculated an external standard curve made with 5 concentrations for the chlorogenic acid (0 $\mu g.ml^{-1}$ - 1000 $\mu g.ml^{-1}$; r^2= 0.9998; y= 0.0329x), caffeic acid (0 $\mu g.ml^{-1}$ - 1000 $\mu g.ml^{-1}$; r^2= 0.999; y= 0.0169x), quercetin (0 $\mu g.ml^{-1}$ – 100 $\mu g.ml^{-1}$; r^2= 0.9998; y= 0.0523x) and artepillin C (0 $\mu g.ml^{-1}$ - 610 $\mu g.ml^{-1}$; r^2= 0.9999; y= 0.0309x), using as a reference the full area of the peaks. The compounds amounts were expressed in $mg.g^{-1}$ of dry mass. The compounds found according to the comparison with the external standard were called "annoted". However, the compounds identified through the comparison of molecular mass with data from the literature were called “identified”. Finally, the unidentified peaks (ni) were called "detected".

4.2.4 HPLC-MS analysis

LC-MS/MS analysis was carried out for identification of the compounds using the SCIEX X500R QTOF system with Turbo V™ source and Electrospray Ionization (ESI). The negative mode was used with the IS voltage set to 4500 V and the following parameters were adopted: mass range 100–1000m/z, fragmentor energy

at 100V, collision energies at 10 and 35 V and the estimated cycles was 1757. The temperature of the source was set at 500°C for ionization mode. The high purity nitrogen was used as the desolvation gas with flow at 6 $L.min^{-1}$ and pressure at 2 bar. The samples (2µL) were eluted using the flow rate of 0.3$ml.min^{-1}$ and a linear gradient of methanol: water: formic acid (15: 84.9: 0.1, v/v/v, solvent A), and methanol and formic acid (99.9: 0.1, v/v, solvent B). The gradient profile started with 0-10 min (0% B), 10-60 min (72.2% B), 60-65 min (72.2% B), 65-67 min (0% B), 67-75 min (0% B).

4.2.5 Extraction and quantification of the essential oil

Fresh leaves of male and female plants of each population were separated for essential oil isolation. For the extraction was used 100g of fresh leaves added to 1L of distilled water, the process was done by hydrodistillation in Clevenger equipment for 2 hours and 30 minutes, in three replications The essential oil content was measured based on the dry of leaves and the yield was expressed as a percentage (%). The samples were stored in a freezer (-5°C) until further analysis.

The composition of the essential oils was performed by gas chromatography (GC), through GC/FID (Agilent 7890A) to quantification and GC/MS (Shimadzu-2010 Plus) for identification of the compounds. The column used was a HP-5MS capillary GC column 30 mm x 0. 25 mm x 0.25 µm and the inject temperature was 250°C. Hydrogen and Helium were used as carrier gas flow for GC/FID (1.5 mL/min) and GC/MS (1 mL/min), respectively. The temperature program was 60°C, rising to 240°C at the rate of 3°C/minute. For GC–MS, the injection volume was 1uL of the sample, 1% (w/v) in hexane, the interface temperature was at 300°C and mass range of m/z 40–400. The mass detector was operated in the ionization mode (electron impact 70 eV) at 3.15 scan s^{-1} and the ion source was maintained at 230 °C. The identification of the compounds was done through the calculation of the linear retention index using a homologous series of alkane: C7-C30 (VAN DEN DOOL, 1963) and in addition, the mass spectrum was compared with data from the literature (ADAMS, 2007). The constituents were quantified by dividing the peak area of each compound by the total area of the identified compounds (%).

4.2.6 Determination of antioxidant activity

4.2.6.1 DPPH radical scavenging assay

For the extracts and essential oil the antioxidant activity by free radical DPPH (2,2-diphenyl-1-pyridyl-hydrazila) was performed according previously described by Brand-Williams et al. (1995). The method is based on the reduction of DPPH radical by the antioxidant compounds of the sample and consequently in change of violet to yellow coloration, proportional to the concentration of reducing substance. Initially, 100 µl of six different concentrations of ethanolic extracts and essential oil of *B. dracunculifolia* leaves were added to 3.9 mL of DPPH methanolic P.A solution (23.66 $\mu g.ml^{-1}$). The reaction was incubated protected from light at room temperature for 30 minutes. After, the solution was subjected to UV-Vis spectrophotometry (Shimadzu®-1800). To determine absorbances at 515 ηm. The DPPH scavenging capacity was expressed as percentage inhibition: AA(%)= ((Abs.control - Abs.sample)/Abs.control)100 where 'Abs.control' is the absorbance of DPPH radical in methanol and 'Abs. sample' is the absorbance of DPPH radical solution + plant extract or essential oil.

To determine IC_{50} values (antioxidant concentration required to reduce by 50% the amount of DPPH), the percent of inhibition was calculated and plotted against the sample concentration. For this, six concentrations of the extracts (3200, 1600, 800, 400, 200 and 100 $\mu g.ml^{-1}$) and essential oil (640, 320, 160, 80, 40 and 20 $\mu g.ml^{-1}$) were prepared.

4.2.6.2 ABTS radical scavenging assay

The antioxidant activity of ethanolic extracts by free radical ABTS (2,2-azinobis- (3-ethyl-benzothiazolin-6-sulfonic acid)) was performed following the method of Re et al. (1999) with some modifications. This analysis was done just for ethanolic extracts (50% v/v) because the solubilization of the essential oil with the analysis reagents was not possible. Initially, the stock solution were prepared with 7mM ABTS solution and 140mM potassium persulfate solution, the contents were mixed and incubated at room temperature in the dark for 16 h. An aliquot (1ml) of this solution was diluted in 100 ml of sodium acetate buffer (20 $mMol.L^{-1}$) pH 4. After, the

solution was adjusted to obtain an absorbance of 0.700 units at 734 ηm. An aliquot of 30µl of plant extracts were added in 3ml of adjusted solution and after 2h of reaction the absorbance was taken at 734 nm using UV/Vis spectrophotometer (Shimadzu®-1800). An analytical curve using Trolox as a standard were calculated for the conversion of the absorbance within the range of 0 $\mu g.ml^{-1}$ - 2500 $\mu g.ml^{-1}$ ($y = -0.0003x + 0.6847$; $r^2 = 0.9993$). The results were expressed as Trolox equivalent antioxidant capacity ($\mu M.g^{-1}$).

4.2.7 Statistical analysis

The experimental design was completely randomized in a 2x2 factorial scheme (two populations and two gender: male and female), with 3 replications. The data were tested with a Bartlett test to verify the homogeneity of the variances. After this, analysis of variance (ANOVA) was applied with mean separation using Tukey test at 5% probability ($p < 0.05$), when the differences were significant. The analyses were performed using the software Assistat (SILVA; AZEVEDO, 2016).

4.3 RESULTS AND DISCUSSIONS

4.3.1 Analysis of the extracts

4.3.1.1 Chemical characterization of the extracts

The chemical constituents from the aqueous ethanolic extracts (50% v/v) of *B. dracunculifolia* from botanical materials analyzed are described in TABLE 4.1. Chromatographic analysis such as LC-MS and HPLC-DAD could annoted three compounds, identified fourteen compounds and detected fourteen compounds. Caffeoylquinic acids with its derivatives and isomers are the main compounds identified in *B. dracunculifolia* leaves, followed by prenylated cinnamic acid derivatives: artepillin C, baccharin and drupanin. Many studies suggest that *B. dracunculifolia* is the main botanical source of Brazilian green propolis, where similar profiles were identified between plant resin and propolis samples, in particular to the composition of prenylated cinnamic acid derivative, flavonoids and chlorogenic acid,

as well as found in this work (KUMAZAWA et al., 2003; PARK et al., 2004; TEIXEIRA et al., 2005; TOMAZZOLI et al., 2020).

Differences in gender of the plant and in growth sites (populations) were not identified in the qualitative composition of *B. dracunculifolia* leaves in this work, but a large difference was found in the quantification of compounds between them by HPLC-DAD (CHART 4.1). Related to the identification of the compounds, similar result was found by Park et al. (2004) where the same compounds were identified for male and female *B. dracunculifolia* leaf bud. However, Teixeira et al. (2005) identified some differences between apices of female and male plants, when evaluated together volatile and nonvolatile compounds, where male plants were chemically more complex than female plants, with 42 identified substances in male and 33 in female apices. This difference can be related to abiotic factors and also to the herbivory predilations between male and female plants, that affect the plant defense and consequently the secondary metabolism (HALL et al., 2017).

TABLE 4.1 - COMPOSITION OF *B. dracunculifolia* LEAVES BY LC-MS/MS.

(to be continued)

Compounds	Rt	M^{-1}	Mass Fragmentation	Mol For	Ref
1- Caffeoylquinic acid isomer	14.57	353.087	191.0557/ 173.0454/ 161.0243/ 135.0451/ 127.0399/ 93.0344/ 85.0294	$C_{16}H_{18}O_9$	1, 3
2- Caffeic Acid	15.45	179.0348	135.0452/ 117.0346	$C_9H_8O_4$	1
3- ni	15.97	595.1656	475.1241/ 457.1136/ 415.1026/ 385.0928/ 355.0820	$C_{27}H_{32}O_{15}$	
4- ni	18.93	415.1601	191.0563/ 149.0457/ 131.0342/ 89.0244	$C_{19}H_{28}O_{10}$	
5- ni	23.60	519.1704	339.1086/ 309.0978/ 279.0871	$C_{22}H_{32}O_{14}$	
6- 3,4-Di-O-(E)-caffeoylquinic acid	27.20	515.1184	353.0873/ 335.0770/ 191.0558/ 179.0346/ 173.0452/ 161.0243/ 135.0450	$C_{25}H_{24}O_{12}$	1, 3
7- 3,5-Di-O-(E)-caffeoylquinic acid	27.97	515.1189	353.0875/ 191.0558/ 179.0347/ 173.0347/ 161.0246/ 135.0451	$C_{25}H_{24}O_{12}$	1, 3
8- 4,5-Di-O-(E)-caffeoylquinic acid	31.06	515.1184	353.0863/ 191.0553/ 179.0341/ 173.0446/ 161.0241/ 135.0446	$C_{25}H_{24}O_{12}$	3, 5
9- Caffeic acid derivative	32.06	487.1598	323.0768/ 263.0558/ 179.0346/ 161.0240	$C_{25}H_{28}O_{10}$	1
10- ni	34.43	361.1651	317.1761/ 287.1657/ 243.1757/ 155.0868	$C_{20}H_{26}O_6$	
11- ni	35.18	361.1649	317.1750/ 287.1653/ 243.1750/ 155.0864	$C_{20}H_{26}O_6$	
12- Tricaffeoylquinic acid	37.60	677.1494	515.1180/ 353.0866/ 335.0767/ 191.0555/ 179.0344/ 161.0240	$C_{34}H_{30}O_{15}$	1
13- Artepillin C derivative – CO_2	41.46	343.1541	299.1629/ 281.1555/ 255.1740/ 241.1589/ 217.1226/ 197.1330/ 173.1334	$C_{20}H_{24}O_5$	1
14- Kaempferide	42.43	299.0551	284.0320/ 255.0293/ 227.0343/212.0474/ 200.0475/ 136.9877	$C_{16}H_{12}O_6$	1, 2, 4
15- Kaempferide isomer	43.27	299.0552	284.0323/ 255.0294/ 227.0346	$C_{16}H_{12}O_6$	
16- ni	43.33	329.0659	314.0424/ 29.0189/ 271.0240/ 255.0293	$C_{17}H_{14}O_7$	
17- ni	44.11	407.2064	389.1969/ 359.1861/ 315.1963/ 287.1649/ 243.1751/ 217.1228/ 175.1126/ 111.0451	$C_{22}H_{32}O_7$	
18- 4-Hydroxy 3-prenylcinnamic acid- Drupanin	45.75	231.1021	187.1126/ 132.0578	$C_{14}H_{16}O_3$	1, 2, 3

(finish)

19- ni	45.81	389.1957	359.1860/ 315.1960/ 269.1540/ 241.1590	$C_{22}H_{30}O_6$	
20- ni	46.76	327.1228	321.1004/ 163.0399/ 145.0294/ 119.0510	$C_{19}H_{20}O_5$	
21- ni	47.98	391.2112	345.1697/ 332.1620/ 315.1595/ 301.1805/ 271.1698/ 243.1746/ 205.1229	$C_{22}H_{32}O_6$	
22- ni	48.60	315.1592	297.1490/ 253.1590/ 241.1590/ 198.1044/ 186.1045	$C_{19}H_{24}O_4$	
23- Methyl-Kaempferide	49.54	313.0709	298.0479/283.0240/ 255.0289/ 227.0345/ 163.0032/ 135.0085	$C_{17}H_{14}O_6$	
24- ni	50.27	295.0967	149.0604/ 119.0499/ 105.0708	$C_{18}H_{16}O_4$	
25- ni	51.11	267.1019	163.0398/ 145.0291/ 119.0499/ 117.0342	$C_{17}H_{16}O_3$	
26- Coumaric acid derivative	53.09	315.1591	297.1491/ 253.1595/ 201.1281/ 271.1700/ 146.0734	$C_{19}H_{24}O_4$	1
27- Artepillin C derivative – OCH_2	53.83	329.1745	299.1624/ 328.1879/ 255.1754	$C_{20}H_{26}O_4$	1
28- ni	54.93	331.1901	301.1799	$C_{20}H_{28}O_4$	
29- Artepillin C	57.46	299.1642	299.1646/ 255.1746/ 200.1199/ 145.0654	$C_{19}H_{24}O_3$	1, 2, 3
30- 3-Prenyl-4-(dihydrocinnamoyloxy)-cinnamic acid- Baccharin	60.67	363.1587	187.1123/ 149.0603/ 131.0498	$C_{23}H_{24}O_4$	1
31- (E)-3-{-4-hydroxy-3-[(E)-4-(2,3-dihydrocinna-moyl oxy)-3-methyl-2-bute-nyl]-5-prenylphenyl}-2-propenoic acid	65.17	447.2159	297.1486/ 253.1588/ 198.1042/ 149.0602/ 105.0707	$C_{28}H_{32}O_5$	1, 2

SOURCE: The author (2019).
SUBTITLE: [1] Szliszka et al. (2013); [2] Carvalho et al. (2011); [3] De Moura et al. (2011); [4]Falcão et al. (2013); [5] Fernandes-Silva et al. (2013). Rt= Retention time (min); M^{-1}= Molecular mass; Mol For= Molecular formula; Ref= References.

4.3.1.2 Chemical content of the extracts

The major compounds identified in the leaves regardless of gender and population were 3,5-Di-O-(E)-caffeoylquinic acid (21.93 to 10.87 $mg.g^{-1}$), 4,5-Di-O-(E)-caffeoylquinic acid (15.25 to 3.72 $mg.g^{-1}$) and artepillin C (18.95 to 3.29 $mg.g^{-1}$), but the interaction between non-volatile constituents of female and male *B. dracunculifolia* leaves and their place of production was also investigated.

Factor analysis applied to compounds above 0.6 $mg.g^{-1}$ identified interaction between gender and population for the compounds: Caffeoylquinic acid isomer, caffeic acid, 3,5-Di-caffeoylquinic acid, 4,5-Di-caffeoylquinic acid and kaempferide (TABLE 4.2). The detected interaction refers to the amount of production these compounds linked to the plant gender in that specific population, but despite that, all presented higher content in male plants from PPA. However, the interaction between populations and gender was not significant for the compounds: 3,4-Di-caffeoylquinic acid, tricaffeoylquinic acid, drupanin, artepillin C and p-coumaric acid, this means that these factors act independently. For this compounds isolated differences were found, with again higher levels in male plants and in the population PPA, with the exception of p-coumaric acid, which showed no statistical difference between the treatments

(TABLE 4.3; TABLE 4.4). The chromatogram profiles (HPLC-DAD) are shown in FIGURE 4.1.

TABLE 4.2 - MEANS AND STANDARD DEVIATION OF COMPOSITION CONTENT mg.g^{-1} OF MALE AND FEMALE LEAVES OF *B. dracunculifolia* PLANTS FROM POPULATION OF PALMEIRA (PPA) AND POPULATION OF PIRAQUARA (PPI) CHARACTERIZED BY HPLC-DAD.

Populations	Caffeoylquinic derivative		Caffeic acid		3,5-Di-caffeoylquinic acid		4,5-Di-caffeoylquinic acid		Kaempferide	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
PPA	3.06 ± 0.23 aA	1.61 ± 0.36 aB	2.65 ± 0.15 aA	1.45 ± 0.13 aB	21.93 ± 1.84 aA	16.73 ± 0.77 aB	15.25 ± 0.87 aA	10.42 ± 0.66 aB	0.59 ± 0.06 aA	0.51 ± 0.05 aB
PPI	1.44 ± 0.15 bA	1.15 ± 0.05 bB	1.18 ± 0.07 bA	0.95 ± 0.08 bB	11.72 ± 0.79 bA	10.87 ± 0.47 bA	9.68 ± 0.64 bA	3.72 ± 0.15 bB	0.51 ± 0.05 bA	0.22 ± 0.01 bB

SOURCE: The author (2019).
SUBTITLE: Means followed by the same lower case letters in columns and upper case letter in lines are not statiscally different at 5% level of significance according to Tukey test.

TABLE 4.3 - MEANS AND STANDARD DEVIATION OF COMPOSITION CONTENT mg.g^{-1} OF LEAVES OF *B. dracunculifolia* PLANTS FROM POPULATION OF PALMEIRA (PPA) AND POPULATION OF PIRAQUARA (PPI) CHARACTERIZED BY HPLC-DAD.

Populations	Compounds				
	3,4-Di-caffeoylquinic acid	Tricaffeoylquinic acid	Drupanin	Artepillin C	p-coumaric acid
PPA	11.09 ± 3.98 a	4.65 ± 1.07 a	1.68 ± 0.54 a	14.24 ± 4.92 a	2.17 ± 0.35 a
PPI	6.20 ± 1.91 b	2.18 ± 0.88 b	0.99 ± 0.49 b	7.91 ± 4.93 b	2.19 ± 0.71 a

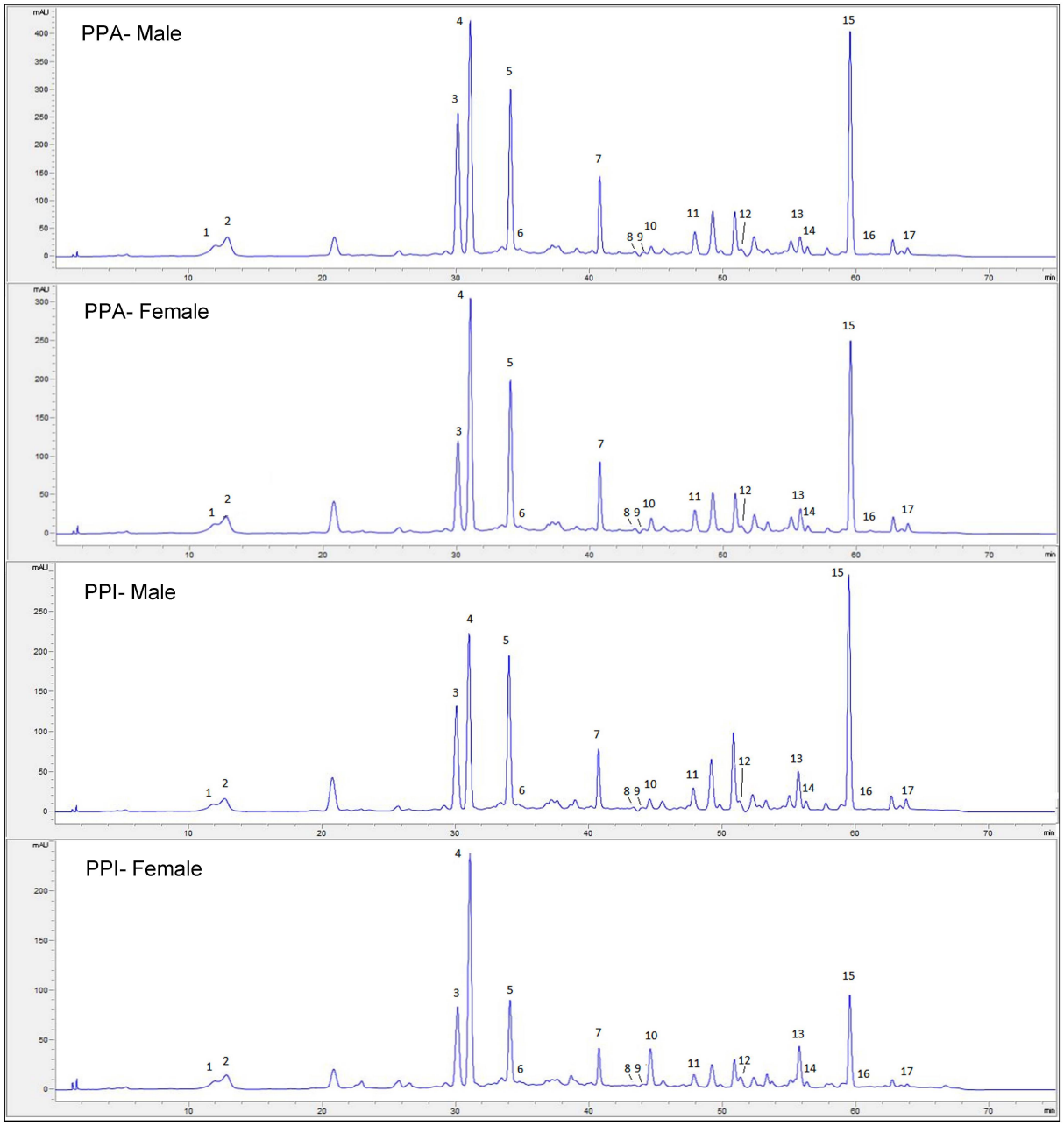
SOURCE: The author (2019).
SUBTITLE: Means followed by the same lower case in columns are not statiscally different at 5% level of significance according to Tukey test.

TABLE 4.4 - MEANS AND STANDARD DEVIATION OF COMPOSITION CONTENT mg.g^{-1} OF LEAVES OF *B. dracunculifolia* PLANTS FROM POPULATION OF PALMEIRA (PPA) AND POPULATION OF PIRAQUARA (PPI) CHARACTERIZED BY HPLC-DAD.

Gender	Compounds				
	3,4-Di-caffeoylquinic acid	Tricaffeoylquinic acid	Drupanin	Artepillin C	p-coumaric acid
Male	11.37 ± 3.71 a	4.32 ± 0.45 a	1.82 ± 0.39 a	15.70 ± 3.40 a	2.41 ± 0.64 a
Female	5.92 ± 1.61 b	2.51 ± 1.22 b	0.85 ± 0.36 b	6.44 ± 3.41 b	1.95 ± 0.31 a

SOURCE: The author (2019).
SUBTITLE: Means followed by the same lower case in columns are not statiscally different at 5% level of significance according to Tukey test.

FIGURE 4.1 - CHROMATOGRAPHIC PROFILES (HPLC-DAD, λ = 316 nm) OF MALE AND FEMALE LEAVES OF *B. dracunculifolia* FROM POPULATION OF PALMEIRA (PPA) AND POPULATION OF PIRAQUARA (PPI).



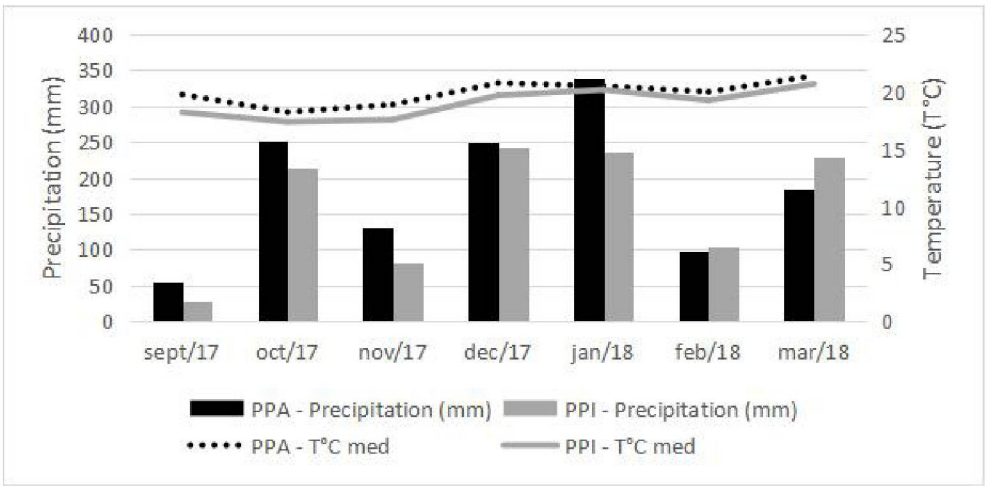
SOURCE: The author (2019)

The peak numbers represent the same compounds: (1) Caffeoylquinic acid isomer; (2) Caffeic Acid; (3) 3,4-Di-O-(E)-caffeoylquinic acid; (4) 3,5-Di-O-(E)-caffeoylquinic acid; (5) 4,5-Di-O-(E)-caffeoylquinic acid; (6) Caffeic acid derivative; (7) Tricaffeoylquinic acid; (8) Artepillin C derivative – CO2; (9) Kaempferide; (10) Kaempferide isomer; (11) Drupanin; (12) Methyl - Kaempferide; (13) Coumaric acid derivative; (14) Artepillin C derivative – OCH2; (15) Artepillin C; (16) Baccharin; (17) (E)-3-{-4-hydroxy-3-[(E)-4-(2,3-dihydrocinnamoyl oxy)-3-methyl-2-butenyl]-5-prenylphenyl}-2-propenoic acid.

The concentration of many secondary metabolites are strongly correlated with plant growth conditions, as synthesis of various metabolites may have different impacts on its accumulation depending on the stimuli (FIGUEIREDO et al., 2008). For example, environmental stresses such as radiation, pathogen attack, nutrient availability, and temperature usually increase phenylpropanoid levels. On the other hand, phenolic levels are extensively affected by nutritional stress (DIXON; PAIVA, 1995; RAMAKRISHNA; RAVISHANKAR, 2011).

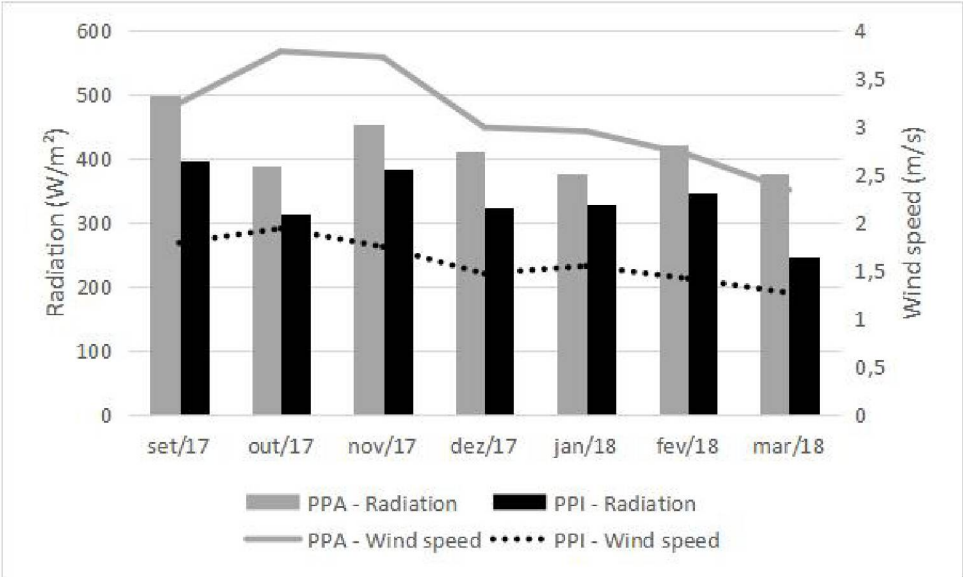
The chemical profile of phenolic compounds is also affected by altitude variation, where the higher the altitude the higher the phenolic content (PANDEY et al., 2018). As previously reported, in this work we identified differences in the compounds produced in the two populations studied, including phenolics compounds (prenylated cinnamic acid derivatives) and flavonols (kaempferide and their derivatives) and this may be related to weather conditions from the two plant growth sites (FIGURE 4.3, FIGURE 4.4). Plants collected in PPA (altitude =1027 meters) presented higher levels of these compounds when compared with PPI (altitude= 891 meters) and consequently plants from PPA were subjected to higher levels of radiation. This may have occurred because plants growing at higher altitudes are subjected to higher UV-B radiation and it affects their development, morphology and physiology and as a protection mechanism plants produce higher contents of phenolic and flavonoids (FROHNMEYER; STAIGER, 2003; JAAKOLA; HOHTOLA; 2010).

FIGURE 4.2 - CLIMATE CONDITIONS OF PRECIPITATION (mm) AND AVERAGE TEMPERATURE (T°C) IN POPULATION OF PALMEIRA (PPA) AND PIRAQUARA (PPI).



SOURCE: Parana Meteorological System - SIMEPAR (2018).

FIGURE 4.3 - CLIMATE CONDITIONS OF RADIATION (W/m²) AND WIND SPEED (m/s) IN POPULATION OF PALMEIRA (PPA) AND POPULATION OF PIRAQUARA (PPI).



SOURCE: Parana Meteorological System - SIMEPAR (2018).

Phenolic compounds protect plant tissues against UV radiation and this can help us to uderstand the increase of phenolic levels. Consequently, the production of these compounds depends of three factors acting simultaneously: light environment, plant physiology and plant biochemistry (BANTIS et al., 2016). Many phenolics like phenolic acids and flavonoids are produced in response to environmental stresses, mainly because of their antioxidant properties (WANG; FREI, 2011). They originate from the phenylpropanoid biosynthetic pathway and for their production a key enzymes, such as phenylalanine ammonia lyase (PAL), is strongly stimulated by environmental stresses, where in most cases, it leads to increased phenolic levels in plants (VOGT, 2010; WANG; FREI, 2011).

Although this work has identified differences in the contents of some compounds according to plant growth site, it is important to highlight that in general the male plants presented higher levels of all compounds studied. The sex-differences in secondary metabolites were very expressive and this may have been caused by different reproductive efforts between males and females (ANTOS; ALEN, 1999; CEPEDA-CORNEJO; DIRZO, 2010). Male plants generally make lower total reproductive investment than females, although their investment during flowering is great (OTERO-ARNAIZ; OYAMA, 2001; TORIMARU; TOMARU, 2006; CEPEDA-CORNEJO; DIRZO, 2010). Female plants have a considerable increase of

investment in reproduction mainly due to ripening of fruit and seeds (TURCOTTE; HOULE, 2001). This higher investment of female metabolism for reproduction may indicate a lower shift of products to secondary metabolism, which is directly related to plant defense.

The pathways of primary and secondary metabolism share common precursors and intermediates and because of this, common molecules are used for synthesis of proteins and defense metabolites and sometimes the tradeoff between plant growth and defense may be inevitable (HERMS; MATTSON, 1992). Previous study estimated that at least 60 percent of total plant biomass is made up of molecules that go through the shikimic acid pathway which originate from amino acid precursors of protein, phenolics, alkaloids, for example (JENSEN, 1986). During plant development, including the phenological stages, there is a strong dependency on protein synthesis for the manufacture of photosynthetic, biosynthetic, regulatory enzymes and structural protein. The same occurs for phenolic synthesis and because of this, growth and defense compete for common substrates (HERMS; MATTSON, 1992).

In dioecious species different resource allocations between gender may also determine unequal patterns of defense and herbivory in the field (HALL et al., 2017). The infestation of different galls on *B. dracunculifolia* is quite discussed and the insect *Baccharopelma dracunculifoliae* (Hemiptera: Psyllidae) is the most frequently gall found in this plant species (Arduin et al., 2005). In the work developed by Bastos et al. (2011) no significant difference between genders of *B. dracunculifolia* were identified about herbivory by different insects: *Baccharopelma dracunculifoliae* galls infestation and *Apis mellifera* visit for resin collection. However, the same work identified the plant growth site may influence *B. dracunculifoliae* galls infestation and resin collecting visits of *A. mellifera*, confirming the relationship between presence of gall insect and production of defense compounds by host plants as a defense strategy. In contrast, Rodrigues (2017) identified significant differences between males and females plants of *B. dracunculifolia*, where males had greater infestation by galling insects and females were most visited by bees for resin collection to produce green propolis.

Many studies about the relationship between galls insects and secondary metabolits from host plant have been quantified and widely discussed in the work developed by Hall et al. (2017). It was found that concentrations of phenolic and

tannin compounds increased significantly in response to galls, whereas the concentration of volatile compounds was not significant, this may occur because of the different roles of these compound classes in plant defence. High concentrations of phenolics and tannins are usually associated with the action against chewing herbivores, whereas volatiles usually induce response to herbivore feeding (FURSTENBERG-HAGG et al., 2013). Because of this, galls strategy is the increase of phenolic and tannins levels to reduce the risk of predation due to herbivory and also reduce the risk of predation with the decrease of volatile emissions (DELVAS et al., 2011; MOILANEN et al., 2016; HALL et al., 2017). Since the work developed by Rodrigues (2017) found higher gall infestation in male plants, this can help us to understand why our work found so much diferences in secondary metabolites of male plants of this species.

4.3.1.3 Antioxidant activities of the extracts

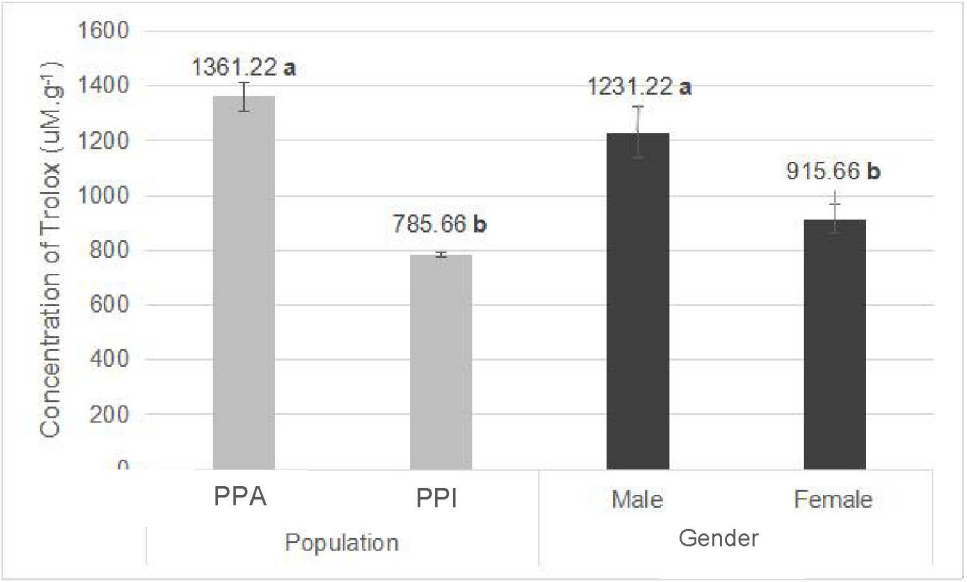
The antioxidant activity of the *B. dracunculifolia* extracts was expressed as IC_{50} ($\mu g.ml^{-1}$) against DPPH and concentration of Trolox ($uM.g^{-1}$) by ABTS, respectively (TABLE 4.5; FIGURE 4.4). The radical scavenging activity can be expressed by the IC_{50}, which is the concentration of antioxidant to reduce the radical by 50% of DPPH, where lower IC_{50} values indicates higher sample activity. For this assay, antioxidant activity varied from 1124.72 ± 50.03 $\mu g.ml^{-1}$ to 2563.63 ± 140.15 $\mu g.ml^{-1}$ of aquous ethanolic extract. We observed an interaction between population and sex of the plant (as the same happened with the analyzed compounds) with lower IC_{50} in male plants from PPA (1124.72 ± 50.03 $\mu g.ml^{-1}$) and consequently highest capacity to neutralize free radicals. The antioxidant activity by ABTS didn´t show factor interaction, but isolated differences between genders and populations were detected, with again higher capacity antioxidant in male plants (1231.22 $uM.g^{-1}$) and in PPA (1361.22 $uM.g^{-1}$).

TABLE 4.5 - MEANS AND STANDARD DEVIATION FROM IC50 OF ANTIOXIDANT ACTIVIT (µg.ml^{-1}) BY DPPH OF MALE AND FEMALE LEAVES OF *B. dracunculifolia* FROM POPULATION OF PALMEIRA (PPA) AND POPULATION OF PIRAQUARA (PPI).

Populations	IC_{50} - DPPH (µg.ml^{-1})	
	Male	Female
PPA	1124.72 ± 50.03 bB	1397.21 ± 40.12 bA
PPI	1604.68 ± 45.25 aB	2563.63 ± 140.15 aA

SOURCE: The author (2019).
SUBTITLE: Means followed by the same lower case letters in columns and upper case letter in lines are not statiscally different at 5% level of significance according to Tukey test.

FIGURE 4.4 - MEANS AND STANDARD DEVIATION OF ANTIOXIDANT ACTIVITY BY ABTS (µm.g^{-1}) OF MALE AND FEMALE LEAVES OF *B. dracunculifolia* FROM POPULATION OF PALMEIRA (PPA) AND POPULATION OF PIRAQUARA (PPI). MEANS FOLLOWED BY THE SAME LOWER CASE LETTERS FOR POPULATIONS AND THE SAME LOWER CASE LETTERS FOR GENDER ARE NOT STATISCALLY DIFFERENT AT 5% LEVEL OF SIGNIFICANCE ACCORDING TO TUKEY TEST.



SOURCE: The author (2019).

Phenolics and flavonoids are important compounds for the free radical scavenging activity, indicating the active role in the robust antioxidant efficacy of the plant (PANDEY et al., 2018). Food, beverage and cosmetic industries have been increasingly interested in natural sources of antioxidant molecules. Phenolics can be used as natural source of antioxidants as they have the ability to scavenge free radicals by single-electron transfer, besides being distributed in various food sources (HIRANO et al., 2001). Some works reported strong antioxidant activity for the caffeoylquinic acid derivatives emphasizing that its content as the major antioxidant

metabolites isolated from Asteraceae plant species (FRAISSE et al., 2011; MIJANGOS-RAMOS et al., 2018). The antioxidant capacity of caffeoylquinic acids can be related to their chemical structure, where catechol moiety combined with unsaturated ester moiety gives their free radical-scavenging activity (PAREJO et al., 2004). In addition, studies have been correlating antioxidant capacity levels with the anti-inflammatory activity of these purified compounds, showing that they may be related to their capacity as radical scavengers (MIJANGOS-RAMOS et al., 2018).

Another compound well known for its antioxidant activity is one of prenylated cinnamic acid derivative, artepillin C. A previous study by Shimizu et al. (2004) not only proved the antioxidant capacity of this isolated compound, but also showed its bioavailable antioxidant, i.e. artepillin C has the capacity to pass though the intestinal absorption system in the free form and avoid oxidative damage in DNA cells, for example in hepatocytes. Unlike artepillin C, most phenolics have low bioavailability due to their low intestinal absorption rate. This happens because these compounds are usually converted to conjugate forms (atoms of the chemical compound are connected through simple covalent bonds which increases their stability) during intestinal absorption and fail to exhibit this ability. On the other hand, the active forms are the free form aglycone of phenolics (MUROTA; TERAO, 2003).

The chemical structure of artepillin C consists of a simple phenol (single ring) and with two prenyl groups. Simple phenols are relatively refractory to conjugation when compared to other phenol, e.g. flavonoids, and additionally prenyl groups make conjugation even harder (MUROTA; TERAO, 2003; SHIMIZU et al., 2004). Previous work described that prenyls increase affinity for cell membranes and this similarly happens with artepillin C, which facilitates its antioxidant effect on membranous lipid peroxidation (KASAI et al., 2002). In this context, due to its affinity for cell membranes and because it is not easily conjugated, artepillin C is used in treatments of degenerative diseases, for exemple, to prevented renal and pulmonary cancers (KIMOTO et al., 2000; KIMOTO et al., 2001).

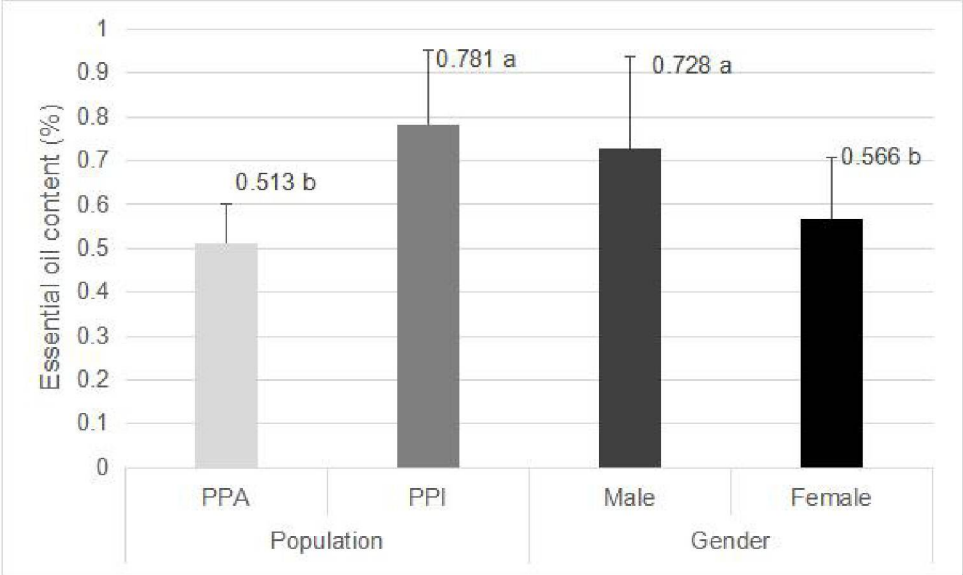
Therefore, plants of *B. dracunculifolia* with higher contents of artepillin C and dicaffeyolquinic acids have the potential to produce a green propolis more beneficial to human health due to increased antioxidant activity, in addition to increasing its market value added.

4.3.2 Analysis of the essential oils

4.3.2.1 Essential oil content (%)

Interaction between population e gender was not significant ($p>0.05$) for essential oil content, this means that these factors act independently in this study. However, isolated differences were verified for population and gender. The essential oil contents of populations ranged from 0.513 - 0.781% and plants from PPI produced higher amounts (0.781%) when compared to PPA. The production of essential oil between female and male plants ranged from 0.566 - 0.728% and male plants produced higher amounts (0.728%) when compared to female plants (FIGURE 4.5).

FIGURE 4.5 - ESSENTIAL OIL CONTENT (%) OF FEMALE AND MALE PLANTS OF *B. dracunculifolia* FROM POPULATION OF PALMEIRA (PPA) AND POPULATION OF PIRAQUARA (PPI). COEFFICIENT OF VARIATION = 16.51%. MEANS FOLLOWED BY THE SAME LOWER CASE LETTERS FOR POPULATIONS AND THE SAME LOWER CASE LETTERS FOR GENDER ARE NOT STATISCALLY DIFFERENT AT 5% LEVEL OF SIGNIFICANCE ACCORDING TO TUKEY TEST.



SOURCE: The author (2019).

B. dracunculifoilia reproduction time is different between the sexes, the male starts flowering earlier, and this can be explained by the competition between males for pollination opportunities. In general, males flowering time are induced by pollen donation (selection pressure) where males that produce earlier flowers increase reproductive success (ESPIRITO-SANTO et al., 2003). As the higher terpene content

may be involved with pollinator activity (MAFFEI, 2010), this can help us understand the higher essential oil content in male plants.

In a previous study carried out with male and female plants of *B. dracunculifolia,* the essential oil contents were similar between genders, with no statistical difference between them, where males produced 0.81% and females 0.85% of essential oil (BESTEN et al., 2012). Similarly, for the essential oil of leaves of *Pistacia atlantica* Desf., the average yield for male (0.12%) and female (0.11%) collected from june to october did not differ during the season and for plant gender (GOURINE et al., 2010). The same was observed in the study developed by Mwang'ingo et al. (2010), where no significant variation in the essential oil content was verified between male (6.985%) and female (6.905%) plants of the species *Osyris lanceolata.* Previous study developed with the same dioecious species *O. lanceolata* suggested that both sexes have similar physiological requirements and this variation is not important to its survival (MWANG'INGO et al., 2007).

However, male and female plants may have diferrent physiological requirement and variations in some aspects between them can be expected (MWANG'INGO et al., 2010). Gender differences were noticed in the work developed by Radoukova et al. (2018) for the plant *Juniperus communis* L., where the interaction between plant sex and habitat was highly significant, the essential oil content ranged from 0.28% to 1.21%, with higher levels in female plants. In contrast, the study developed with *Juniperus scopulorum* Sarg., the essential oil content in male trees was greater than in female trees (ZHELJAZKOV et al., 2013). As previously discussed, male plants generally making lower total reproductive investment than females that have a considerable increase of investment in reproduction mainly due to ripening of fruit and seeds (OTERO-ARNAIZ; OYAMA, 2001; TURCOTTE; HOULE, 2001; TORIMARU; TOMARU, 2006; CEPEDA-CORNEJO; DIRZO, 2010). Once the pathways of primary and secondary metabolism share common precursors and intermediates this higher investment of female metabolism for reproduction may indicate a lower shift of products to secondary metabolism (HERMS; MATTSON, 1992), and consequently leading to lower terpene production, what can help us understand the high levels of essential oil in males when compared to females plants.

In addition, local plant growth also influenced the production of essential oil, where plants from PPI produced higher essential oil content (0.781%) (FIGURE 4.5).

These differences between the two populations may be related to the biotic and abiotic factors which include, environmental conditions, edaphic factors, mechanical or chemical injuries, climate, diseases, pests and genetic factors (FIGUEIREDO et al., 2008). The highest essential oil content from PPI may be related to low precipitation especially during january/2018 and february/2018, period that preceded the leaf harvest in march/2018 (FIGURE 4.2, FIGURE 4.3). Increasing of the essential oil production in many plants is a response to low precipitation, once the prolonged water stress can cause several harmful reactions to the plant including the production of reactive oxygen species (ROS) which damage the overall physiology of the plant (LOPES et al., 2001; CHAI et al., 2005; JEYARAMRAJAA et al., 2005).

Unlike the results of essential oils, the extract analysis by HPLC-DAD (TABLE 4.1, TABLE 4.2) identified higher production of secondary metabolites in PPA plants. This may have happened due to different metabolic pathways for the synthesis of terpenes and phenolic acids. The synthesis of terpenes can occur by the 2-C-methyl-D-erythritol 4-phosphate and by the mevalonate (VRANOVÁ et al., 2013; PAZOUKI et al., 2016). While the synthesis of phenolic compounds ican occur via the shikimic acid pathway and the malonic pathway, the shikimic acid pathway produces most plant phenolics (TAIZ; ZEIGER et al., 2013). Thus, these different metabolic pathways may affected the levels of terpenes and phenolics in inverse correlation depending on plant stimulus.

4.3.2.2 Chemical composition of essential oils

A total of 31 chemical constituents were identified in the essential oils of male and female plants of *B. dracunculifolia* from the two studied populations. The essential oils were predominantly composed of oxygenated sesquiterpenes (36.24 - 52.44%), monoterpene hydrocarbons (15.75 - 43.24%) and sesquiterpenes hydrocarbons (9.34 - 18.38%). The major compounds were: *(E)*-nerolidol (13.03 - 19.68%), β-pinene (9.81 - 20.97%), limonene (6.17 - 18.21%) and spathulenol (6.79 - 12.02%) for all botanical materials (TABLE 4.6). Similar chemical composition has been reported from studies with *B. dracunculifolia* plants from different growth sites of Brazil, where the compounds *(E)*-nerolidol and spathulenol were indicated as the majority and β-pinene and limonene presenting low levels (QUEIROGA et al., 2008; BESTEN et al., 2012; BARBOSA et al., 2015).

TABLE 4.6 - COMPOSITION OF ESSENTIAL OIL% BY GC/MS (MEAN AND STANDARD DEVIATION) OF FRESH LEAVES OF FEMALE AND MALE PLANTS OF *B. dracunculifolia* FROM POPULATION OF PALMEIRA (PPA) AND POPULATION OF PIRAQUARA (PPI).

Compounds	IR^{lit}	IR^{cal}	Populations			
			PPA		PPI	
			Male	Female	Male	Female
1- α-tujene	924	925	0.13 ± 0.03	-	0.15 ± 0.04	0.21 ± 0.01
2- α-pinene	932	932	3.44 ± 0.70	1.89 ± 0.38	3.52 ± 1.09	5.58 ± 0.29
3- sabinene	969	972	0.25 ± 0.07	0.16 ± 0.05	0.16 ± 0.10	0.42 ± 0.05
4- β-pinene	974	976	14.21 ± 2.62	9.81 ± 1.69	18.64 ± 4.34	20.97 ± 0.85
5- myrcene	988	989	1.36 ± 0.30	0.84 ± 0.47	1.77 ± 0.28	2.11 ± 0.09
6- limonene	1024	1026	8.09 ± 0.67	6.17 ± 0.89	18.21 ± 2.25	13.95 ± 0.34
Monoterpenes hydrocarbons			**25.25**	**15.75**	**42.46**	**43.24**
7- β-elemene	1389	1388	0.42 ± 0.06	0.46 ± 0.04	0.21 ± 0.04	0.26 ± 0.02
8- (E)-β-cariofilene	1417	1413	1.86 ± 0.27	2.8 ± 0.11	1.70 ± 0.24	1.52 ± 0.23
9- aromadendrene	1439	1432	0.34 ± 0.04	0.31 ± 0.01	0.38 ± 0.05	0.22 ± 0.02
10- trans-muurola-3,5-dieno	1443	1451	0.11 ± 0.01	0.12 ± 0.01	-	0.13 ± 0.01
11- α-humulene	1446	1452	0.57 ± 0.08	0.71 ± 0.05	0.43 ± 0.05	0.34 ± 0.05
12- cabreuva oxide B	1462	1456	0.59 ± 0.08	0.67 ± 0.04	0.54 ± 0.07	0.36 ± 0.04
13- cabreuva oxide D	1467	1479	0.14 ± 0.01	0.15 ± 0.01	-	0.12 ± 0.01
14- germacrene D	1484	1474	4.10 ± 0.55	4.34 ± 0.12	2.48 ± 0.25	2.20 ± 0.22
15- germacrene A	1497	1508	0.18 ± 0.02	0.19 ± 0.29	-	-
16- bicyclogermacrene	1500	1490	5.93 ± 0.81	6.38 ± 0.12	3.40 ± 0.35	2.66 ± 0.25
17- α-muurolene	1500	1495	0.36 ±0.03	0.28 ± 0.02	0.23 ± 0.02	0.13 ± 0.01
18- γ-cadinene	1513	1508	0.46 ±0.05	0.44 ± 0.03	0.36 ± 0.03	0.28 ± 0.01
19- δ-cadinene	1522	1519	1.9 ± 0.15	1.53 ± 0.13	1.60 ± 0.13	1.12 ± 0.06
Sesquiterpene hydrocarbons			**17.4**	**18.38**	**11.34**	**9.34**
20- (*E*)-nerolidol	1561	1564	19.21 ± 0.23	19.68 ± 0.89	15.74 ± 2.05	13.03 ± 0.31
21- spathulenol	1577	1570	7.87 ± 0.52	12.02 ± 0.58	6.79 ± 1.17	10.34 ± 0.33
22-thujopsan-2-α-ol	1575	1586	3.46 ± 0.14	4.8 ± 0.64	2.93 ± 0.52	3.40 ± 0.11
23- globulol	1583	1590	3.02 ± 0.27	2.35 ± 0.35	2.34 ± 0.46	1.24 ± 0.12
24- ledol	1602	1594	2.02 ± 0.17	2.57 ± 0.33	1.68 ± 0.35	1.86 ± 0.06
25- 1-epi-cubenol	1627	1621	0.56 ± 0.04	0.54 ± 0.08	0.49 ± 0.04	0.53 ± 0.03
26- epi-α-muurolol	1640	1635	2.3 ± 0.19	2.13 ± 0.31	1.61 ± 0.31	1.42 ± 0.05
27- α-muurolol	1644	1640	0.69 ± 0.06	0.75 ± 0.13	0.52 ± 0.13	0.58 ± 0.01
28- α-cadinol	1652	1647	2.55 ± 0.14	2.61 ± 0.37	1.68 ± 0.44	1.50 ± 0.05
29- germacrene-trien-1-α-ol	1677	1685	1.66 ± 0.20	2.79 ± 0.35	1.42 ± 0.34	1.99 ± 0.02
30- shyobunol	1680	1688	1.23 ± 0.15	1.27 ± 0.02	0.69 ± 0.15	0.67 ± 0.05
31- isobiclogermacrenal	1733	1724	0.46 ± 0.06	0.93 ± 0.11	0.37 ± 0.11	0.59 ±0.01
Oxygenated sesquiterpene			**46.17**	**52.44**	**36.27**	**37.15**
Identified compounds %	-	-	89.47	89.69	90.04	89.73

SOURCE: The author (2019).

SUBTITLE: RI^{lit}: retention index of the literature. RI^{cal}: calculated retention index. – trace element <0.1%.

The essential oils from male and female plants showed the same compounds, but with quantitative variations. The same was observed in the work developed by Besten et al. (2012) with *B. dracunculifolia*, where similarities between the essential oil of male and female was verified, but also with quantitative variations.

Interaction between population e gender was not significant ($p>0.05$) for the chemical composition of essential oils, this means that these factors act independently on this study. However, isolated differences were verified for population and gender for some compounds. These analyses considered only the chemical constituents with averages higher than 3.0% in at least one of the botanical materials (TABLE 4.7). Plants from PPI produced higher levels of β-pinene (19.80%), limonene (16.08%) and α-pinene (4.55%) when compared to PPA. On the other hand, plants from PPA produced higher levels of *(E)*-nerolidol (19.45%), bicyclogermacrene (6.15%), germacrene D (4.22%), thujopsan-2-α-ol (4.13%), globulol (2.68%) and α-cadinol (2.58%) when compared with PPI. Similarly, the isolated gender effects (TABLE 4.8) were significant for some compounds studied, where females produced higher contents of spathulenol (11.18%) and thujopsan-2-α-ol (3.9%) than males plants and male plants produced higher levels of globulol than female plants (2.68%).

TABLE 4.7 - MEANS AND STANDARD DEVIATION OF ESSENTIAL OIL COMPOSITION % OF FRESH LEAVES OF *B. dracunculifolia* PLANTS FROM POPULATION OF PALMEIRA (PPA) AND POPULATION OF PIRAQUARA (PPI) CHARACTERIZED BY GC/FID.

Compounds	Populations		CV (%)
	PPA	PPI	
α-pinene	2.66 ± 0.98 b	4.55 ± 1.33 a	19.24
β-pinene	12.71 ± 2.45 b	19.80 ± 3.07 a	16.23
limonene	7.13 ± 1.27 b	16.08 ± 2.74 a	10.97
germacrene D	4.22 ± 0.41 a	2.34 ± 0.26 b	10.85
bicyclogermacrene	6.15 ± 0.61 a	3.03 ± 0.49 b	10.76
(E)-nerolidol	19.45 ± 1.62 a	14.38 ± 2.4 b	9.69
thujopsan-2-α-ol	4.13 ± 0.84 a	3.16 ± 0.42 b	11.58
globulol	2.68 ± 0.46 a	1.79 ± 0.67 b	14.54
α-cadinol	2.58 ± 0.25 a	1.58 ± 0.30 b	14.41

SOURCE: The author (2019).
SUBTITLE: Means followed by the same lower case in lines are not statiscally different at 5% level of significance according to Tukey test.

TABLE 4.8 - MEANS AND STANDARD DEVIATION OF ESSENTIAL OIL COMPOSITION % OF FRESH LEAVES OF *B. dracunculifolia* PLANTS FROM TWO GENDERS CHARACTERIZED BY GC/FID.

Compounds	Gender		CV(%)
	Male	Female	
spathulenol	7.33 ± 1.00 b	11.18 ± 1.39 a	11.36
thujopsan-2-α-ol	3.19 ± 0.44 b	4.10 ± 0.87 a	11.58
globulol	2.68 ± 0.50 a	1.79 ± 0.65 b	14.54

SOURCE: The author (2019).
SUBTITLE: Means followed by the same lower case in lines are not statiscally different at 5% level of significance according to Tukey test.

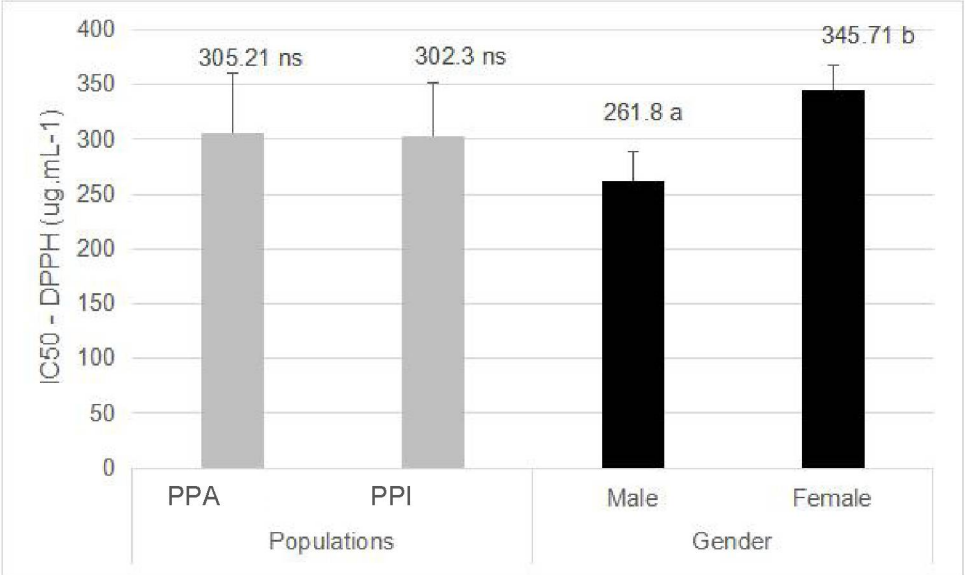
Although the precipitation in PPA and PPI was not discrepant during the plant development period, the precipitation in PPI (average) was lowest, with water deficit mainly in the periods of vegetative development (Sept/17 - Nov/17). Moreover, it is possible to notice that the monoterpenes: α-pinene, β-pinene and limonene were produced in higher amounts in PPI and the sesquiterpenes: *(E)*-nerolidol, thujopsan-2-α-ol, globulol and α-cadinol in PPA. Previous study by Ormeno et al. (2007) found that monoterpenes seem to protect plants against drought periods more than sesquiterpenes, where the monoterpenes contents were maintained during this period. According to this study, this can occur because sesquiterpenes are probably replaced by monoterpenes during water stress period, once the drought could impede cyclization of sesquiterpene precursor. In general, plants produce larger amounts of terpene due to the low allocation of carbon for the plant development, which suggests a trade-off between plant growth and defence (STAUDT; BERTIN, 1998).

On the other hand, the production of certain sesquiterpenes can be increased when plants are exposed to certain abiotic stresses such as light (MAES; DEBERGH, 2003). The UV radiation can cause important effects in plant growth and may affect the synthesis of terpenes (LIU et al., 2017). Since that plants from PPA were subjected to higher radiation, this may also help us understand the higher sesquiterpene yields of these essential oils. For example the high contents of *(E)*-nerolidol is due to its known scavenging activity (VINHOLES et al, 2014) and may act as an antioxidant compound on leaves under stressful conditions.

4.3.2.3 Antioxidant activity of essential oils

For antioxidant activity of *B. dracunculifolia* essential oils, expressed as IC_{50} ($\mu g.ml^{-1}$), the interaction between population and gender was also not significant ($p>0.05$). However, isolated differences between male and female plants were identified (FIGURE 4.6). Essential oils from male plants showed lower IC_{50} (261.8 $\mu g.ml^{-1}$) and consequently higher capacity to neutralize free radicals when compared to essential oils from female plants. Differences in antioxidant activity between male and female plants have been reported by other authors (EMAMI et al., 2007; ZUCCOLOTTO et al., 2019). Previous study developed with essential oils of the of leaves of *Juniperus communis* subsp. *hemisphaerica* verified the DPPH scavenging activity of the males plants with higher potential than oil of female plants for use as preserving food materials (EMAMI et al., 2007). On the other hand, in work developed with different species of the genus *Bacharis* as *B. aracatubaensis*, *B. burchellii* and *B. organensis*, no significant differences in the antioxidant activity (by ORAC method) of essential oils between male and female plants were observed (ZUCCOLOTTO et al., 2019).

FIGURE 4.6 - MEANS AND STANDARD DEVIATION FROM IC_{50} OF ANTIOXIDANT ACTIVIT ($\mu g.ml^{-1}$) BY DPPH OF MALE AND FEMALE LEAVES OF *B. dracunculifolia* FROM POPULATION OF PALMEIRA (PPA) AND POPULATION OF PIRAQUARA (PPI). COEFFICIENT OF VARIATION= 9.24%. MEANS FOLLOWED BY THE SAME LOWER CASE LETTERS FOR POPULATIONS AND THE SAME LOWER CASE LETTERS FOR GENDER ARE NOT STATISCALLY DIFFERENT AT 5% LEVEL OF SIGNIFICANCE ACCORDING TO TUKEY TEST. NS= NOT SIGNIFICANT.



SOURCE: The author (2019).

The antioxidant activity of essential oils and their use as a natural antioxidant is widely known (AMORATI et al., 2013). However, the antioxidant activity of essential oil may be a result of the synergistic effect between two or more compounds, as previously reported (XAVIER et al., 2016). This synergism may have influenced the high antioxidant capacity of the essential oils of male plants especially due to the high levels of compounds with known antioxidant activity such as *(E)*-nerolidol (NETO et al., 2013; VINHOLES et al, 2014) and limonene (ROBERTO et al., 2009). In addition, significant differences in spathulenol contents in female plants was found and this may be related to possible low antioxidant activity of the compound, since a previous study verified decrease in the contents of spathulenol under NaCl stress, process that produce oxidative damages in plant cells (TAARIT et al., 2009).

These results show important differences between male and female plants of this versatile aromatic species, where its use is restricted not only to the plant but also to the essential oil and the production of green propolis. With this study it is possible to verify a great potential of male plants to produce significant amounts of the high added-value compound artepillin C, caffeoylquinic acids, higher essential oil content and antioxidant acitivity (essential oil and plant extract). However, further work is needed to verify the predilection of *Apis mellifera* bees for male plants, aiming at producing high quality green propolis for the market.

4.4 CONCLUSIONS

Caffeoylquinic acids (3,4-Di-Caffeoylquinic; 3,5-Di-Caffeoylquinic; 4,5-Di-Caffeoylquinic) and artepillin C are the main compounds identified in the extracts of *B. dracunculifolia* and *(E)*-nerolidol, β-pinene and limonene in essential oils;

The secondary metabolism of the species is affected by the gender of the plant and by the growth sites (populations).

Male plants produce higher levels of non-volatile compounds (in the both growth sites), higher amounts of essential oil content and higher antioxidant capacity for both extracts and essential oil than female plants.

Artepilin C, the most important compound for the commercialization of green propolis, present higher content in the male leaves of the two populations studied, when compared to the female plants.

REFERENCES

ADAMS, R. P. **Identification of essential oil components by gas chromatography/mass spectrometry**. Carol Stream, Illinois: Allured Publishing Corporation, 2007.

AGA, H. et al. Isolation and identification of antimicrobial compounds in Brazilian propolis. **Bioscience, Biotechnology, and Biochemistry**, v. 58, n. 5, p. 945-946, 1994.

AHN, M. et al. Suppression of tumor-induced angiogenesis by Brazilian propolis: Major component artepillin C inhibits in vitro tube formation and endothelial cell proliferation. **Cancer Letters**, v. 252, p. 235-243, 2007.

AMORATI, R.; FOTI, M. C.; VALGIMIGLI, L. Antioxidant activity of essential oils. **Journal of Agricultural and Food Chemistry**, v. 61, p. 10835−10847, 2013.

ANTOS, J. A.; ALLEN, G. A. Patterns of reproductive effort in male and female shrubs of *Oemleria cerasiformis:* a 6 year study. **Journal of Ecology**, v. 87, p. 77-84, 1999.

ARDUIN, M. I.; FERNANDES, G. W.; KRAUS, J. E. Morphogenesis of galls induced by *Baccharopelma dracunculifoliae* (Hemiptera: Psyllidae) on *Baccharis dracunculifolia* (Asteraceae) leaves. **Brazilian Journal of Biology**, v. 65, p. 559, 2005.

BANKOVA, V.; CASTRO, S. DE.; MARCUCCI, M. Propolis: recent advances in chemistry and plant origin. **Apidologie**, v. 31, p. 3–15, 2000.

BANO, U. et al. Effect of plant growth regulators on essential oil yield in aromatic plants. **Journal of Chemical and Pharmaceutical Research**, v. 8, p. 733-739, 2016.

BANSKOTA, A. H. et al. Chemical constituents of Brazilian propolis and their cytotoxic activities. **Journal of Natural Products**, v. 61, p. 896-900, 1998.

BANTIS, F.; OUZOUNIS, T.; RADOGLOU, K. Artificial LED lighting enhances growth characteristics and total phenolic content of *Ocimum basilicum*, but variably affects transplant success. **Scientia Horticulturae**, v. 198, p. 277–283, 2016.

BARBOSA, L. N. et al. In vitro antibacterial and chemical properties of essential oils including native plants from Brazil against pathogenic and resistant bacteria. **Journal of Oleo Science**, v. 64, p. 289-298, 2015.

BARROSO, G. M.; BUENO, O. L. Flora ilustrada catarinense plantas compostas-subtribo: Baccharidinae. Itajaí: **Herbário Barbosa Rodrigues (HBS)**, 2002.

BASTOS, E. M. A. F. et al. Interaction between *Apis mellifera* L. and *Baccharis dracunculifolia* DC, that favours green propolis production in Minas Gerais. **Brazilian Journal of Biology**, v. 71, n. 3, p. 727-734, 2011.

BESTEN, M. A. et al. Chemical composition similarity between the essential oils isolated from male and female specimens of each five *Baccharis* species. **Journal of the Brazilian Chemical Society**, v. 23, n. 6, p. 1041-1047, 2012.

BESTEN, M. A. et al. Chemical composition of volatiles from male and female specimens of *Baccharis trimera* collected in two distant regions of southern Brazil: a comparative study using chemometrics. **Química Nova**, v. 36, p. 1096-1100, 2013.

BOLDT, P. E. *Baccharis* (Asteraceae). **A Review of its taxonomy, phytochemistry, ecology, economic status, natural enemies and potential for its biological control in the United States**. Washington: USDA. Agricultural Research Service Grassland, 1989.

BOUARAB-CHIBANE, L. et al. Antibacterial properties of polyphenols: characterization and QSAR (Quantitative Structure–Activity Relationship) Models. **Frontiers in Microbiology**, v. 10, p. 1-23, 2019.

BRAND-WILLIAMS, W.; CUVELIER, M. E.; BERSET, C. Use of a free radical method to evaluate antioxidant activity. **LWT - Food Science and Technology**, v. 28, p. 25-30, 1995.

BÚFALO, M. C. et al. In vitro cytotoxic activity of *Baccharis dracunculifolia* and propolis against HEp-2 cells. **Natural Product Research**, v. 24, p. 1710–1718, 2010.

CARVALHO, A. A. et al. In vivo antitumoural activity and composition of an oil extract of Brazilian propolis. **Food Chemistry**, v.126, p. 1239–1245, 2011.

CEPEDA-CORNEJO, V.; DIRZO, R. Sex-Related differences in reproductive allocation, growth, defense and herbivory in three dioecious neotropical palms. **PLOS ONE**, v. 5, Issue 3, 2010.

CHAABAN, A. et al. Insecticide activity of *Baccharis dracunculifolia* essential oil against *Cochliomyia macellaria* (Diptera: Calliphoridae). **Natural Product Research**, v. 32, p. 2954-2958, 2017.

CHAI, T. T. et al. Water stress-induced oxidative damage and antioxidant responses in micropropagated banana plantlets. **Biologia Plantarum**, v. 49, p. 153-156, 2005.

CORNELISSEN, T.; STILING, P. Sex-biased herbivory: a meta-analysis of the effects of gender on plant-herbivore interactions. **OIKOS**, v. 111, p. 488-500, 2005.

DELVAS, N. et al. Phenolic compounds that confer resistance to spruce budworm. **Entomologia Experimentalis et Applicata**, v. 141, p. 35–44, 2011.

DE MOURA, S. A. L. et al. Aqueous extract of brazilian green propolis: primary components, evaluation of inflammation and wound healing by using subcutaneous implanted sponges. **Evidence-Based Complementary and Alternative Medicine**, v. 2011, p. 1-8, 2011.

DE SOUSA, J. P. et al. Seasonal variation of the *(E)*-Nerolidol and other volatile compounds within ten different cultivated populations of *Baccharis dracunculifolia* D.C. (Asteraceae). **Journal of Essential Oil Research**, v. 21, p. 308-314, 2009.

DIXON, R.; PAIVA, N. Stress-induced phenylpropanoid metabolism. **The Plant Cell**, v. 7, p. 1085-1097, 1995.

DUDLEY, L. S. Ecological correlates of secondary sexual dimorphism in *Salix glauca* (Salicaceae). **American Journal of Botany**, v. 93, p. 1775–1783, 2006.

EMAMI, S. A.; JAVADI, B.; HASSANZADEH, M. K. Antioxidant ctivity of the essential oils of different parts of *Juniperus communis* subsp. hemisphaerica and *Juniperus oblonga.* **Pharmaceutical Biology**, v. 45, n. 10, p. 769-776, 2007.

ESPIRITO-SANTO, M.M. et al. Sexual differences in reproductive phenology and their consequences for the demography of *Baccharis dracunculifolia* (Asteraceae), a dioecious tropical shrub. **Annals of Botany**, v. 91, p.13-19, 2003.

FALCÃO, S. et al. Phenolic profiling of Portuguese propolis by LC–MS spectrometry: uncommon propolis rich in flavonoid glycosides. **Phytochemical Analysis**, v. 24, p. 309-318, 2013.

FERESIN G. E. et al. Constituents of the Argentinian medicinal plant *Baccharis grisebachii* and their antimicrobial activity. **Journal of Ethnopharmacology**, v. 89, p. 73-80, 2003.

FERNANDES-SILVA, C. et al. Chemical profiling of six samples of Brazilian propolis. **Quimica Nova**, v. 36, n. 2, p. 237-240, 2013.

FERRONATTO, R. et al. Antimicrobial activity of essential oils produced by *Baccharis dracunculifolia* D.C. and *Baccharis uncinella* D.C. (Asteraceae). **Revista Brasileira de Farmacognosia**, v. 17, p. 224-230, 2007.

FIGUEIREDO, A. C. et al. Factors affecting secondary metabolite production in plants: volatile components and essential oils. **Flavour and Fragrance Journal**, v. 23, p. 213-226, 2008.

FILHO, A. A. S. et al. "In vitro trypanocidal activity evaluation of crude extract and isolated compounds from *Baccharis dracunculifolia* DC (Asteraceae)," **Journal of Pharmacy and Pharmacology**, v. 56, n. 9, p. 1195–1199, 2004.

FILHO, A. A. S. et al. Antimicrobial activity of the extract and isolated compounds from *Baccharis dracunculifolia* D.C. (Asteraceae). **Zeitschrift fur Naturforschung**, v. 63, n. 1-2, p. 40–46, 2008.

FLORÃO, A. et al. Essential oils from *Baccharis* species (Asteraceae) have anti-inflammatory effects for human cells. Journal of Essential Oil Research, v.24, p. 561-570, 2012.

FRAISSE, D. et al. Caffeoyl derivatives: major antioxidant compounds of some wild herbs of the Asteraceae family. **Food and Nutrition Sciences**, v. 2, p. 181–192, 2011.

FROHNMEYER, H.; STAIGER, D. Ultraviolet-B radiation-mediated responses in plants. Balancing damage and protection. **Plant Physiology**, v. 133, p. 133:1420-1428, 2003.

FURSTENBERG-HAGG, J.; ZAGROBELNY, M.; BAK, S. Plant defence against insect herbivores. **International Journal of Molecular Sciences**, v. 14, p. 10242–10297, 2013.

GOURINE, N. et al. Seasonal variation of chemical composition and antioxidant activity of essential oil from *Pistacia atlantica* Desf. Leaves. **Journal of the American Oil Chemists' Society**, v. 87, p. 157-166, 2010.

HALL, C. R.; CARROLL, A. R.; KITCHING, R. L. A meta-analysis of the effects of galling insects on host plant secondary metabolites. **Arthropod-Plant Interactions**, v. 11, n. 4, p. 463-473, 2017.

HERMS, D. A.; MATTSON, W. J. The dilemma of plants: to grow or defend. **The Quarterly Review Of Biology**, v.67, n. 3, 1992.

HIRANO, R. et al. Antioxidant ability of various flavonoids against DPPH radicals and LDLoxidation. **Journal of Nutritional Science and Vitaminology**, v. 47, p. 357–362, 2001.

JAAKOLA, L.; HOHTOLA, A. Effect of latitude on flavonoid biosynthesis in plants. **Plant, Cell & Environment**, v. 33, p. 1239-1247, 2010.

JENSEN, R. A. The shikimate/arogenate pathway: Link between carbohydrate metabolism and secondary metabolism. **Physiologia Plantarum**, v. 66, p. 164-168, 1986.

JEYARAMRAJA, P. R. et al. Water deficit induced oxidative damage in tea (*Camellia sinensis*) plants. **Journal of Plant Physiology**, v. 162, p. 413-419, 2005.

KASAI, S. et al. Design and synthesis of antiangiogenic/heparin-Binding arginine dendrimer mimicking the surface of endostatin. **Bioorganic & Medicinal Chemistry Letters**, v. 12, p. 951–954, 2002.

KIMOTO, T. S. et al. Renal carcinogenesis induced by ferric nitrilotriacetate in mice, and protection from it by Brazilian propolis and artepillin C. **Pathology International**, v. 50, p. 679–689, 2000.

KIMOTO, T. S. et al. Pulmonary carcinogenesis induced by ferric nitrilotriacetate in mice and protection from it by Brazilian propolis and artepillin C. **Virchows Archiv**, v. 438, p. 259-270, 2001.

KUMAZAWA, S. et al. Direct evidence for the plant origin of Brazilian propolis by the observation of honeybee behavior and phytochemical analysis. **Chemical and Pharmaceutical Bulletin**, v. 51, n. 6, p. 740-742, 2003.

LAPCZYNSKI, A. et al. Fragrance material review on nerolidol (isomer unspecified). **Food and Chemical Toxicology**, v. 46, p. S247–S250, 2008.

LEMOS, M. et al. *Baccharis dracunculifolia* the main botanical source of Brazilian green propolis, Displays Antiulcer Activity. **Journal of Pharmacy and Pharmacology**, v. 59, p. 603–608, 2007.

LI, X.; JIANG, S.; MAN, C. Metabolomic analysis of female and male plants of *Pistacia chinensis* Bunge. **Pakistan Journal of Botany**, v. 48, n. 5, p. 1971-1977, 2016.

LIU, H. et al. UV-B irradiation differentially regulates terpene synthases and terpene content of peach. **Plant, Cell & Environment**, v. 40, p. 2261–2275, 2017.

LOAYZA, I. et al. Essential oils of *Baccharis salicifolia, Baccharis latifolia* and *Baccharis dracunculifolia*. **Phytochemistry**, v. 38, p. 381-389, 1995.

LOPES, R. C. et al. Different hydric influencing essential oil production from *Plygonum punctatum* Ell. **Revista Brasileira de Plantas Medicinais**, v. 3, p. 7-10, 2001.

MAES, K.; DEBERGH, P. C. Volatiles emitted from *in vitro* grown tomato shoots during abiotic and biotic stress. Plant Cell, Tissue and Organ Culture, v. 75, p. 73-78, 2003.

MAFFEI, M.E. Sites of synthesis, biochemistry and functional role of plant volatiles. **South African Journal of Botany**, v. 76, p. 612-631, 2010.

MESSERLI, S. et al. Artepillin C (ARC) in Brazilian green propolis selectively blocks oncogenic PAK1 signaling and suppresses the growth of NF tumors in mice. **Phytotherapy Research**, v. 23, p. 423-427, 2009.

MIJANGOS-RAMOS, I. F. et al. Bioactive dicaffeoylquinic acid derivatives from the root extract of *Calea urticifolia.* **Revista Brasileira de Farmacognosia**, v. 28, p. 339–343, 2018.

MISHIMA, S. et al. Identification of caffeoylquinic acid derivatives from Brazilian propolis as constituents involved in induction of granulocytic differentiation of HL-60 cells. **Bioorganic & Medicinal Chemistry**, v. 13, n. 20, p. 5814-5818, 2005.

MOILANEN, J. et al. Biological activity of ellagitannins: effects as anti-oxidants, pro-oxidants and metal chelators. **Phytochemistry**, v. 125, p. 65–72, 2016.

MUNARI, C. C. et al. Mutagenicity and antimutagenicity of *Baccharis dracunculifolia* extract in chromosomal aberration assays in chinese hamster ovary cells. **Planta Medica**, v. 74, p. 1363– 1367, 2008.

MUROTA, K.; TERAO, J. Antioxidative flavonoid quercetin: implication of its intestinal absorption and metabolism. **Archives of Biochemistry and Biophysics**, v. 417, p. 12-17, 2003.

MWANG'INGO, P. L. et al. Sex distribution, reproductive biology and regeneration in the dioecious species *Osyris lanceolata* (African sandalwood) in Tanzania. **Tanzania Journal of Forestry and Nature Conservation**, v. 76, p. 118-133, 2007.

MWANG'INGO, P. L.; KIBODYA, G.; MNG'ONG'O, A. R. Oil yield and quality variation between sexes in *Osyris lanceolata* (African sandalwood) and its value as a fodder plant in Tanzania. **Southern Forests**, v. 72, n. 2, p. 69–74, 2010.

NAKANISHI, H. et al. Efficient radical scavenging ability of artepillin C, a major component of Brazilian propolis, and the mechanism. **Organic and Biomolecular Chemistry**, v. 1, n. 9, p. 1452–1454, 2003.

NEGI, P. S. Plant extracts for the control of bacterial growth: Efficacy, stability, and safety issues for food application**. International Journal of Food Microbiology**, v. 156, p. 7–17, 2012.

NETO, J. et al. Antioxidant effects of nerolidol in mice hippocampus after open field test. **Neurochemical Research**, v. 38, 1861–1870, 2013.

ORMENO, E. et al. Water deficit stress induces different monoterpene and sesquiterpene emission changes in Mediterranean species. Relationship between terpene emissions and plant water potential. **Chemosphere**, v. 67, n. 2, p. 276-284, 2007.

OTERO-ARNAIZ, A.; OYAMA, K. Reproductive phenology, seed-set and pollination in *Chamaedorea alternans*, an understorey dioecious palm in a rain forest in Mexico. **Journal of Tropical Ecology**, v. 17, p. 745-754, 2001.

PANDEY, G. et al. Altitudinal variation of berberine, total phenolics and flavonoid content in *Thalictrum foliolosum* and their correlation with antimicrobial and antioxidant activities. **Journal of Ayurveda and Integrative Medicine**, v. 9, p. 169-176, 2018.

PAREJO, I. et al. Bioguided isolation and identification of the nonvolatile antioxidant compounds from fennel (*Foeniculum vulgare* Mill.) waste. **Journal of Agricultural and Food Chemistry**, v. 52, p. 1890-1897, 2004.

PARK, Y. K. et al. Chemical constituents in *Baccharis dracunculifolia* as the main botanical origin of southeastern Brazilian propolis. **Journal of Agricultural and Food Chemistry**, v. 52, p. 1100-1103, 2004.

PARREIRA, N. A. et al. Antiprotozoal, schistosomicidal, and antimicrobial activities of the essential oil from the leaves of *Baccharis dracunculifolia*. **Chemistry & Biodiversity**, v. 7, p. 993-1001, 2010.

PAULINO, N. et al. Anti-inflammatory effects of a bioavailable compound, Artepillin C, in Brazilian propolis. **European Journal of Pharmacology**, v. 587, n. 1-3, p. 296-301, 2008.

PAZOUKI, L.; NIINEMETS, Ü. Multi-substrate terpene synthases: their occurrence and physiological significance. **Frontiers in Plant Science**, v. 7, p. 1-16, 2016.

QUEIROGA, C. L. et al. Comparison of the chemical composition of the essential oil and the water soluble oil of *Baccharis dracunculifolia* DC. (Asteraceae). **Journal of Essential Oil Research**, v. 20, p. 111-114, 2008.

RADOUKOVA, T. et al. Differences in essential oil yield, composition, and bioactivity of three juniper species from Eastern Europe. **Industrial Crops & Products**, v. 124, p. 643-652, 2018.

RAMAKRISHNA, A.; RAVISHANKAR, G. Influence of abiotic stress signals on secondary metabolites in plants. **Plant Signaling & Behavior**, v. 6, n. 11, p. 1720-1731, 2011.

RE, T. et al. Antioxidant activity applying an improved ABTS radical cation decolorization assay. **Free Radical Biology and Medicine**, v. 26, n. 9-10, p. 1231-1237, 1999.

ROBERTO, D. et al. Antioxidant activity of limonene on normal murine lymphocytes: relation to H2O2 modulation and cell proliferation. **Basic & Clinical Pharmacology & Toxicology**, v. 106, p. 38–44, 2009.

RODRIGUES, D. M. ***Baccharis dracunculifolia:*** **formação de parto apícola, estudos das interações com *Apis mellifera* e insetos galhadores na produção de própolis verde**. 2017. 26 f. Dissertação (Mestrado em Produtos Naturais e Sintéticos) - Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto (SP), 2017. Disponível em: https://www.teses.usp.br/teses/disponiveis/60/60138/tde-22052018-150650/publico/Dissertacao_corrigida_simplificada.pdf. Acesso em 05 jul. 2019.

SALAZAR, G. J. T. et al. Phytochemical characterization of the *Baccharis dracunculifolia* DC (Asteraceae) essential oil and antibacterial activity evaluation. **Industrial Crops and Products**, v. 122, p. 591-595, 2018.

SANTOS, V. M. C. S. et al. Seasonal variation of vegetative growth, essential oil yield and composition of menthol mint genotypes at southern Brazil. **Bioscience Journal**, v. 28, p. 790–798, 2012.

SHIMIZU, K. et al. Antioxidative bioavailability of artepillin C in Brazilian propolis. **Archives of Biochemistry and Biophysics**, v. 424, p. 181-188, 2004.

SILVA, F. A. S.; AZEVEDO, C. A. V. The assistat software version 7.7 and its use in the analysis of experimental data. **African Journal of Agricultural Research**, v. 11, p. 3733–3740, 2016.

STAUDT, M.; BERTIN, N. Light and temperature dependence of the emission of cyclic and acyclic monoterpenes from holm oak (*Quercus ilex* L.) leaves. **Plant, Cell & Environment**, v. 21, p. 385-395, 1998.

SUZUKI, A. A. Reproductive investment in male and female *Eurya japonica* (Theaceae) at tree and branch levels. **American Journal of Botany**, v. 92, p. 2003-2009, 2005.

SZLISZKA, E. et al. Chemical composition and anti-Inflammatory effect of ethanolic extract of Brazilian green propolis on activated J774A.1 Macrophages. **Evidence-Based Complementary and Alternative Medicine**, v. 2013, p. 1-13, 2013.

TAARIT, M. B. et al. Plant growth, essential oil yield and composition of sage (*Salvia officinalis* L.) fruits cultivated under salt stress conditions. **Industrial Crops and Products**, v. 30, p. 333-337, 2009.

TAIZ, L.; ZEIGER, E. **Plant Physiology**, Sunderland: Sinauer Associates Inc, 2013.

TEIXEIRA, É. W. et al. Plant origin of green propolis: bee behavior. Plant Anatomy and Chemistry. **Evidence-Based Complementary and Alternative Medicine**, v. 2, p. 85-92, 2005.

TOMAZZOLI, M. M. et al. Botanical source investigation and evaluation of the effect of seasonality on Brazilian propolis from *Apis mellifera* L. **Scientia Agricola**, v. 77, n.6, 2020.

TORETI, V. C. et al. Recent progress of propolis for its biological and chemical compositions and its botanical prigin. **Evidence-Based Complementary and Alternative Medicine**, v. 2013. p. 1-13, 2013.

TORIMARU, T.; TOMARU, N. Relationships between flowering phenology, plant size, and female reproductive output in a dioecious shrub, *Ilex leucoclada* (Aquifoliaceae). **Canadian Journal of Botany Revue canadienne de botanique**, v. 84, p. 1860-1869, 2006.

TURCOTTE, J.; HOULE, G. Reproductive costs in *Salix planifolia* ssp. Planifolia in subarctic Québec, Canada. **Écoscience**, v. 8, n. 4, p. 506-512, 2001.

VAN DEN DOOL, H. KRATZ, P. D. A generalisation of the retention index system including linear temperature programmed gas-liquid chromatography. **Journal of Chromatography**, v. 11, p. 463-471, 1963.

VINHOLES, J. et al. Assessment of the antioxidant and antiproliferative effects of sesquiterpenic compounds in in vitro Caco-2 cell models. **Food Chemistry**, v. 156, p. 204–211, 2014.

VOGT, T. Phenylpropanoid biosynthesis. **Molecular Plant**, v. 3, p. 2-20, 2010.

VRANOVÁ, E.; COMAN, D.; GRUISSEM, W. Network analysis of the MVA and MEP pathways for isoprenoid synthesis. **Annual Review of Plant Biology**, v. 64, p. 665–700, 2013.

WANG, Y.; FREI, M. Stressed food – The impact of abiotic environmental stresses on crop quality. **Agriculture, Ecosystems and Environment**, v. 141, p. 271-286, 2011.

XAVIER, M. N. et al. Chemical composition from essential oil of *Cardiopetalum calophyllum* Schltdl. (Annonaceae) and their antioxidant, antibacterial and antifungal activities. **Revista Virtual de Química**, v. 8, p. 1433-1448, 2016.

ZHELJAZKOV, V.D.; ASTATKIE, T.; JELIAZKOVA, E. Year-round variations in essential oil content and composition of male and female Juniper. **Hortscience**, v. 48, n. 7, p. 883-886, 2013.

ZUCCOLOTTO, T. et al. Chemical, antioxidant, and antimicrobial evaluation of essential oils and an anatomical study of the aerial parts from *Baccharis* species (Asteraceae). **Chemistry & Biodiversity**, v. 16, Issue 4, 2019.

5 PROPAGAÇÃO SEXUADA E ASSEXUADA DE *Baccharis dracunculifolia* DC.

RESUMO

Baccharis dracunculifolia DC. é uma planta pioneira nativa do Brasil que possui grande importância devido aos seus metabólitos secundários e ao seu potencial na recuperação de áreas degradadas. O principal objetivo deste estudo foi avaliar as técnicas de propagação de *B. dracunculifolia* (1) por reprodução sexual: curva de maturação das sementes para determinação do ponto de maturidade fisiológica e definção da melhor temperatura para a germinação; (2) por reprodução assexuada: a influência do sexo da planta matriz no enraizamento de estacas caulinares de acordo com diferentes concentrações de ácido indol butírico (AIB). Para a reprodução por sementes, indivíduos do sexo feminino de uma população natural foram monitorados periodicamente com relação ao seu desenvolvimento reprodutivo e as sementes foram coletadas para avaliação do acúmulo máximo de matéria seca para a determinação da maturidade fisiológica. O teste de germinação foi realizado com 4 temperaturas (20, 25, 30 e 20-30°C), além da condução dos testes de velocidade de germinação (IVG) e de primeira contagem. Para a propagação vegetativa, estacas caulinares (8 cm) de indivíduos machos e fêmeas foram tratadas com solução de AIB nas concentrações: 0, 1500, 3000, 4500 e 6000 $mg.L^{-1}$. Após 120 dias as estacas foram avaliadas quanto a mortalidade, sobrevivência, enraizamento, brotação, retenção foliar, calogênese e número e comprimento médio de raízes (cm). A maturidade fisiológica das sementes ocorreu aos 40 dias após a antese, sendo este o melhor momento para a coleta das sementes no campo. As temperaturas de 25°C e 20-30°C podem ser utilizadas nos testes de germinação, uma vez que apresentaram melhores resultados de % de germinação, IVG e primeira contagem. A primeira contagem foi identificada 4 dias após a semeadura e a última contagem após 11 dias. O enraizamento de estacas de *B. dracunculifolia* é muito baixo. O fator sexo não possui influência nos parâmetros avaliados, no entanto, doses crescentes de AIB influenciam positivamente o enraizamento, n° de raízes e comprimento médio das raízes e negativamente a formação de calos.

Palavras-chave: Alecrim-do-campo. Germinação. Enraizamento. Ácido indol butírico.

5 SEXUAL AND ASSEXUADED PROPAGATION OF *Baccharis dracunculifolia* DC.

ABSTRACT

Baccharis dracunculifolia DC. is a pioneer plant native from Brazil that has great importance due to its secondary metabolites and for its potential in the recovery of degraded areas. The main goal of this study was to evaluate the propagation techniques of *B. dracunculifolia,* (1) by sexual reproduction: maturation curve to determine the physiological maturity point for seed collection and define better temperature for germination; (2) by asexual reproduction: the influence of the plant sex on stem cuttings rooting according to different concentrations of indole butyric acid (IBA). For the reproduction by seeds, female individuals from a natural population were periodic monitoring for their reproductive development and their seeds were collected for the evaluation of maximum dry mass accumulation to determine physiological maturity. The germination test was performed by testing 4 temperatures (20°C, 25°C, 30 and 20-30°C), in addition to the germination speed test (GSI) and first count. For the vegetative propagation, stem cuttings (8 cm) of male and female individuals were treated with 0, 1500, 3000, 4500 and 6000 $mg.L^{-1}$ of IBA solution. After 120 days the cuttings were evaluated for mortality, survival, rooting, sprouting, leaf retention, callogenesis and number and average length of roots (cm). The physiological maturity of seeds occurred at 40 days after anthesis, being this the best time for seed collection in the field. The temperatures of 25°C and 20-30°C can be used in the germination tests of the species, for best germination percentage, GSI and first count results. The first count being identified 4 days after sowing and the last count after 11 days. The rooting of *B. dracunculifolia* cuttings is very low. Sex did not influence the parameters evaluated, however, increasing doses of IBA positively influence rooting, number of roots and average length of roots and negatively the calluses formation.

Keywords: Alecrim-do-campo. Germination. Rooting. Indole butyric acid.

5.1 INTRODUCTION

Baccharis dracunculifolia DC. (Asteraceae) is a medicinal species native from Brazil widely distributed in South America. The plant is a perennial woody shrub (2-3 meters high), dioecious, propagated by seeds, the flowers are in multifloro chapter and the fruits are glabrous achenes with length of 1.5mm (BARROSO; BUENO, 2002). *B. dracunculifolia* is well known as *Apis mellifera* main bee pasture for the production of Brazilian green propolis, a product with numerous beneficial properties for human health (BARROSO; BUENO, 2002; PARK et al., 2004; SFORCIN et al., 2012; BANKOVA et al., 2016). Its flowers are in multifloro chapter and the fruits are glabrous achenes with length of 1.5mm. Among the plants of the genus *Baccharis* found in Brazil, *B. dracunculifolia* is the species with the most characteristics of anthropic pioneer (BARROSO; BUENO, 2002), and has a high rate of natural regeneration which emphasizes its ecological importance in the recovery of degraded areas (FERREIRA et al., 2009).

Despite the ecological importance of the plant and economic importance of its products (green propolis and essential oil), there is limited information about propagation of the species. Previous study developed by Gomes; Fernandes (2002) with *B. dracunculifolia* seeds found that the optimum conditions for seed germination are at temperature between 15 to 20ºC in the presence of light. In addition, a study by Santos et al. (2016) identified orthodox characteristic of seeds of the species. However, important information such as the optimal point of harvest of seeds is absent and it is an essential factor for obtaining high quality plant material. The optimal point of seed harvesting is established by the point of physiological maturity in the maturation curve, identified by the maximum dry mass accumulation (MARCOS-FILHO, 2015).

Due to the lack of vegetative propagation recommendations for *B. dracunculifolia* seed reproduction as the usual method, though, for other species of the genus *Baccharis* (*Baccharis trimera*, *Baccharis articulata* and *Baccharis stenocephala*) vegetative propagation by cuttings has been shown to be effective (BONA et al., 2005). Vegetative propagation by cuttings is the most widely used reproduction method in the commercial production of various medicinal, fruit and ornamental crops and is characterized by maintaining genetic stability (HARTMANN

et al., 2014), that for medicinal plants can mean to maintain uniformity for production of secondary metabolites.

Dioecious species, such as *B. dracunculifolia*, may present morphological and physiological differences between male and female plants, due to developed resource allocation strategies to maximize reproductive success (ESPIRITO-SANTO et al., 2003; BESTEN et al., 2013; ROBINSON et al., 2014; LI et al., 2016). Previous studies of vegetative propagation in dioecious species identified the influence of plant sex on rooting of stem cuttings (CARVALHO et al., 2007; WENDLING et al., 2016). This may be related to the phenolic contents in the plant, since it has been reported that *meta*-diphenols, *ortho*-diphenols and polyphenols inhibit auxin decarboxylation enhancing rooting, but on the other hand, monophenols act as cofactors of IAA-oxidase and as a consequence increase IAA decarboxylation (BANDURSKI et al., 1995).

Auxins are important phytohormones that regulate adventitious root induction (STEFFENS; RASMUSSEN, 2016). The best known endogenous auxins are Indole-acetic acid (IAA) and indole-butyric acid (IBA), the difference between them is stability, IAA is relatively labile and due to lower IBA instability, it has a higher ability to induce adventitious roots and has lower sensitivity to oxidation and is the most applied auxin for root induction in woody species (NORDSTRÖM et al., 1991; LUDWIG-MÜLLER, 2000; STUEPP et al., 2017).

The main goal of this study was therefore to evaluate the propagation techniques of *Baccharis dracunculifolia* species: (1) by sexual reproduction: maturation curve to determine the physiological maturity point for seed collection and define better condition (temperature) for germination; (2) by asexual reproduction: the influence of the plant sex on stem cuttings rooting according to different concentrations of IBA.

5.2 MATERIAL AND METHODS

5.2.1 Seed propagation

5.2.1.1 Monitoring of seed maturation

B. dracunculifolia plants were monitored between January and April 2017, during the flowering and seed production of the specie. These plants were present in a natural population located in southern Brazil in the Atlantic araucaria forest, in the municipality of Palmeira, PR, Brazil (25°19'80''S - 49°48'35''W, altitude 1027 meters) located in the area of 'Reserva Particular do Patrimônio Natural (RPPN) Butuquara', with the licenses (09.18) of the Environmental Institute of Paraná (IAP) for collecting and transporting plant material. In this region of Brazil the climate is classified as Cfb according to Alvares et al. (2013). All female individuals (n= 15) in the reproductive stage were monitored. The species was confirmed from herbarium analysis in the Municipal Botanical Museum of Curitiba - MBM Herbarium, with the voucher specimen deposited with the number: MBM-370.693. The registration number of the species in the '*Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado* - SisGen' is AC74344

For each female individual, 10 branches were marked and monitored weekly until 50% of inflorescences were in anthesis (open flowers). After this, field observations were reduced to twice a week in order to monitor seed formation. The seeds were collected for maturation study when they presented complete formation and the hardening of the integument were identified.

5.2.1.2 Dry mass analysis

For the analysis of dry mass, 20 fruits per branch of the selected matrices were collected in each collection performed after the anthesis, i.e. 19, 26, 29, 33, 36 e 40 days after anthesis (DAA), totaling six samples. After this, the seeds were separated from the branches and homogenized by the manual method (Brasil, 2009) and four repetitions of 200 seeds (achenes + papus) were weighed using an analytical balance (0.001g) and packed in Kraft paper bags. The bags containing the seeds were dried in a forced air circulation oven at 65°C for 48 hours until a constant weight was reached and the final weight was recorded. The dry mass was attributed to the final weight of seeds and the result was expressed as a percentage (%).

Seed development was monitored through a stereoscopic microscope and the germination test was evaluated with the sample that reached the maximum dry matter accumulation, that is, reached the physiological maturity.

5.2.1.3 Seed germination test

The seeds (achenes + papus) were separated through sieve friction, using a circular sieve with 1.8 and 1.6 mm of diameter and after homogenized by the manual method (Brasil, 2009) and stored under refrigeration (5°C). The germination test was installed with seeds that presented maximum dry mass accumulation, therefore, at 40 DAA. The test was conducted in germination chamber using 50 intact seeds sown in transparent gerbox boxes (11.0 x 11.0 x 3.5 cm), with four replications. The substrate was blotter paper moisturised with water 2.5 times the dry substrate mass (BRASIL, 2009). The seeds were disinfected using sodium hypochlorite solution at 1% (v/v) for 3 minutes. Germination tests were conducted at temperatures: 20, 25, 30 and 20-30°C. For treatment using alternating temperatures, the lowest temperature was maintained for 16h at night and the highest for 8h during the day. Lighting was maintained during the high temperature period. Seed germination was evaluated every 24h after observation of the first normal seedling until germination became constant and were defined as the first and last count of the test. The determination of germination percentage was defined by: G= (N/A) x 100 where 'N' is number of normal seedlings and 'A' is number of seeds in the treatment.

In parallel with the germination test, the tests of first count and germination speed were also performed. The germination speed index was performed according Maguire (1962), defined by: GSI= (G1/N1)+(G2/N2)+...(Gn/Nn) where 'G1; G2; Gn' are number of normal seedlings calculated at first count, second count and last count, respectively and 'N1; N2; Nn' are number of days of first count, second count and last count, respectively.

5.2.1.4 Data analysis

The experimental design was completely randomized with four replications. The data were submitted to the Bartlett test to verify the homogeneity of variances. Analysis of variance (ANOVA) was applied with mean separation using Tukey test at 5% probability ($p < 0.05$), when significant, with the support of the statistical software ASSISTAT® (SILVA; AZEVEDO, 2016). The data on seed maturation process were analyzed by the regression model with the best fit for the obtained curve.

5.2.2 Vegetative propagation - Stem cuttings

5.2.2.1 Plant material collection site

The plant material for stem cutting production was collected in a natural population of southern Brazil (25°30'635"S - 49°02'58"W, altitude 891 meters) in April 2018, in the larger population than the seed collection population. The climate of the region is also classified as Cfb according to Alvares et al. (2013). The braches with leaves were collected from 25 female and 25 male individuals in the field during the morning (7-8am) and then were transported to the greenhouse in the moisture black polyethylene bags. The species and the sex of plant were confirmed through botanical identification in the Municipal Botanical Museum of Curitiba - MBM Herbarium (deposit number for males: MBM-371.866; deposit number for females: MBM-379.293).

5.2.2.2 Preparation and evaluation of stem cuttings

Initially, female and male branches were separately disinfected with sodium hypochlorite at 0.5 % (v/v) for 15 minutes followed by washing in water for 5 minutes. Then, apical stem cuttings measuring 8cm long, average diameter of 3.0 mm and with 4-6 leaves were prepared for each plant gender. All ready cuttings were kept in water and in the upright position to prevent dehydration. The apical stem cuttings were immersed in the following concentrations of indole butyric acid - IBA (aquous ethanolic solutions, 50% (v/v): 0, 1500, 3000, 4500 and 6000 $mg.L^{-1}$, for 10 seconds. The test solution (0 $mg.L^{-1}$) was made with distilled water. Subsequently, the cuttings were placed in 120 cm^3 polypropylene tubes with MecPlant® commercial substrate, composed by pine bark, vermiculite, macro and micronutrients, with moisture content between 54 - 58% and density of $375g.L^{-1}$. The cuttings were kept in a greenhouse setting at 25 ± 2ºC and 95% relative humidity, under intermittent fogging for 5 seconds every 30 minutes.

After 120 days the cuttings were evaluated for mortality (dead/necrosed cuttings), survival (live/tender cuttings neither root induction nor calluses formation), rooting, sprouting, leaf retention, callogenesis and number and average length of roots (cm).

5.2.2.3 Data analysis

The experimental design was completely randomized in a 2x5 factorial scheme (two genders: male and female cuttings; five IBA concentrations: 0, 1500, 3000, 4500 and 6000 $mg.L^{-1}$), with 4 replicates and 16 cuttings as experimental unit.

For the variable average length of roots the normal distribution was assumed and before perform the analysis the data were transformed with the function log(x+0.01), as the graphical analysis of the residuals indicated the escape of suppositions for variance analysis. After this, analysis of variance were applied to test the effect of each experimental term (main effects and interaction).

The variables mortality, survival, rooting, sprouting, leaf retention and callogenesis, as they are binary variables (presence and absence of characteristic), it was aplied the generalized linear models (GLM) to them with the Quasi-binomial specification, which estimates as a dispersion, making the model more flexible to accommodate overdispersion. For the variable number of roots, as a count variable, it was also aplied the generalized linear models but with the Quasi-Poisson specification, which is more flexible because it has a dispersion parameter. For all these variables the *deviance* analysis was performed using the F statistic for testing model terms. Regression analysis were used to study the effect of doses of IBA.

All analyses were performed using the R statistical computing software (R CORE TEAM, 2019).

5.3 RESULTS AND DISCUSSIONS

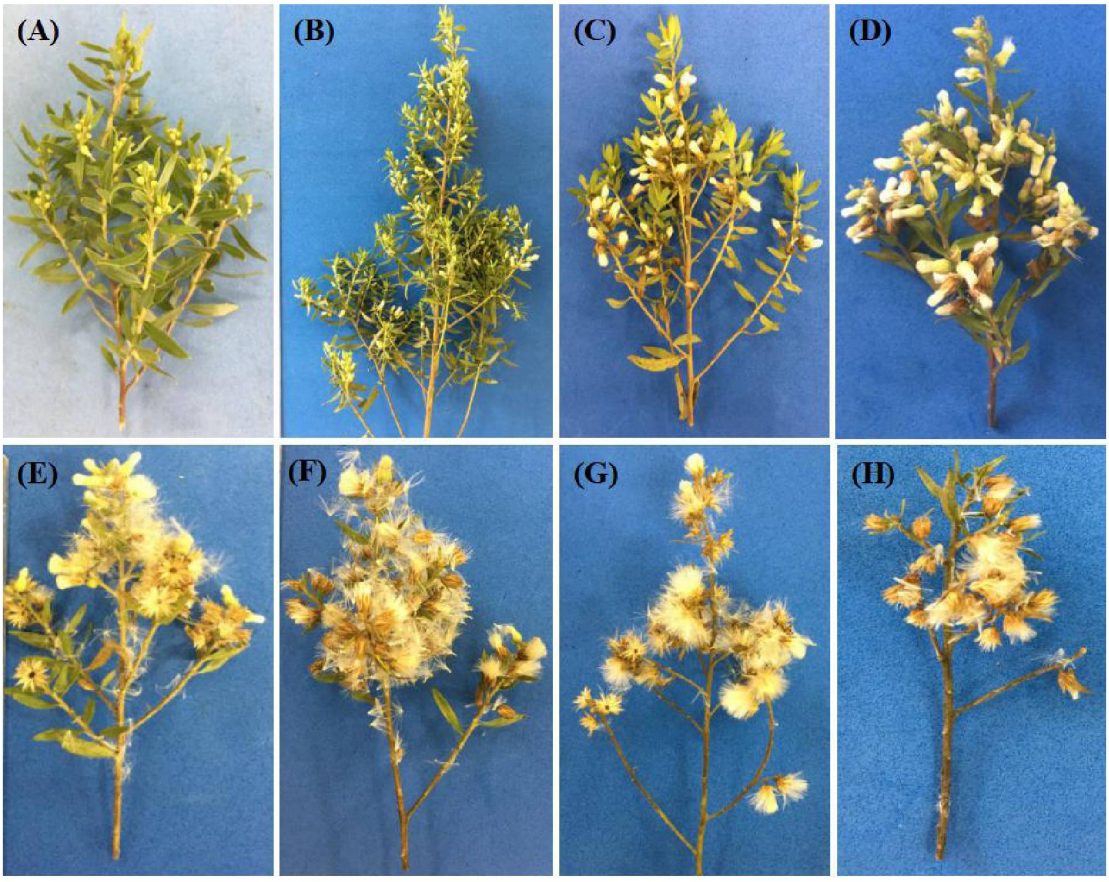
5.3.1 Seed propagation

5.3.1.1 Monitoring of seed maturation

The maturation process of *Baccharis dracunculifolia* fruits, from the appearance of the first flower buds to the complete seed formation and physiological detachment of the mother plant, was 61 days. The visual characteristics of the *B. dracunculifolia* branches and seeds during the physiological maturity process is presented in FIGURE 5.1. The physiological maturity of the seeds is reached when the seeds presented their maximum dry mass accumulation, when there was no

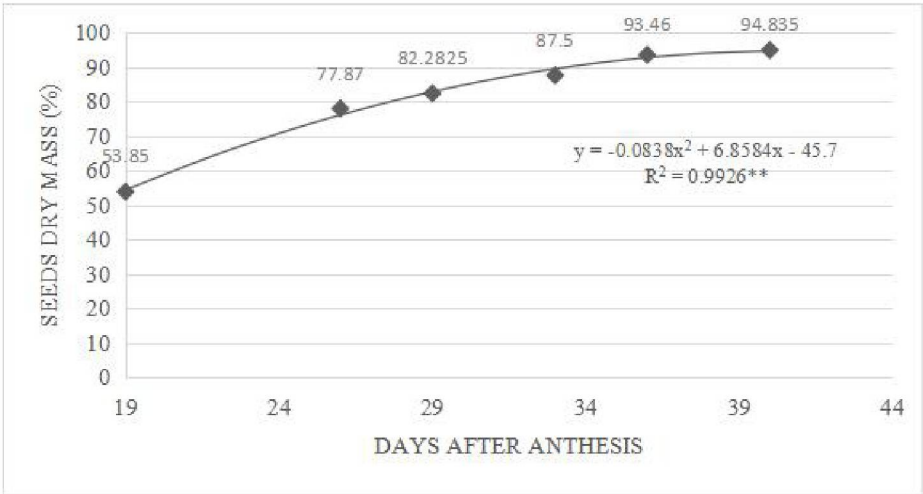
more significant transfer of reserves from the mother plant to the embryos (MARCOS-FILHO, 2005). Nineteen days after anthesis – DAA, (50% of inflorescences were open flowers) the seed had accumulated 53.85% of dry mass and in 36 DAA achieve 93.46% that remained practically constant until 40 DAA (94.83%), after more five days no more seeds were identified to collect. The increase of dry mass for seeds verified significant effect of quadratic order and the maximum point of dry mass accumulation of *B. dracunculifolia* seeds occurred at 40 DAA (FIGURE 5.2).

FIGURE 5.1 - CHARACTERISTIC OF *B. dracunculifolia* BRANCHES AND SEEDS DURING PHYSIOLOGICAL MATURITY: (A) FLOWER BUDS; (B) ANTHESIS AND (C) 19 DAYS; (D) 26 DAYS; (E) 29 DAYS; (F) 33 DAYS; (G) 36 DAYS AND (H) 40 DAYS AFTER ANTHESIS.



SOURCE: The author (2017).

FIGURE 5.2 - DRY MASS ACCUMULATION (%) IN *B. dracunculifolia* SEEDS THROUGHOUT MATURATION PROCESS. (**) SIGNIFICANCE P ≤0.01 ACCORDING TO THE F TEST.



SOURCE: The author (2019).

For other Asteraceae family's species similiar results were found, where seed physiological maturity point was achieved between 32 to 49 DDA (SILVEIRA et al., 2002; GRZYBOWSKI et al., 2016; FRANCISCO et al., 2019). Usually, the initial dry mass accumulation of seeds is slow, followed by rapid and constant accumulation, until the maximum accumulation is reached and maintained. Eventually, the dry mass may have a slight decline (CARVALHO; NAKAGAWA, 2012). The final of the accumulation of dry mass is mainly characterized by the cessation of the vascular connection between the seed and the mother plant, later the seed water content decreases and the it acclimatizes with the environment (HAY; PROBERT, 2013). However, the high dry mass indice (period of maintenance) is influenced by the environment, where unfavourable conditions of relative humidity and temperature may contribute to the acceleration of the respiratory process and after this, seeds begin the deterioration process by the oxidation of the reserve substances (MARCOS-FILHO, 2015).

For *B. dracunculifolia*, the type of anemochory dispersal seeds (ALMEIDA et al., 2008) may have been decisive for its rapid dispersion after reaching physiological maturity, where after five days no more seeds were identified for the harvest, and because of this, it was not possible to identify declines in seed dry mass accumulation. Therefore, changes in fruit and seed color may be a strong indication of seed maturity prior to dispersal (HAY; PROBERT, 2013). In this study, the

appearance of *B. dracunculifolia* fruits at physiological maturity point (40 DAA) is presented in Figure 5.3.

FIGURE 5.3 - DETAILS OF *B. dracunculifolia* BRANCHES AND FRUITS AT THE POINT OF PHYSIOLOGICAL MATURITY.



SOURCE: The author (2017).

5.3.1.2 Seed germination test

After the identification of the physiological maturity point of *B. dracunculifolia* seeds, it was possible to study the germination process of the species more accurately. TABLE 5.1 shows the seed germination percentages at the four temperatures tested (20, 25, 30 and 20-30°C). According to the test, seeds submitted to alternating temperature of 20-30°C and constant of 25°C had the higher germination germination percentage 53.50% and 43.00%, respectively than seeds expose to 20 or 30°C. The seeds submitted to the most extreme constant temperatures in this test (20 and 30°C) presented lower germination, which does not fully reproduce the temperature behavior common to the collection region, which may have influenced this inferior result in this study.

TABLE 5.1 - MEAN AND STANDARD ERROR OF GERMINATION (%) AND GERMINATION SPEED INDEX (GSI) OF *B. dracunculifolia* SEEDS, BY DIFFERENT INCUBATION TEMPERATURES AND USING BLOTTER PAPER AS SUBSTRATE.

Temperature	Germination (%)	GSI
20°C	23.50 ± 2.06 bc	1.67 ±0.21 bc
25°C	43.00 ± 5.44 ab	3.40 ± 0.52 ab
30°C	19.50 ± 6.70 c	1.51 ± 0.53 c
20-30°C	53.50 ± 4.11 a	3.89 ± 0.29 a
CV (%)	28.06	29.71

SOURCE: The author (2019).
SUBTITLE: Means followed by the same letter in colluns do not differ by Tukey test at 5% of probability.

A study by Santos et al. (2016) tested the germination of *B. dracunculifolia* seeds submitted to 15 and 20°C and found similar results to ours, where at 20°C with light supply reached 50% germination rate, however, the authors did not test higher temperatures. On the other hand, Gomes; Fernandes (2002) tested four different constant temperatures (15, 20, 25 and 30°C) on the same species and reported close to 80% of germination at 15 and 20°C with light supply and 15°C without light supply. Importantly, the two previous studies with seed of the species were collected from natural populations from different regions, i.e. São Paulo state (SANTOS et al., 2016) and Minas Gerais state (GOMES; FERNANDES, 2002) - both from southeastern Brazil - subjected to different weather conditions and possibly different genetic material. Furthermore, many wild species have different flowering times both within the population and within individuals, which may cause a significant portion of immature seeds (HAY; PROBERT, 2013) and consequently different germination rates.

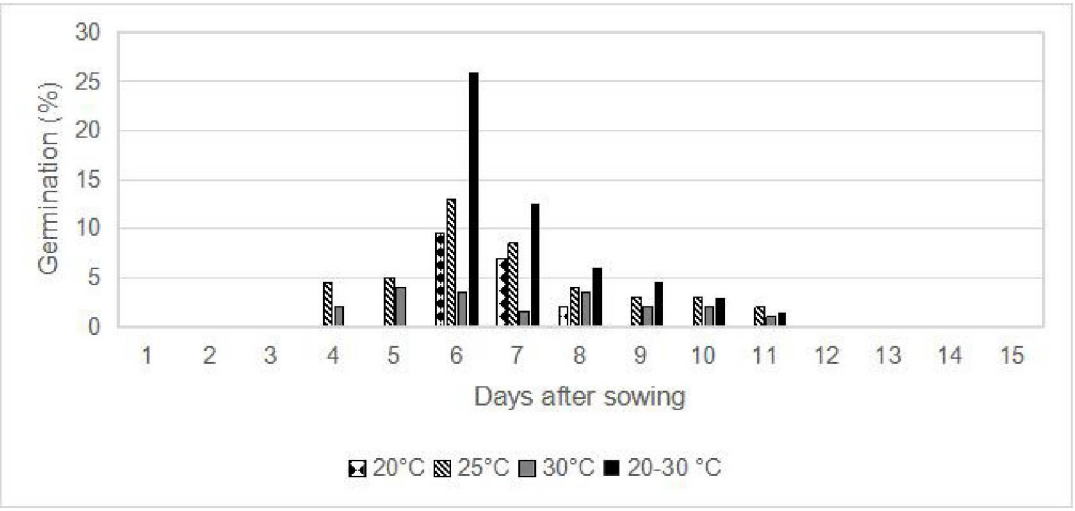
According to a study by Grzybowski et al. (2016) with seeds of *Vernonanthura discolor*, a native plant from Brazil, it ws reported that the seeds can be germinated at 20 or 25°C with supply of light, or at 25 °C in the dark, and this can be explained due to its pioneer plant characteristics, where its seeds may or may not be shaded by other plants. Similarly, *B. dracunculifolia* is also considered a pioneer plant, being one of the first plants to establish in the forest canopy (BARROSO; BUENO, 2002), and previous studies have indicated that the seeds can be germinated with or without supply of light depending on the temperature (GOMES; FERNANDES, 2002; SANTOS et al., 2016). However, light supply is recommended in routine analysis of

seeds in order to promote the development of essential plant structures, to reduce microbial attack and facilitate evaluation (BRASIL, 2009). The germination of *B. dracunculifolia's* seeds in this study was therefore tested with the light supply only.

Previous study developed by Santos et al. (2016) with The viability of *B. dracunculifolia* seeds on medium-term storage for 3, 6, and 9 months at 6°C and during 45 days at -20°C and moisture content reduced to 3.3% concluded that the viability of the seeds have not changed (SANTOS et al., 2016). Seeds are generally classified into two groups for storage purposes, namely orthodox and recalcitrant seeds (ROBERTS, 1973). Orthodox seeds can be stored for relatively long periods at negative temperatures (usually -15°C) and for short-term periods about 5 years or less at a storage temperature of 0-5°C. However, recalcitrant seeds do not resist desiccation and moisture should be maintained between 20-50% during storage depending on the species (BONNER, 1990).

The germination per day of *B. dracunculifolia* seeds submitted to different temperatures are presented (FIGURE 5.4). The constant temperatures of 25 and 30°C showed the first count 4 days after sowing, while the temperature of 20°C and alternating 20-30°C were germinated after 6 days. No germination of *B. dracunculifolia* seeds was observed after 11 days of sowing and it is therefore recommended that the germination test be performed between the 4 and 11 days after sowing.

FIGURE 5.4 - PERCENTEGE OF GERMINATION (NORMAL SEEDLINGS) PER DAY OF *B. dracunculifolia* SEEDS AT 20, 25, 30 AND 20-30°C.



SOURCE: The author (2019)

Vigor tests in seeds, as first germination count and speed germination, are applied to demonstrate that physiologically aged or more deteriorated individuals have slow germination, period ranging from the onset of water absorption to the emergence of the primary root, and this is importante because uniformity and germination speed of seeds are two factors that directly affect seedling establishment in the field (MARCOS-FILHO, 2015). In this sense, in this study the seeds germinated at temperatures of 25 and 20-30°C had the highest rates of germination speed index (TABLE 5.1), emphasizing greater vigor compared to constant temperatures of 20 and 30°C.

The germination process consists of three phases: Phase I) Rapid increase in water absorption by imbibition; Phase II) Lag phase of germination that is is characterized by active metabolic activity (e.g. mitochondria "maturation", protein synthesis, storage reserve metabolism and specific enzymatic activity) and lower water uptake; Phase III) Radicle protrusion and fresh weight gain related to the water absorption (HARTMANN et al., 2014). Seed germination speed is a very important vigor attribute and is an indicator of physiological quality, as delayed root emergence maybe one of the first signs of deterioration (MATTHEWS et al., 2012). Delayed root protrusion is considered one of the first signs of deterioration, which is directly proportional to the duration of the lag phase (Phase II) of germination and may indicate incomplete metabolic repair (MARCOS-FILHO, 2005; MATTHEWS; KHAJEH-HOSSEINI, 2007).

The definition of the physiological maturity point of seeds is essential for obtaining quality seedlings and consequently for the success of cultivation. Considering that most green propolis beekeepers in Brazil produce their own seedlings of *B. dracunculifolia* by seed, the lack of information about its harvesting time may reduce the success of quality seedling production. In addition, the definition of the ideal temperature for the germination of the species, through parameters such as germination percentage and vigor tests, can also help to determine the best time for sowing, bringing economic impacts to beekeepers and to medicinal plant producers.

5.3.2 Stem cuttings propagation

In the study of stem cuttings propagation of *B. dracunculifolia*, the deviance analysis are shown in TABLE 5.2. No significant effects of gender and IBA were identified for the variables studied, however, the tests revealed that for rooting, calluses, number of roots and average lenght of roots there was a significant dose-dependent effect in *B. dracunculifolia* stem cuttings treated with different IBA concentrations, emphasizing the effect of this auxin on root emission. It is important to note that the mortality variable, although not showing significant statistical differences between treatments, were quite high for this species, ranging from 62.5 to 100% for male cuttings and from 50 to 100% for female cuttings. Cuttings considered dead (necrotic) are presented in FIGURE 5.5 C.

TABLE 5.2 - SUMMARIES OF DEVIANCE ANALYSIS CHART OF ROOTING, SPROUTING, LEAVE RETENTION, CALLUSES, MORTALITY, SURVIVAL, NUMBER OF ROOTS AND AVERAGE LENGTH OF ROOTS OF *B. dracunculifolia* STEM CUTTINGS.

Variation source	DF	Rooting		Sprouting		Leave retention		Calluses	
		Deviance	Pr(>F)	*Deviance*	Pr(>F)	*Deviance*	Pr(>F)	*Deviance*	Pr(>F)
Gender (F1)	1	45.351	0.4247	65.857	0.5861	74.783	0.3401	61.339	0.3841
IBA (F2)	4	29.101	0.0021 **	56.741	0.1618	68.824	0.4961	40.857	0.0121*
F1 x F2	4	24.017	0.1764	45.908	0.1057	60.191	0.3098	39.401	0.8923

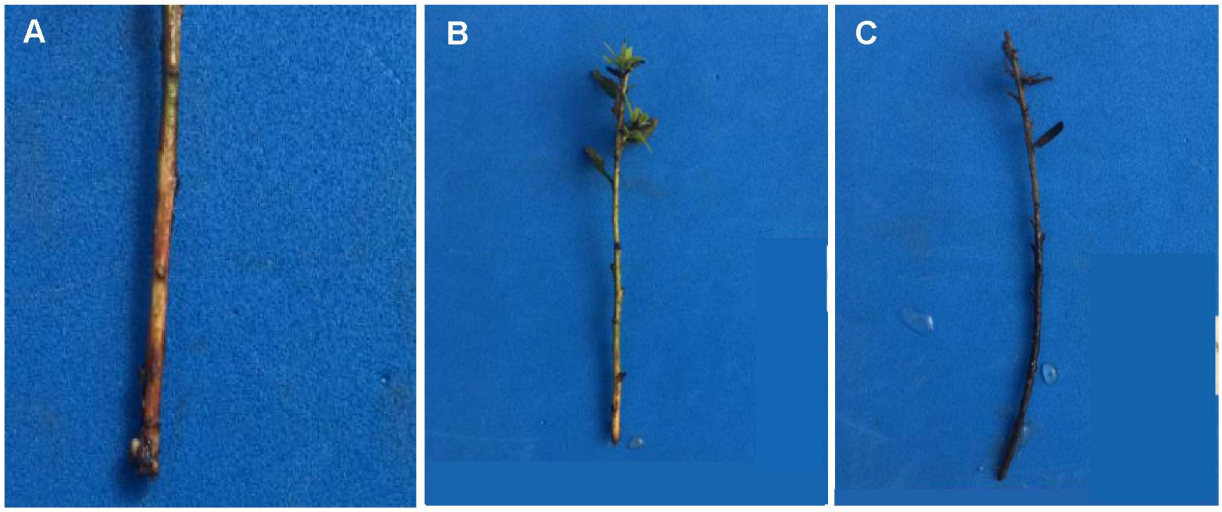
Variation source	DF	Mortality		Survival		Number of roots		Average lenght of roots	
		Deviance	Pr(>F)	*Deviance*	Pr(>F)	*Deviance*	Pr(>F)	SS	Pr(>F)
Gender (F1)	1	91.747	0.7601	79.863	0.3	203.906	0.0361	19.9146	0.9003
IBA (F2)	4	87	0.7222	74.774	0.6007	105.256	0.0025 **	9.8109	0.0479*
F1 x F2	4	79	0.5075	65.653	0.3123	73.572	0.1473	6.8566	0.2603

SOURCE: The author (2019).
SUBTITLE: (**) significant at 1% probability according to the F test; (*) significant at 5% probability according to the F test; DF = degree of freedom; SS = sum of the squares; F1 = Factor 1; F2 = Factor 2. Pr(>F)= probability of observing a value for Statistics F greater than or equal to statistics F calculated.

There are no other reports in the literature about vegetative propagation of *B. dracunculifoli*a, but a study with species of the same genus was developed by Bona et al. (2005), who evaluated the effect of different parts of the branch and different substrates in the stem cuttings of *Baccharis trimera*, *Baccharis articulata* and

Baccharis stenocephala, species commonly known as 'carqueja'. The authors verified that the apical and median parts of the branches are indicated for the propagation of *B. articulata* and *B. stenocephala*, whereas for *B. trimera* any part of the branch can be used. Related to substrate use, sand is not recommended for both species.

FIGURE 5.5 - *Baccharis dracunculifolia* STEM CUTTINGS AFTER 120 DAYS FROM PLANTING: A) CALLUSES FORMATION; B) ALIVE CUTTINGS (NEITHER CALLUSES NOR ROOT FORMATION); C) DEAD CUTTINGS (NECROSED).

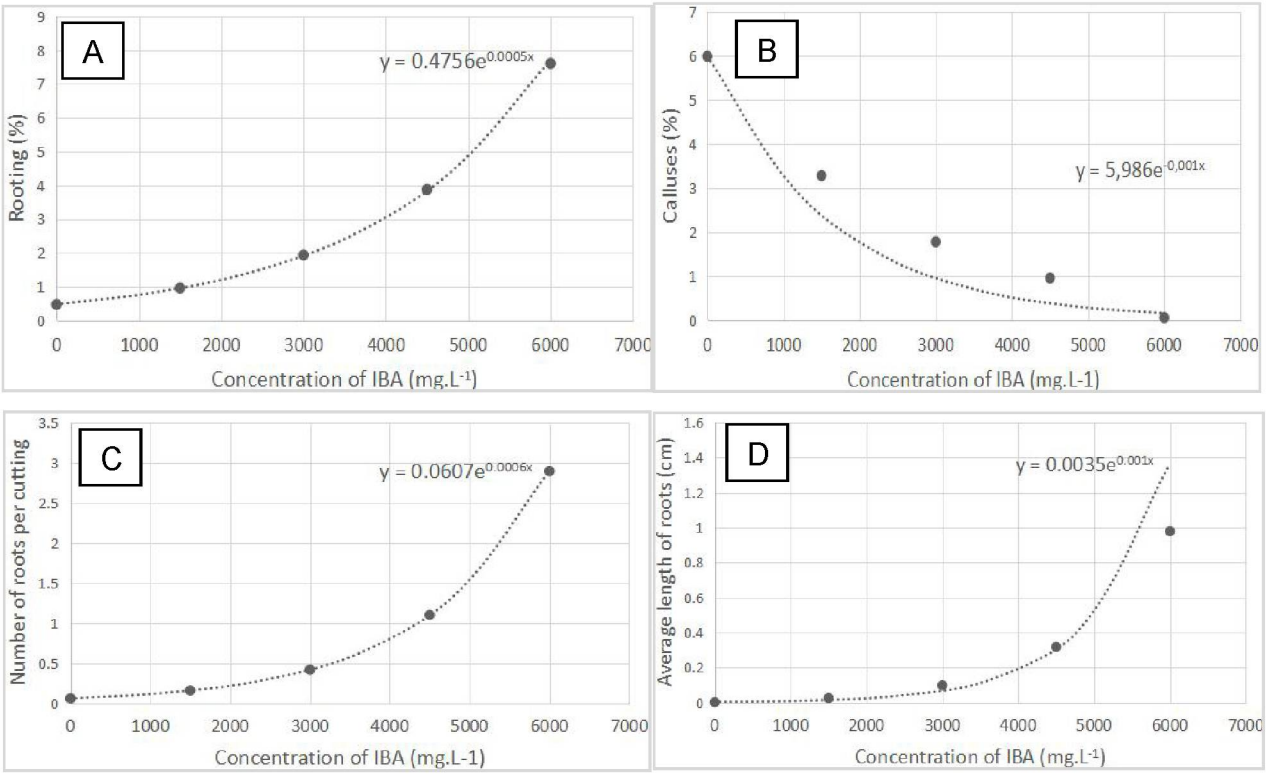


SOURCE: The author (2019).

Despite the fact that vegetative propagation is usually more costly than reproduction by seeds (HARTMANN et al., 2014), for *B. dracunculifolia* species, this method of reproduction is interesting because it will help to select superior clones with respect to secondary metabolites production, justifying the higher cash value of propagation costs. In addition, through vegetative propagation it is possible to select male and female individuals, which is also interesting for *B. dracunculifolia*, since, as presented in chapter 3, males can produce higher contents of bioactive compounds than females.

The present study found a low number of rooted cuttings, even at the highest doses of IBA. It was possible to verify that there is an increasing percentage of rooting with the increase of IBA doses, where without the application of auxin the percentage of *B. dracunculifolia* stem cuttings taking root was 0.471%, while the application of 6000 $mg.L^{-1}$ increased that percentage to 7.606%. The regression of root formation in function of IBA is presented in FIGURE 5.6 A and FIGURE 5.7.

FIGURE 5.6 - REGRESSION ANALYSIS FOR THE VARIABLES OF ROOTING (A), CALLUSES (B), AVERAGE NUMBER OF ROOTS PER CUTTINGS (C), AVERAGE LENGHT OF ROOTS (D) IN *B. DRACUNCULIFOLIA* STEM CUTTINGS TREATED WITH INCREASING CONCENTRATIONS OF IBA ($mg.L^{-1}$).



SOURCE: The author (2019)

The number of roots per cutting and length (cm) presented a positive exponential increment with the increase of IBA doses (FIGURE 5.6 C, FIGURE 5.6 D). In addition to rooting, other factors such as the number and length of roots are also important for the formation of quality seedlings (HARTMANN et al., 2014). The higher production of roots per cut increases its surface area and, consequently, the plant's efficiency in absorbing minerals available in the soil solution (ZONTA et al., 2006).

For the variable calluses was possible to verify the reduction of the percentage of calluses formation in *B. dracunculifolia* cuttings with the increase of IBA doses, we can observe that without the application of IBA the percentage of calluses formation is 5.98%, while with the increasing application of IBA up to 6000 $mg.L^{-1}$ the percentage decreases to 0.509%. The regression of calluses formation in function of IBA (negative function) is presented FIGURE 5.6 B. Calluses usually develops in the basal part of the cuttings and are definied as a irregular mass of parenchyma cells

and its growth proliferates primarily from cells the vascular cambium, but also from cells of the cortex and pith (HARTMANN et al., 2014).

FIGURE 5.7- EFFECT OF DIFFERENT IBA TREATMENTS (mg.L^{-1}) ON *B. dracunculifolia* STEM ROOT FORMATION.



SOURCE: The author (2019).

However, variations in the rooting potential of plant species within the same genus are expected. In a study by Bona et al. (2005), it was reported that for *B. trimera* and *B. stenocephala* high rooting rate was found, 76.1 and 83.3%, respectivaly, even without the application of exogenous auxin. The same was verified for species of other genera, such as *Piper* spp., where the species *Piper crassinervium* had a high rooting rate of 75.83% (NUNES GOMES; KRINSKI, 2019), whereas *P. umbellatum* reached maximum values of 37.5% (MATTANA et al., 2009) and *P. amalago* only 22.92% rooting (NUNES GOMES; KRINSKI, 2016). These variations may be related to several factors that act in isolated or concomitant ways,

such as the amount of endogenous auxin and other rooting promoters intrinsic to each species, the type of cuttings, time of collection of plant material and conditions of humidity, temperature and luminosity (ZUFFELLATO-RIBAS ; RODRIGUES, 2001).

The root formation process is usually divided into four stages: I) Dedifferentiation, the activation of cells to become competent; II) Formation of root from cells of vascular tissue, where through dedifferentiation become meristematic; III) Meristematic cells (development of root initials) develop into organized root primordia; IV) Root primordia growth and emerge out of the stem tissue and the formation of the vascular tissue between the vascular tissue of the cutting and the root primordia (HARTMANN et al., 2014).

Auxins such as IAA and IBA are important phytohormones that regulate adventitious root induction (STEFFENS; RASMUSSEN, 2016). However, auxin is only part of the stimulus, since for many species that are difficult to root, the root formation has been improved through with the addition of rooting cofactors, which are endogenous substances capable of acting synergistically with the IAA promoting rooting of cuttings (ZUFFELLATO-RIBAS; RODRIGUES, 2001). The complete understanding of the role of these cofactors in the induction of roots is complicated due to the apparent diversity of this group of bioactive substances (FOONG; BARNES, 1981). Previous studies have shown that some exogenous catechol-related polyphenols can act as rooting promoters (ZUFFELLATO-RIBAS; RODRIGUES, 2001), in the same way that some monophenols can act as cofactors of IAA-oxidase reducing root induction (BANDURSKI et al., 1995).

In addition, the rooting of the cuttings of *B. dracunculifolia* presented in this study may also be associated with anatomical barriers for the emission of roots related to, e.g., the accumulation of lignified tissue between the phloem and the cortex (XAVIER et al., 2009). The formation of a continuous sclerenchyma ring between the phloem and the cortex occurs in species that are difficult-to-root, while the easy-to-root types are characterized by discontinuity of this sclerenchyma ring or or for the least amount of its cell layers (HARTMANN et al., 2014).

This is the first study of propagation of *B. dracunculifolia* through stem cuttings but there is a need for optimization of the methodology due to the high mortality and low rooting of the cuttings, even in concentrations considered high of IBA. Although the analysis indicates increase in the rooting of the cuttings with the application of this auxin, tests with increased doses would not be the most indicated, since

application of auxins in excess may cause blackening of the stem and death of the cuttings (HARTMANN et al., 2014).

However, some factors may be involved in this low performance and may help to justify the reduction of cuttings survival and rooting, such as the phenological phase of the mother-plant, given that for difficult-to-root species the phenological phase can affect rooting ability, such as the trasition from vegetative to floral phase, in view of competing sink between flowering and rooting (HARTMANN et al., 2014; RASMUSSEN et al., 2015). In this sense, it is suggested that further studies of the vegetative propagation of *B. dracunculifolia* be developed taking account the seasonal behavior of propagation by cuttings of the species. Another alternative is the production of mini-cuttings using propagules originated from seeds, in view of the rejuvenation of plant material and high concentration of endogenous auxin, as previously described for the species *Araucaria angustifolia* (PIRES et al., 2013) and *Eucalyptus* (XAVIER et al., 2009). In addition, the application of rejuvenation techniques such as the propagation of epicornic buds and collecting branches from different parts of the plant can be an alternative to increase the cuttings rooting rate (ZUFFELLATO-RIBAS; RODRIGUES, 2001).

5.4 CONCLUSIONS

The physiological maturity of *Baccharis dracunculifolia* seeds produced from sexual reproduction occur at 40 days afte0r anthesis, being the best time for seed collection in the field. The constant temperature of 25° C and alternating 20-30°C can be used in the germination tests of the species, for best germination percentage, germination speed index and first count results. The first count is identified at 4 days after sowing and the last count after 11 days.

Baccharis dracunculifolia is a difficult-to-root species. The sex factor of plants does not influence the rooting, however, the increment in the concentration of IBA increases the rooting of this species. This is the first report of propagation of *B. dracunculifolia* using stem cuttings, although the optimization of the methodology is needed.

REFERENCES

ALMEIDA, S. R. et al. Florística e síndromes de dispersão de um remanescente de Floresta Ombrófila Mista em sistema faxinal. **Ambiência - Revista do Setor de Ciências Agrárias e Ambientais**, v. 4, n. 2, p. 289-297, 2008.

ALVARES, C. A. et al. Koppen's climate classification map for Brazil. **Meteorologische Zeitschrift**, v. 22, n. 6, p. 711–728, 2013.

BANDURSKI, R. S. et al. **Auxin biosynthesis and metabolism**. Dordrecht: Kluwer Academic Publishers, 1995.

BANKOVA, V. et al. Standard methods for *Apis mellifera* propolis research. **Journal of Apicultural Research**, v. 56, n. SI3, p. 1-49, 2016.

BARROSO, G. M.; BUENO, O. L. Flora ilustrada catarinense plantas compostas-subtribo: Baccharidinae. Itajaí: Herbário Barbosa Rodrigues (HBS), 2002.

BESTEN, M. A. et al. Chemical composition of volatiles from male and female specimens of *Baccharis trimera* collected in two distant regions of southern brazil: a comparative study using chemometrics. **Química Nova**, v. 36, n. 8, p. 1096-1100, 2013.

BONA, C. M. et al. Estaquia de três espécies de *Baccharis*. **Ciência Rural**, v. 35, n. 1, p. 223-226, 2005.

BONNER, F. T. Storage of seeds: Potential and limitations for germplasm conservation. **Forest Ecology and Management**, v.35, p. 35-43, 1990.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. **Regras para análise de sementes.** Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. Brasília: MAPA/SDA, 2009. 395p.

CARVALHO, M. N.; NAKAGAWA, J**. Sementes: ciência, tecnologia e produção**. Jaboticabal: Funep, 2012.

CARVALHO, R. I. N. et al. Enraizamento de estacas de carqueja em função de diferentes Substratos e posições do ramo em plantas masculinas e Femininas. **Scientia agraria**, v. 3, n. 3, p. 269-274, 2007.

ESPIRITO-SANTO, M. M. et al. Sexual differences in reproductive phenology and their consequences for the demography of *Baccharis dracunculifolia* (Asteraceae), a dioecious tropical shrub. **Annals of Botany**, v. 91, p.13-19, 2003.

FRANCISCO, F. et al. Maturation, processing and seed storage of *Elephantopus mollis* Kunth. **Acta Scientiarum. Agronomy**, v. 41, p. 1-12, 2019.

FERREIRA, M. J. et al. Avaliação da regeneração natural em nascentes perturbadas no município de Lavras, MG. **Ciência Florestal**, v. 19, n. 2, p.109-129, 2009.

FOONG, T. W.; BARNES, M. F. Rooting "cofactors" in rhododendron: the fractionation and activity of components from an easy-to-root and a difficult-to-root variety. **Biochemie und Physiologie der Pflanzen**, v. 176, p. 507-523, 1981.

GOMES, V.; FERNANDES, G. W. Germinação de aquênios de *Baccharis dracunculifolia* D.C. (Asteraceae). **Acta Botanica Brasilica**, v. .16, n .4, p. 421-427, 2002.

GRZYBOWSKI, C. R. S. et al. Maturation and germination of *Vernonanthura discolor* seeds. **Ciência e Agrotecnologia**, v. 40, n. 2, p. 164-172, 2016.

HARTMANN, H. T. et al. **Plant propagation principles and practices**. Harlow: Pearson Education Limited, 2014.

HAY, F. R.; PROBERT, R. J. Advances in seed conservation of wild plant species: a review of recent research. **Conservation Physiology .Conservation Physiology**, v. 1, n. 1, p. 1-11, 2013.

LI, X.; JIANG, S.; MAN, C. Metabolomic analysis of female and male plants of *Pistacia chinensis* Bunge. **Pakistan Journal of Botany**, v. 48, n. 5, p. 1971-1977, 2016.

LUDWIG-MÜLLER, J. Indole-3-butyric acid in plant growth and development. **Plant Growth Regulation**, v. 32, p. 219-230, 2000.

MAGUIRE, J. D. Speed of germination - aid in selection and evolution for seedling emergence and vigor. **Crop Science**, v. 2, n. 1, p. 176-177, 1962.

MARCOS-FILHO, J. **Fisiologia de sementes de plantas cultivadas**. Londrina: ABRATES, 2015.

MATTANA, R. S. et al. Propagação vegetativa de plantas de pariparoba [*Pothomorphe umbellata* (L.) Miq.] em diferentes substratos e número de nós das estacas. **Revista Brasileira de Plantas Medicinais**, v. 11, p. 325-329, 2009.

MATTHEWS, S.; KHAJEH-HOSSEINI, M. Length of the lag period of germination and metabolic repair explain vigour differences in seed lots of maize. **Seed Science and Technology**, v. 35, p. 200-212, 2007.

MATTHEWS, S. et al. Evaluation of seed quality: from physiology to international standardization. **Seed Science Research**, v. S1: p. S69-S73, 2012.

NORDSTRÖM, A. C.; JACOBS, F. A.; ELIASSON, L. Effect of exogenous indole-3-acetic acid and indole-3-butyric acid on internal levels of the respective auxins and their conjugation with aspartic acid during adventitious root formation in pea cuttings. **Plant Physiology**, v. 96, p. 856-861, 1991.

NUNES GOMES, E.; KRINSKI, D. Propagação vegetativa de *Piper amalago* (Piperaceae) em função de tipos de estaca e substratos. **Cultura Agronômica**, v. 25, p. 199-210, 2016.

NUNES GOMES, E.; KRINSKI, D. Enraizamento de estacas caulinares de *Piper crassinervium* Kunth sob diferentes concentrações de ácido indolbutírico. **Revista de Agricultura Neotropical**, v. 6, n. 1, p. 92-97, 2019.

PARK, Y. K, et al. Chemical constituents in *Baccharis dracunculifolia* as the main botanical origin of southeastern brazilian propolis. **Journal of Agricultural and Food Chemistry**, v. 52, p. 1100-1103, 2004.

PIRES, P. P.; WENDLING, I.; BRONDANI, G. Ácido indol butírico e ortotropismo na miniestaquia de *Araucaria angustifolia*. **Revista Árvore**, v. 37, p. 393-399, 2013.

R CORE TEAM. **R: A language and environment for statistical computing**. R foundation for statistical computing, Vienna, Austria, 2019.

RASMUSSEN, A. et al. Adventitious rooting declines with the vegetative to reproductive switch and involves a changed auxin homeostasis. **Journal of Experimental Botany**, v. 66, n. 5, p. 1437-1452, 2015.

RASMUSSEN, A.; SMITH, T. E.; HUNT, M. A. Cellular stages of root formation, root system quality and survival of *Pinus elliottii* var. *elliottii* x *P. caribaea* var. *hondurensis* cuttings in different temperature environments. **New Forests**, v. 38, p. 285-294, 2009.

ROBERTS, E. H. Predicting the storage life of seeds. **Seed Science and Technology**, v. 1, p. 499-514, 1973.

ROBINSON, K. M. et al. *Populus tremula* (European aspen) shows no evidence of sexual dimorphism. **BMC Plant Biology**, v. 14, p. 1-14, 2014.

SANTOS, C. D. L.; FIGUEIRA, G. M.; BELINI, C. M. B. Seed conservation methods and evaluation of germination rate in *Baccharis dracunculifolia* DC. (Asteraceae). **Acta Horticulturae**, v. 1125, p. 263-268, 2016.

SFORCIN, J. M. et al. ***Baccharis dracunculifolia*: uma das principais fontes vegetais da própolis brasileira**. São Paulo: Editora UNESP, 2012.

SILVA, F. A. S.; AZEVEDO, C. A. V. The assistat software version 7.7 and its use in the analysis of experimental data. **African Journal of Agricultural Research**, v. 11, p. 3733–3740, 2016.

SILVEIRA, M. A. M.; VILLELA, F. A.; TILLMANN, M. A. A. Maturação fisiológica de sementes de calêndula (*Calendula officinalis* L.). **Revista Brasileira de Sementes**, v. 24, n. 2, p. 31-37, 2002.

STEFFENS, B.; RASMUSSEN, A. The physiology of adventitious roots. **Plant Physiology**, v. 170, p. 603-617, 2016.

STUEPP, C. A. et al. The use of auxin quantification for understanding clonal tree propagation. **Forests**, v. 8, n. 27, p. 1-15, 2017.

WENDLING, I.; STUEPP, C. A.; ZUFFELLATO-RIBAS, K. C. Rooting of *Araucaria angustifolia*: Types of cuttings and stock plants sex. **Revista Árvore**, v. 40, n. 6, p. 1013-1021, 2016.

XAVIER, A.; WENDLING, I.; SILVA, R. L. **Silvicultura clonal: princípios e técnicas**. Viçosa: Universidade Federal de Viçosa, 2009.

ZONTA, E. et al. **O Sistema radicular e suas interações com o ambiente edáfico**. Viçosa: Sociedade Brasileira de Ciência do Solo, 2006.

ZUFFELLATO-RIBAS, K. C.; RODRIGUES, J. D. **ESTAQUIA: Uma abordagem dos principais aspectos fisiológicos**. Curitiba: Universidade Federal do Paraná, 2001.

GENERAL CONCLUSIONS

The essential oil of *Baccharis dracunculifolia* show quantitative changes in its compounds, however, there were no changes in the chemical composition.

Differences in the yield and level of compounds of essential oils between populations may be due related to the genetic and other biotic and abiotic factors besides those evaluated in this study. This quantitative differences also reflected in their antioxidant activities;

The populations evaluated were divided into three main groups, according to the levels of their essential oil compounds;

Differences in content, of the compounds and in antioxidant activity of essential oils are detected throughout the year, emphasizing the importance of the seasonal chemical characterization of the species;

Essential oils can be separated into two groups according to the collection time: 1) autumn and summer, flowering phase, with high levels of *(E)*-nerolidol; 2) winter and spring, vegetative phase, high levels in β-pinene;

The secondary metabolism of *B. dracunculifolia*, i.e essential oil and extracts, was affected by the gender of the plant and by the growth sites (populations);

Male plants produced higher level of compounds in the extracts (non-volatile compounds), higher essential oil content and higher antioxidant capacity for both extracts and essential oil than female plants;

Artepilin C, the most important compound for the commercialization of green propolis, was present higher content in male leaves of the two populations studied, when compared to the female leaves plants;

The physiological maturity of seeds occur at 40 days after anthesis, and this is the best time for seed collection in the field;

The constant temperature of 25° C and alternating 20-30°C can be used in the germination tests of the species, for best germination percentage, germination speed index and first count results. The first count being identified at 4 days after sowing and the last count after 11 days;

The sex factor does not influence the rooting of stem cuttings, but the application of IBA is essential for the rooting of cuttings;

Although the application of increasing doses of IBA helped in the rooting of cuttings, the proportion of rooted cuttings was very low;

This is the first report of propagation of *B. dracunculifolia* stem cuttings, although the optimization of the methodology is needed.

FINAL CONSIDERATIONS

The essential oil yield and chemical composition (quantitative) among the populations of *Baccharis dracunculifolia* studied show variability, and the quantitative differences observed are possibly related to the genetic and other biotic and abiotic factors besides those evaluated in this study. These quantitative variations are reinforced when its chemical profile are evaluated seasonally, where both the phenological stages and climatic variations may have influenced the levels of certain terpenic compounds. However, with the seasonal study it was possible to understand in which periods the plant produced higher levels of important compounds such as *(E)*-Nerolidol, that has several applications in pharmacology, in the food industry and as an ingredient in fine fragrances and cosmetics. Similarly, the antioxidant activity showed variations due to the levels of these compounds, which was decisive for its greater or lesser activity. In this sense, the overall chemical composition and the content of specific compounds are the main factors affecting the type of industry in which the final product will be applied and consequently their final market value.

Differences in secondary metabolism of male and female plants of this versatile aromatic species were also presented in this study and it was possible to verify a great potential of male plants to produce significant amounts of the high added-value compounds as artepillin C and caffeoylquinic acids and also higher essential oil content and antioxidant activity. Artepillin C being the most important compound and with higher added value for the commercialization of green propolis, the management of male *B. dracunculifolia* plants in the field can be a strategy to increase the quality and the market value of this final product.

However, further studies are needed to verify the predilection of *Apis mellifera* bees by male plants instead of female ones in the collection of resin to produce green propolis, aiming at producing high quality product for the market. Therefore, the understanding of chemical diversity in wild populations and, subsequently, the selection of highly productive accessions for domestication, cultivation and breeding are some of the initial steps to successfully satisfy the industrial demands.

Until then, the management of *B. dracunculifolia* in the field is extractive both with regard to essential oil and the production of green propolis. The commercial cultivation of the species is still incipient, the reports of this type of practice are few and, as far as we know, it is carried out by beekeepers producing green propolis that

propagate the plant by seeds with the aim of increasing their green propolis production. Thus, information about physiological maturity point of seeds can help the producers to harvest the seed at the correct point thus increasing the success of quality seedling production. In addition, the definition of the ideal temperature for the germination of the species, can also help determine the best time for sowing, bringing economic impacts to beekeepers and producers of medicinal plants.

It is important to note that this is the first study of propagation of B. dracunculifolia stem cuttings, however, the application of IBA is necessary for rooting and this fact becomes important in view of the high content of compounds with high added value produced by male leaves of the species. Thus, it is suggested further studies taking into account the collection of cuttings at different times of the year, tests with cuttings originating from different positions on the branch and application of rejuvenation techniques such as production of mini-cuttings and propagation of epicornic buds and application of micropropagation techniques.

GENERAL REFERENCES

ABDEL-LATEIF, K. S.; ELDEAB, H. A.; MAGHRABI, I. A. The plant natural products: their antioxidants, free radical scavengers, DNA protection and antimicrobial activities. **Journal of Bioprocessing and Biotechniques**, v. 6, p. 2-7, 2016.

ADAMS, R. P. **Identification of essential oil components by gas chromatography/mass spectrometry**. Carol Stream: Allured Publishing Corporation, 2007.

AGA, H. et al. Isolation and identification of antimicrobial compounds in brazilian propolis. **Bioscience, Biotechnology, and Biochemistry**, v. 58 n. 5, p. 945 946, 1994.

AHMAD, P.; SARWAT, M.; SHARMA, S. Reactive oxygen species, antioxidants and signaling in plants. **Journal of Plant Biology**, v. 51, p. 167-173, 2008.

AHN, M. et al. Suppression of tumor-induced angiogenesis by Brazilian propolis: Major component artepillin C inhibits in vitro tube formation and endothelial cell proliferation. **Cancer Letters**, v. 252, p. 235-243, 2007.

AIDI WANNES, W.; MARZOUK, B. Maturational effect on essential oil yield and composition of *Myrtus communis* var. baetica fruit. **Journal of Essential Oil Bearing Plants**, v.15, p. 847–853, 2012.

ALMEIDA, S. R. et al. Florística e síndromes de dispersão de um remanescente de Floresta Ombrófila Mista em sistema faxinal. **Ambiência - Revista do Setor de Ciências Agrárias e Ambientais**, v. 4, n. 2, p. 289-297, 2008.

ALVARES, C. A. et al. Koppen's climate classification map for Brazil. **Meteorologische Zeitschrift**, v. 22, n. 6, p. 711–728, 2013.

AMORATI, R.; FOTI, M. C.; VALGIMIGLI, L. Antioxidant activity of essential oils. **Journal of Agricultural and Food Chemistry**, v. 61, p. 10835−10847, 2013.

ANTOS, J. A.; ALLEN, G. A. Patterns of reproductive effort in male and female shrubs of *Oemleria cerasiformis:* a 6 year study. **Journal of Ecology**, v. 87, p. 77-84, 1999.

ARDUIN, M. I.; FERNANDES, G. W.; KRAUS, J. E. Morphogenesis of galls induced by *Baccharopelma dracunculifoliae* (Hemiptera: Psyllidae) on *Baccharis dracunculifolia* (Asteraceae) leaves. **Brazilian Journal of Biology**, v. 65, p. 559, 2005.

ARRUDA, D. C. et al. Antileishmanial activity of the terpene nerolidol. **Antimicrobial Agents and Chemotherapy**, v. 5, p. 1679-1687, 2005.

BANDURSKI, R. S. et al. **Auxin biosynthesis and metabolism**. Dordrecht: Kluwer Academic Publishers, 1995.

BANKOVA, V. et al. Standard methods for *Apis mellifera* propolis research. **Journal of Apicultural Research**, v. 56, n. SI3, p. 1-49, 2016.

BANKOVA, V.; CASTRO, S. DE.; MARCUCCI, M. Propolis: recent advances in chemistry and plant origin. **Apidologie**, v. 31, p. 3–15, 2000.

BANO, U. et al. Effect of plant growth regulators on essential oil yield in aromatic plants. **Journal of Chemical and Pharmaceutical Research**, v. 8, p. 733-739, 2016.

BANSKOTA, A. H. et al. Chemical constituents of brazilian propolis and their cytotoxic activities. **Journal of Natural Products**, v. 61, p. 896-900, 1998.

BANTIS, F.; OUZOUNIS, T.; RADOGLOU, K. Artificial LED lighting enhances growth characteristics and total phenolic content of *Ocimum basilicum*, but variably affects transplant success. **Scientia Horticulturae**, v. 198, p. 277–283, 2016.

BARBOSA, L. N. et al. In vitro antibacterial and chemical properties of essential oils including native plants from Brazil against pathogenic and resistant bacteria. **Journal of Oleo Science**, v. 64, p. 289-298, 2015.

BARROSO, G. M.; BUENO, O. L. **Flora ilustrada catarinense plantas compostas-subtribo: Baccharidinae**. Itajaí: Herbário Barbosa Rodrigues, 2002.

BASTOS, E. M. A. F. et al. Interaction between *Apis mellifera* L. and *Baccharis dracunculifolia* DC, that favours green propolis production in Minas Gerais. **Brazilian Journal of Biology**, v. 71, n. 3, p. 727-734, 2011.

BESTEN, M. A. et al. Chemical composition of volatiles from male and female

specimens of *Baccharis trimera* collected in two distant regions of southern Brazil: a comparative study using chemometrics. **Química Nova**, v. 36, p. 1096-1100, 2013.

BESTEN, M. A. et al. Chemical composition similarity between the essential oils isolated from male and female specimens of each five *Baccharis* species. **Journal of the Brazilian Chemical Society**, v. 23, n. 6, p. 1041-1047, 2012.

BITTENCOURT, M. L. F. et al. Metabolite profiling, antioxidant and antibacterial activities of Brazilian propolis: Use of correlation and multivariate analyses to identify potential bioactive compounds. **Food Research International**, v. 76, p. 449-457, 2015.

BOLDT, P. E. ***Baccharis* (Asteraceae). A Review of its taxonomy, phytochemistry, ecology, economic status, natural enemies and potential for its biological control in the United States**. Washington: USDA. Agricultural Research Service Grassland, 1989.

BONA, C. M. et al. Estaquia de três espécies de *Baccharis*. **Ciência Rural**, v. 35, n. 1, p. 223-226, 2005.

BONNER, F. T. Storage of seeds: Potential and limitations for germplasm conservation. **Forest Ecology and Management**, v.35, p. 35-43, 1990.

BOTREL, P. et al. Teor e composição química do óleo essencial de *Hyptis marrubioides* Epl., Lamiaceae em função da sazonalidade. **Acta Scientiarum. Agronomy**, v. 32, p. 533-538, 2010.

BOUARAB-CHIBANE, L. et al. Antibacterial properties of polyphenols: characterization and QSAR (Quantitative Structure–Activity Relationship) models. **Frontiers in Microbiology**, v. 10, p. 1-23, 2019.

BRAND-WILLIAMS, W.; CUVELIER, M. E.; BERSET, C. Use of a free radical method to evaluate antioxidant activity. **LWT- Food Science and Technology**, v. 28, p. 25-30, 1995.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. **Regras para análise de sementes.** Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. Brasília: MAPA/SDA, 2009. 395p.

BUDEL, J.M.; DUARTE, C.A.M.; FARAGO, N.I.; MATZENBACHE. O progresso da pesquisa sobre o gênero *Baccharis*, Asteraceae: I - Estudos botânicos. **Revista Brasileira de Farmacognosia**, v. 15, n. 3, p. 268-271, 2005.

BÚFALO, M. C. et al. In vitro cytotoxic activity of *Baccharis dracunculifolia* and propolis against HEp-2 cells. **Natural Product Research**, v. 24, p. 1710–1718, 2010.

CARVALHO, A. A. et al. *In vivo* antitumoural activity and composition of an oil extract of Brazilian propolis. **Food Chemistry**, v.126, p. 1239–1245, 2011.

CARVALHO, M. N.; NAKAGAWA, J**. Sementes: ciência, tecnologia e produção**. Jaboticabal: Funep, 2012.

CARVALHO, R. I. N. et al. Enraizamento de estacas de carqueja em função de diferentes substratos e posições do ramo em plantas masculinas e femininas. **Scientia agraria**, v. 3, n. 3, p. 269-274, 2007.

CASAGRANDE, M. et al. Influence of time, temperature and solvent on the extraction of bioactive compounds of *Baccharis dracunculifolia*: *In vitro* antioxidant activity, antimicrobial potential, and phenolic compound quantification. **Industrial Crops and Products**, v. 125, p. 207–219, 2018.

CEPEDA-CORNEJO, V.; DIRZO, R. Sex-related differences in reproductive allocation, growth, defense and herbivory in three dioecious neotropical palms. **PLOS ONE**, v. 5, Issue 3, 2010.

CHAABAN, A. et al. Insecticide activity of *Baccharis dracunculifolia* essential oil against *Cochliomyia macellaria* (Diptera: Calliphoridae). **Natural Product Research**, v. 32, p. 2954-2958, 2017.

CHAI, T. T. et al. Water stress-induced oxidative damage and antioxidant responses in micropropagated banana plantlets. **Biologia Plantarum**, v. 49, p. 153-156, 2005.

CHAN, W. K. et al. Nerolidol: a sesquiterpene alcohol with multi-faceted pharmacological and biological activities. **Molecules**, v. 21, p. 1-40, 2016.

CORNELISSEN, T.; STILING, P. Sex-biased herbivory: a meta-analysis of the effects of gender on plant-herbivore interactions. **OIKOS**, v. 111, p. 488-500, 2005.

DA SILVA, A.C.R. et al. Biological activities of α-pinene and β-pinene enantiomers. **Molecules**, v. 17, p. 6305-6316, 2012.

DAJIC-STEVANOVIC, Z.; PLJEVLJAKUSIC, D. **Challenges and decision making in cultivation of medicinal and aromatic plants**. Dordrecht: Springer, 2015.

DELVAS, N. et al. Phenolic compounds that confer resistance to spruce budworm. **Entomologia Experimentalis et Applicata**, v. 141, p. 35–44, 2011.

DE-MOURA, S. A. L. et al. Aqueous extract of brazilian green propolis: primary components, evaluation of inflammation and wound healing by using subcutaneous implanted sponges. **Evidence-Based Complementary and Alternative Medicine**, v. 2011, p. 1-8, 2011.

DE SOUSA, J. P. et al. Seasonal variation of the *(E)*-nerolidol and other volatile compounds within ten different cultivated populations of *Baccharis dracunculifolia* D.C. (Asteraceae). **Journal of Essential Oil Research**, v. 21, p. 308-314, 2009.

DIXON, R.; PAIVA, N. Stress-Induced phenylpropanoid metabolism. **The Plant Cell**, v. 7, p. 1085-1097, 1995.

DORMAN H. D. et al. *In vitro* evaluation of antioxidant activity of essential oils and their components. **Flavour and Fragrance Journal**, v. 15, p. 12-16, 2000.

DUDAREVA, M.; PICHERSKY, E.; GERSHENZON, J. Biochemistry of plant volatiles. **Plant Physiology**, v. 135, p. 1893–1902, 2004.

DUDLEY, L. S. Ecological correlates of secondary sexual dimorphism in *Salix glauca* (Salicaceae). **American Journal of Botany**, v. 93, p. 1775–1783, 2006.

EMAMI, S. A.; JAVADI, B.; HASSANZADEH, M. K. Antioxidant activity of the essential oils of different parts of *Juniperus communis* subsp. hemisphaerica and *Juniperus oblonga.* **Pharmaceutical Biology**, v. 45, n. 10, p. 769-776, 2007.

ESPIRITO-SANTO, M. M. et al. Sexual differences in reproductive phenology and their consequences for the demography of *Baccharis dracunculifolia* (Asteraceae), a dioecious tropical shrub. **Annals of Botany**, v. 91, p.13-19, 2003.

FALCÃO, S. et al. Phenolic Profiling of portuguese propolis by LC–MS spectrometry: uncommon propolis rich in flavonoid glycosides. **Phytochemical Analysis**, v. 24, p. 309-318, 2013.

FERESIN G. E. et al. Constituents of the Argentinian medicinal plant *Baccharis grisebachii* and their antimicrobial activity. **Journal of Ethnopharmacology**, v. 89, p. 73-80, 2003.

FERNANDES-SILVA, C. et al. Chemical profiling of six samples of brazilian propolis. **Quimica Nova**, v. 36, n. 2, p. 237-240, 2013.

FERREIRA, M. J. et al. Avaliação da regeneração natural em nascentes perturbadas no município de Lavras, MG. **Ciência Florestal**, v. 19, n. 2, p.109-129, 2009.

FERRONATTO, R. et al. Antimicrobial activity of essential oils produced by *Baccharis dracunculifolia* D.C. and *Baccharis uncinella* D.C. (Asteraceae). **Revista Brasileira de Farmacognosia**, v. 17, p. 224-230, 2007.

FIGUEIREDO, A. C. et al. Factors affecting secondary metabolite production in plants: volatile components and essential oils. **Flavour and Fragrance Journal**, v. 23, p. 213-226, 2008.

FILHO, A. A. S. et al. "In vitro trypanocidal activity evaluation of crude extract and isolated compounds from *Baccharis dracunculifolia* DC (Asteraceae)," **Journal of Pharmacy and Pharmacology**, v. 56, n. 9, p. 1195–1199, 2004.

FILHO, A. A. S. et al. Antimicrobial activity of the extract and isolated compounds from *Baccharis dracunculifolia* D. C. (Asteraceae). **Zeitschrift fur Naturforschung**, v. 63, n. 1-2, p. 40–46, 2008.

FLORÃO, A. et al. Essential oils from *Baccharis* species (Asteraceae) have anti-inflammatory effects for human cells. **Journal of Essential Oil Research**, v. 24, p. 561-570, 2012.

FOONG, T. W.; BARNES, M. F. Rooting "cofactors" in Rhododendron: The fractionation and activity of components from an easy-to-root and a difficult-to-root variety. **Biochemie und Physiologie der Pflanzen**, v. 176, p. 507-523, 1981.

FRAISSE, D. et al. Caffeoyl derivatives: major antioxidant compounds of some wild herbs of the Asteraceae family. **Food and Nutrition Sciences**, v. 2, p. 181–192, 2011.

FRANCISCO, F. et al. Maturation, processing and seed storage of *Elephantopus mollis* Kunth. **Acta Scientiarum. Agronomy**, v. 41, p. 1-12, 2019.

FRIZZO, C. D. et al. Essential oil variability in *Baccharis uncinella* DC and *Baccharis dracunculifolia* DC growing wild in southern Brazil, Bolivia and Uruguay. **Flavour and Fragrance Journal**, v. 23, p. 99-106, 2008.

FROHNMEYER, H.; STAIGER, D. Ultraviolet-B radiation-mediated responses in plants. Balancing damage and protection. **Plant Physiology**, v. 133, p. 133:1420-1428, 2003.

FUKUDA. M, et al. Studies on the constituents of the leaves of *Baccharis dracunculifolia* (Asteraceae) and their cytotoxic activity. **Chemical & Pharmaceutical Bulletin**, v. 54, p. 1465–1468, 2006.

FURSTENBERG-HAGG, J.; ZAGROBELNY, M.; BAK, S. Plant defence against insect herbivores. **International Journal of Molecular Sciences**, v. 14, p. 10242–10297, 2013.

GHASEMI, M. et al. Increase of chamazulene and α-bisabolol contents of the essential oil of german chamomile (*Matricaria chamomilla* L.) using salicylic acid treatments under normal and heat stress conditions. **Foods**, v. 5, p. 1-14, 2016.

GOMES, V.; FERNANDES, G. W. Germinação de aquênios de *Baccharis dracunculifolia* D.C. (Asteraceae). **Acta Botanica Brasilica**, v. .16, n .4, p. 421-427, 2002.

GOURINE, N. et al. Seasonal variation of chemical composition and antioxidant activity of essential oil from *Pistacia atlantica* Desf. leaves. **Journal of the American Oil Chemists' Society**, v. 87, p. 157-166, 2010.

GRZYBOWSKI, C. R. S. et al. Maturation and germination of *Vernonanthura discolor* seeds. **Ciência e Agrotecnologia**, v. 40, n. 2, p. 164-172, 2016.

HALL, C. R.; CARROLL, A. R.; KITCHING, R. L. A meta-analysis of the effects of galling insects on host plant secondary metabolites. **Arthropod-Plant Interactions**, v. 11, n. 4, p. 463-473, 2017.

HARTMANN, H. T. et al. **Plant propagation principles and practices**. Harlow: Pearson Education Limited, 2014.

HAY, F. R.; PROBERT, R. J. Advances in seed conservation of wild plant species: a review of recent research. **Conservation Physiology .Conservation Physiology**, v. 1, n. 1, p. 1-11, 2013.

HERMS, D. A.; MATTSON, W. J. The dilemma of plants: to grow or defend. **The Quarterly Review Of Biology**, v.67, n. 3, 1992.

HIRANO, R. et al. Antioxidant ability of various flavonoids against DPPH radicals and LDLoxidation. **Journal of Nutritional Science and Vitaminology**, v. 47, p. 357–362, 2001.

HUSSAIN, A. I. et. al. Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. **Food Chemistry**, v. 108, p. 986-995, 2008.

JAAKOLA, L.; HOHTOLA, A. Effect of latitude on flavonoid biosynthesis in plants. **Plant, Cell & Environment**, v. 33, p. 1239-1247, 2010.

JENSEN, R. A. The shikimate/arogenate pathway: Link between carbohydrate metabolism and secondary metabolism. **Physiologia Plantarum**, v. 66, p. 164-168, 1986.

JEYARAMRAJA, P. R. et al. Water deficit induced oxidative damage in tea (*Camellia sinensis*) plants. **Journal of Plant Physiology**, v. 162, p. 413-419, 2005.

KASAI, S. et al. Design and synthesis of antiangiogenic/heparin-binding arginine dendrimer mimicking the surface of endostatin. **Bioorganic & Medicinal Chemistry Letters**, v. 12, p. 951–954, 2002.

KIMOTO, T. S. et al. Renal carcinogenesis induced by ferric nitrilotriacetate in mice, and protection from it by Brazilian propolis and artepillin C. **Pathology International**, v. 50, p. 679–689, 2000.

KIMOTO, T. S. et al. Pulmonary carcinogenesis induced by ferric nitrilotriacetate in mice and protection from it by Brazilian propolis and artepillin C. **Virchows Archiv**, v. 438, p. 259-270, 2001.

KLOPELL, F. C. et al. Nerolidol, an antiulcer constituent from essential oil of *Baccharis dracunculifolia* DC (Asteraceae). **Zeitschrift für Naturforschung**, v. 62c, p. 537–542, 2007.

KUMAZAWA, S. et al. Direct evidence for the plant origin of brazilian propolis by the observation of honeybee behavior and phytochemical analysis. **Chemical and Pharmaceutical Bulletin**, v. 51, n. 6, p. 740-742, 2003.

LAGE, T. C. A. et al. Chemical composition and acaricidal activity of the essential oil of *Baccharis dracunculifolia* De Candole (1836) and its constituents nerolidol and limonene on larvae and engorged females of *Rhipicephalus microplus* (Acari: Ixodidae). **Experimental Parasitology**, v. 148, p. 24-29, 2015.

LAPCZYNSKI, A. et al. Fragrance material review on nerolidol (isomer unspecified). **Food and Chemical Toxicology**, v. 46, p. S247–S250, 2008.

LEITE, A.M. et al. Inhibitory effect of β-pinene, α-pinene and eugenol on the growth of potential infectious endocarditis causing Gram-positive bacteria.Brazilian. **Journal of Pharmaceutical Sciences,** v. 43, n. 1, p. 121-126, 2007.

LEMOINE, N. P.; BURKEPILE, D. E.; PARKER, J. D. Variable effects of temperature on insect herbivory. **PeerJ**, v. 2, p. e376, 2014.

LEMOS, M. et al. *Baccharis dracunculifolia* the main botanical source of brazilian green propolis, displays antiulcer activity. **Journal of Pharmacy and Pharmacology**, v. 59, p. 603–608, 2007.

LI, X.; JIANG, S.; MAN, C. Metabolomic analysis of female and male plants of *Pistacia chinensis* Bunge. **Pakistan Journal of Botany**, v. 48, n. 5, p. 1971-1977, 2016.

LIU, H. et al. UV-B irradiation differentially regulates terpene synthases and terpene content of peach. **Plant, Cell & Environment**, v. 40, p. 2261–2275, 2017.

LLUSIA, J. et al. Effects of UV radiation and water limitation on the volatile terpene emission rates, photosynthesis rates, and stomatal conductance in four Mediterranean species. **Acta Physiologiae Plantarum**, v. 34, p. 757–769, 2012.

LOAYZA, I. et al. Essential oils of *Baccharis salicifolia, Baccharis latifolia* and *Baccharis dracunculifolia*. **Phytochemistry**, v. 38, p. 381-389, 1995.

LOPES, R. C. et al. Different hydric influencing essential oil production from *Plygonum punctatum* Ell. **Revista Brasileira de Plantas Medicinais**, v. 3, p. 7-10, 2001.

LORETO, F.; VELIKOVA, V. Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. **Plant Physiology**, v. 127, p. 1781-1787, 2001.

LUDWIG-MÜLLER, J. Indole-3-butyric acid in plant growth and development. **Plant Growth Regulation**, v. 32, p. 219-230, 2000.

LUSHCHAK, V. I. Free radicals, reactive oxygen species, oxidative stress and its classification. **Chemico-Biological Interactions**, v. 224, p. 164-175, 2014.

MAES, K.; DEBERGH, P. C. Volatiles emitted from in vitro grown tomato shoots during abiotic and biotic stress. Plant Cell, Tissue and Organ Culture, v. 75, p. 73-78, 2003.

MAFFEI, M.E. Sites of synthesis, biochemistry and functional role of plant volatiles. **South African Journal of Botany**, v. 76, p. 612–631, 2010.

MAGUIRE, J. D. Speed of germination - aid in selection and evolution for seedling emergence and vigor. **Crop Science**, v. 2, n. 1, p. 176-177, 1962.

MARCOS-FILHO, J. **Fisiologia de sementes de plantas cultivadas**. Londrina: ABRATES, 2015.

MARTINEZ-CORREA, H. Á. et al. Extracts from the leaves of *Baccharis dracunculifolia* obtained by a combination of extraction processes with supercritical CO2, ethanol and water. **The Journal of Supercritical Fluids**, v. 63, p. 31-39, 2012.

MATTANA, R. S. et al. Propagação vegetativa de plantas de pariparoba [*Pothomorphe umbellata* (L.) Miq.] em diferentes substratos e número de nós das estacas. **Revista Brasileira de Plantas Medicinais**, v. 11, p. 325-329, 2009.

MATTHEWS, S. et al. Evaluation of seed quality: from physiology to international standardization. **Seed Science Research**, v. S1: p. S69-S73, 2012.

MATTHEWS, S.; KHAJEH-HOSSEINI, M. Length of the lag period of germination and metabolic repair explain vigour differences in seed lots of maize. **Seed Science and Technology**, v. 35, p. 200-212, 2007.

MESSERLI, S. et al. Artepillin C (ARC) in brazilian green propolis selectively blocks oncogenic PAK1 signaling and suppresses the growth of NF Tumors in mice. **Phytotherapy Research**, v. 23, p. 423-427, 2009.

MIJANGOS-RAMOS, I. F. et al. Bioactive dicaffeoylquinic acid derivatives from the root extract of *Calea urticifolia.* **Revista Brasileira de Farmacognosia**, v. 28, p. 339–343, 2018.

MIRANDA, C. A. S. F. et al. Essential oils from leaves of various species: antioxidant and antibacterial properties on growth in pathogenic species. **Revista Ciência Agronômica**, v. 47, p. 213-220, 2016.

MIRJALILI, M. H. et al. Essential oil variation of *Salvia officinalis* aerial parts during its phenological cycle. **Chemistry of Natural Compounds**, v. 42, p. 19–23, 2006.

MISHIMA, S. et al. Identification of caffeoylquinic acid derivatives from Brazilian propolis as constituents involved in induction of granulocytic differentiation of HL-60 cells. **Bioorganic & Medicinal Chemistry**, v. 13, n. 20, p. 5814-5818, 2005.

MOILANEN, J. et al. Biological activity of ellagitannins: effects as anti-oxidants, pro-oxidants and metal chelators. **Phytochemistry**, v. 125, p. 65–72, 2016.

MUNARI, C. C. et al. Mutagenicity and Antimutagenicity of *Baccharis dracunculifolia* Extract in Chromosomal Aberration Assays in Chinese Hamster Ovary Cells. **Planta Medica**, v. 74, p. 1363– 1367, 2008.

MUROTA, K.; TERAO, J. Antioxidative flavonoid quercetin: implication of its intestinal absorption and metabolism. **Archives of Biochemistry and Biophysics**, v. 417, p. 12-17, 2003.

MWANG'INGO, P. L. et al. Sex distribution, reproductive biology and regeneration in the dioecious species *Osyris lanceolata* (African sandalwood) in Tanzania. **Tanzania Journal of Forestry and Nature Conservation**, v. 76, p. 118-133, 2007.

MWANG'INGO, P. L.; KIBODYA, G.; MNG'ONG'O, A. R. Oil yield and quality variation between sexes in *Osyris lanceolata* (African sandalwood) and its value as a fodder plant in Tanzania. **Southern Forests**, v. 72, n. 2, p. 69–74, 2010.

NAKANISHI, H. et al. Efficient radical scavenging ability of artepillin C, a major component of Brazilian propolis, and the mechanism. **Organic and Biomolecular Chemistry**, v. 1, n. 9, p. 1452–1454, 2003.

NEGI, P. S. Plant extracts for the control of bacterial growth: Efficacy, stability, and safety issues for food application**. International Journal of Food Microbiology**, v. 156, p. 7–17, 2012.

NETO, J. D. N. et al. Antioxidant effects of nerolidol in mice hippocampus after open field test. **Neurochemical Research**, v. 38, p. 1861-1870, 2013.

NOGUEIRA NETO, J. et al. Antioxidant effects of nerolidol in mice hippocampus after open field test. **Neurochemical Research**, v. 38, 1861–1870, 2013.

NORDSTRÖM, A. C.; JACOBS, F. A.; ELIASSON, L. Effect of exogenous indole-3-acetic acid and indole-3-butyric acid on internal levels of the respective auxins and their conjugation with aspartic acid during adventitious root formation in pea cuttings. **Plant Physiology**, v. 96, p. 856-861, 1991.

NUNES GOMES, E.; KRINSKI, D. Enraizamento de estacas caulinares de *Piper crassinervium* Kunth sob diferentes concentrações de ácido indolbutírico. **Revista de Agricultura Neotropical**, v. 6, n. 1, p. 92-97, 2019.

NUNES GOMES, E.; KRINSKI, D. Propagação vegetativa de *Piper amalago* (Piperaceae) em função de tipos de estaca e substratos. **Cultura Agronômica**, v. 25, p. 199-210, 2016.

ORMENO, E. et al. Water deficit stress induces different monoterpene and sesquiterpene emission changes in Mediterranean species. Relationship between terpene emissions and plant water potential. **Chemosphere**, v. 67, n. 2, p. 276-284, 2007.

OTERO-ARNAIZ, A.; OYAMA, K. Reproductive phenology, seed-set and pollination in *Chamaedorea alternans*, an understorey dioecious palm in a rain forest in Mexico. **Journal of Tropical Ecology**, v. 17, p. 745-754, 2001.

PACCIARONI, A. V. et al. Bioactive Constituents of *Conyza albida*. **Planta Medica**, v. 66, p. 720-723, 2000.

PANDEY, G. et al. Altitudinal variation of berberine, total phenolics and flavonoid content in *Thalictrum foliolosum* and their correlation with antimicrobial and antioxidant activities. **Journal of Ayurveda and Integrative Medicine**, v. 9, p. 169-176, 2018.

PAREJO, I. et al. Bioguided isolation and identification of the nonvolatile antioxidant compounds from fennel (*Foeniculum vulgare* Mill.) waste. **Journal of Agricultural and Food Chemistry**, v. 52, p. 1890-1897, 2004.

PARK, Y. K, et al. Chemical constituents in *Baccharis dracunculifolia* as the main botanical origin of southeastern Brazilian propolis. **Journal of Agricultural and Food Chemistry**, v. 52, p. 1100-1103, 2004.

PARREIRA, N. A. et al. Antiprotozoal, schistosomicidal, and antimicrobial activities of the essential oil from the leaves of *Baccharis dracunculifolia*. **Chemistry & Biodiversity**, v. 7, p. 993-1001, 2010.

PAULINO, N. et al. Anti-inflammatory effects of a bioavailable compound, Artepillin C, in Brazilian propolis. **European Journal of Pharmacology**, v. 587, n. 1-3, p. 296-301, 2008.

PAZOUKI, L.; NIINEMETS, Ü. Multi-substrate terpene synthases: their occurrence and physiological significance. **Frontiers in Plant Science**, v. 7, p. 1-16, 2016.

PEGORINI, F.; MARANHÃO, L. T.; ROCHA, L. D. Organização estrutural das folhas de *Baccharis dracunculifolia* DC. Asteraceae. **Revista Brasileira de Farmácia**, v. 89, p. 272-275, 2008.

PEREIRA, C. B. et al. Chemical composition and biological activities of *Baccharis milleflora* essential oil. **Latin American Journal of Pharmacy**, v. 35, p. 2225-2233, 2016.

PERRY, N. B. et al. Essential oils from dalmatian sage (*Salvia officinalis* L.):

variations among individuals, plant parts, seasons, and sites. **Journal of Agricultural and Food Chemistry**, v. 47, p. 2048-2054, 1999.

PIRBALOUTI, A.G.; MAHDAD, E.; CRAKER, L. Effects of drying methods on qualitative and quantitative properties of essential oil of two basil landraces. **Food Chemistry**, v. 141, p. 2440-2449, 2013.

PIRES, P. P.; WENDLING, I.; BRONDANI, G. Ácido indol butírico e ortotropismo na miniestaquia de *Araucaria angustifolia*. **Revista Árvore**, v. 37, p. 393-399, 2013.

QUEIROGA, C. L. et al. Comparison of the chemical composition of the essential oil and the water soluble oil of *Baccharis dracunculifolia* DC. (Asteraceae). **Journal of Essential Oil Research**, v. 20, p. 111-114, 2008.

R CORE TEAM. **R: A language and environment for statistical computing**. R Foundation for Statistical Computing, Vienna, Austria, 2019.

RADOUKOVA, T. et al. Differences in essential oil yield, composition, and bioactivity of three juniper species from Eastern Europe. **Industrial Crops & Products**, v. 124, p. 643-652, 2018.

RAHMAN, K. Studies on free radicals, antioxidants, and co-factors. **Clinical Interventions in Aging**, v. 2, p. 219-236, 2007.

RAMAKRISHNA, A.; RAVISHANKAR, G. Influence of abiotic stress signals on secondary metabolites in plants. **Plant Signaling & Behavior**, v. 6, n. 11, p. 1720-1731, 2011.

RASMUSSEN, A. et al. Adventitious rooting declines with the vegetative to reproductive switch and involves a changed auxin homeostasis. **Journal of Experimental Botany**, v. 66, n. 5, p. 1437-1452, 2015.

RASMUSSEN, A.; SMITH, T. E.; HUNT, M. A. Cellular stages of root formation, root system quality and survival of *Pinus elliottii* var. *elliottii* x *P. caribaea* var. *hondurensis* cuttings in different temperature environments. **New Forests**, v. 38, p. 285-294, 2009.

RAVEN, P. H.; EVERT, R. F.; EICHHORN, S. E**. Biology of plants**. New York: W. H. Freeman and Company, 2005.

RE, T. et al. Antioxidant activity applying an improved ABTS radical cation decolorization assay. **Free Radical Biology and Medicine**, v. 26, n. 9-10, p. 1231-1237, 1999.

RIGOTTI, M. ***Baccharis dracunculifolia* D.C.: diversidade genética e química de populações.** 2011. 94 f. Tese (Doutorado em Agronomia - Horticultura) - Faculdade de Ciências Agronômicas, Universidade Estadual Paulista "Júlio de Mesquita Filho", Botucatu (SP), 2011. Disponível em: https://repositorio.unesp.br/bitstream/handle/11449/133997/000858227.pdf?sequence=1&isAllowed=y. Acesso em 04 mai. 2019.

ROBERTO, D. et al. Antioxidant activity of limonene on normal murine lymphocytes: relation to H2O2 modulation and cell proliferation. **Basic & Clinical Pharmacology & Toxicology**, v. 106, p. 38–44, 2009.

ROBERTS, E. H. Predicting the storage life of seeds. **Seed Science and Technology**, v. 1, p. 499-514, 1973.

ROBINSON, K. M. et al. *Populus tremula* (European aspen) shows no evidence of sexual dimorphism. **BMC Plant Biology**, v. 14, p. 1-14, 2014.

RODRIGUES, D. M. ***Baccharis dracunculifolia:* formação de pasto apícola, estudos das interações com *Apis mellifera* e insetos galhadores na produção de própolis verde**. 2017. 26 f. Dissertação (Mestrado em Produtos Naturais e Sintéticos) - Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto (SP), 2017. Disponível em: https://www.teses.usp.br/teses/disponiveis/60/60138/tde-22052018-150650/publico/Dissertacao_corrigida_simplificada.pdf. Acesso em 05 jul. 2019.

RUBERTO, G.; BARATTA, M. T. Antioxidant activity of selected essential oil components in two lipid model systems. **Food Chemistry**, v. 69, p. 167-174, 2000.

SALAZAR, G. J. T. et al. Phytochemical characterization of the *Baccharis dracunculifolia* DC (Asteraceae) essential oil and antibacterial activity evaluation. **Industrial Crops and Products**, v. 122, p. 591-595, 2018.

SALEM, N. et al. Variation in chemical composition of *Eucalyptus globulus* essential oil under phenological stages and evidence synergism with antimicrobial standards**.** **Industrial Crops & Products**, v. 124, p. 115–125, 2018.

SANTOS, R. F. et al. Composição química e produtividade dos principais

componentes do óleo essencial de *Baccharis dracunculifolia* DC. em função da adubação orgânica. **Revista Brasileira de Plantas Medicinais**, v. 14, p. 224-234, 2012 a.

SANTOS, V. M. C. S. et al. Seasonal variation of vegetative growth, essential oil yield and composition of menthol mint genotypes at southern Brazil. **Bioscience Journal**, v. 28, p. 790–798, 2012 b.

SANTOS, C. D. L.; FIGUEIRA, G. M.; BELINI, C. M. B. Seed conservation methods and evaluation of germination rate in *Baccharis dracunculifolia* DC. (Asteraceae). **Acta Horticulturae**, v. 1125, p. 263-268, 2016.

SANTOS, V. M. C. S. et al. Seasonal variation of vegetative growth, essential oil yield and composition of menthol mint genotypes at southern Brazil. **Bioscience Journal**, v. 28, p. 790–798, 2012.

SANTOS-GOMES, P.C.; FERNANDES-FERREIRA, M. Organ- and season-dependent variation in the essential oil composition of *Salvia officinalis* L. cultivated at two different sites. **Journal of Agricultural and Food Chemistry**, v. 49, p. 2908−2916 ,2001.

SCHONEWALD-COX, M. C. et al. **Genetics and conservation: a reference for managing wild animal and plant populations**. Menlo Park: Benjamin Cummings Publishing Company, 1983.

SELANI, M. M. et al. Wine industry residues extracts as natural antioxidants in raw and cooked chicken meat during frozen storage. **Meat Science**, v. 88, p. 397–403, 2011.

SFORCIN, J. M. et al. *Baccharis dracunculifolia*, **uma das principais fontes vegetais da própolis brasileira**. São Paulo: Unesp, 2012.

SHIMIZU, K. et al. Antioxidative bioavailability of artepillin C in Brazilian propolis. **Archives of Biochemistry and Biophysics**, v. 424, p. 181-188, 2004.

SILVA, E. R. Antioxidant Evaluation of *Baccharis* patens and *Baccharis psiadioides* Essential Oils. **Journal of Essential Oil Research**, v. 21, p. 485-492, 2018.

SILVA, F. A. S.; AZEVEDO, C. A. V. The assistat software version 7.7 and its use in the analysis of experimental data. **African Journal of Agricultural Research**, v. 11, p. 3733–3740, 2016.

SILVA, F. et al. Seasonal variability in the essential oils of wild and cultivated *Baccharis trimera*. **Journal of the Brazilian Chemical Society**, v. 18, n. 5, p. 990-997, 2007.

SILVEIRA, M. A. M.; VILLELA, F. A.; TILLMANN, M. A. A. Maturação fisiológica de sementes de calêndula (*Calendula officinalis* L.). **Revista Brasileira de Sementes**, v. 24, n. 2, p. 31-37, 2002.

SOUZA, J. R. P. et al. Development of holy-thorn under different light intensities and pruning levels. **Horticultura Brasileira**, v. 26, p. 40-44, 2008.

STAUDT, M.; BERTIN, N. Light and temperature dependence of the emission of cyclic and acyclic monoterpenes from holm oak (*Quercus ilex* L.) leaves. **Plant, Cell & Environment**, v. 21, p. 385-395, 1998.

STEFFENS, B.; RASMUSSEN, A. The physiology of adventitious roots. **Plant Physiology**, v. 170, p. 603-617, 2016.

STUEPP, C. A. et al. The use of auxin quantification for understanding clonal tree propagation. **Forests**, v. 8, n. 27, p. 1-15, 2017.

SUZUKI, A. A. Reproductive investment in male and female *Eurya japonica* (Theaceae) at tree and branch levels. **American Journal of Botany**, v. 92, p. 2003-2009, 2005.

SZLISZKA, E. et al. Chemical composition and anti-inflammatory effect of ethanolic extract of Brazilian green propolis on activated J774A.1 Macrophages. **Evidence-Based Complementary and Alternative Medicine**, v. 2013, p. 1-13, 2013.

TAARIT, M. B. et al. Plant growth, essential oil yield and composition of sage (*Salvia officinalis* L.) fruits cultivated under salt stress conditions. **Industrial Crops and Products**, v. 30, p. 333-337, 2009.

TAIZ, L.; ZEIGER, E. **Plant physiology**, Sunderland: Sinauer Associates Inc, 2013.

TEIXEIRA, É. W. et al. Plant origin of green propolis : bee behavior. Plant Anatomy and Chemistry. **Evidence-Based Complementary and Alternative Medicine**, v. 2, p. 85-92, 2005.

TIIVA, P. T, et al. Isoprene emission from a subarctic peatland under enhanced UV-B radiation. **New Phytologist**, v. 176, p. 346–355, 2007.

TINGEY, D. T. et al. Influence of light and temperature on monoterpene emission rates from slash pine. **Plant Physiology**, v. 65, p. 797-801, 1980.

TOMAZZOLI, M. M. et al. Botanical source investigation and evaluation of the effect of seasonality on Brazilian propolis from *Apis mellifera* L. **Scientia Agricola**, v. 77, n.6, 2020.

TORETI, V. C. et al. Recent progress of propolis for its biological and chemical compositions and its botanical origin. **Evidence-Based Complementary and Alternative Medicine**, v. 2013. p. 1-13, 2013.

TORIMARU, T.; TOMARU, N. Relationships between flowering phenology, plant size, and female reproductive output in a dioecious shrub, *Ilex leucoclada* (Aquifoliaceae). **Canadian Journal of Botany Revue canadienne de botanique**, v. 84, p. 1860-1869, 2006.

TURCOTTE, J.; HOULE, G. Reproductive costs in *Salix planifolia* ssp. Planifolia in subarctic Québec, Canada. **Écoscience**, v. 8, n. 4, p. 506-512, 2001.

ULBELEN, A. et al. Terpenoides from *Salvia sclarea*. **Phytochemistry**, v. 36, p. 971-974, 1994.

URBANSKA, K. M. **Differentiation patterns in higher plants**. New York: Academic Press, 1987.

VAITHIYANATHAN, S. et al. Effect of dipping in pomegranate (*Punica granatum*) fruit juice phenolic solution on the shelf life of chicken meat under refrigerated storage (4 °C). **Meat Science**, v. 88 p. 409-414, 2011.

VAN DEN DOOL, H. KRATZ, P. D. A generalisation of the retention index system including linear temperature programmed gas-liquid chromatography. **Journal of Chromatography**, v. 11, p. 463-471, 1963.

VERDI, L. G.; BRIGHENTE, I. M. C.; PIZZOLATTI, M. G. Gênero *Baccharis* (Asteraceae): aspectos químicos, econômicos e biológicos. **Química Nova**, v. 28, p. 85-94, 2005.

VERMA, N.; SHUKLA, S. Impact of various factors responsible for fluctuation in plant secondary metabolites. **Journal of Applied Research on Medicinal and Aromatic Plants**, v. 2, p. 105-113, 2015.

VINHOLES, J. et al. Assessment of the antioxidant and antiproliferative effects of sesquiterpenic compounds in in vitro Caco-2 cell models. **Food Chemistry**, v. 156, p. 204-211, 2014.

VOGT, T. Phenylpropanoid biosynthesis. **Molecular Plant**, v. 3, p. 2-20, 2010.

VRANOVÁ, E.; COMAN, D.; GRUISSEM, W. Network analysis of the MVA and MEP pathways for isoprenoid synthesis**. Annual Review of Plant Biology**, v. 64, p. 665-700, 2013.

WANG, Y.; FREI, M. Stressed food – The impact of abiotic environmental stresses on crop quality. **Agriculture, Ecosystems and Environment**, v. 141, p. 271-286, 2011.

WENDLING, I.; STUEPP, C. A.; ZUFFELLATO-RIBAS, K. C. Rooting of *Araucaria angustifolia*: Types of cuttings and stock plants sex. **Revista Árvore**, v. 40, n. 6, p. 1013-1021, 2016.

XAVIER, A.; WENDLING, I.; SILVA, R. L. **Silvicultura clonal: princípios e técnicas**. Viçosa: Universidade Federal de Viçosa, 2009.

XAVIER, M. N. et al. Chemical composition from essential oil of *Cardiopetalum calophyllum* Schltdl. (Annonaceae) and their antioxidant, antibacterial and antifungal activities. **Revista Virtual de Química**, v. 8, p. 1433-1448, 2016.

YANG, Y. et al. Environmental cues induce changes of steviol glycosides contents and transcription of corresponding biosynthetic genes in *Stevia rebaudiana*. Plant Physiology and Biochemistry, 86, 174-180 (2015).

YU, L.; YAN, J.; SUN Z. D-limonene exhibits anti-inflammatory and antioxidant properties in an ulcerative colitis rat model via regulation of iNOS, COX-2, PGE2 and ERK signaling pathways. **Molecular Medicine Reports**, v. 15, p. 2339-2346, 2017.

ZHELJAZKOV, V.D.; ASTATKIE, T.; JELIAZKOVA, E. Year-round variations in essential oil content and composition of male and female juniper. **Hortscience**, v. 48, n. 7, p. 883-886, 2013.

ZONTA, E. et al. **O Sistema radicular e suas interações com o ambiente edáfico**. Viçosa: Sociedade Brasileira de Ciência do Solo, 2006.

ZUCCOLOTTO, T. et al. Chemical, antioxidant, and antimicrobial evaluation of essential oils and an anatomical study of the aerial parts from *Baccharis* species (Asteraceae). **Chemistry & Biodiversity**, v. 16, Issue 4, 2019.

ZUFFELLATO-RIBAS, K. C.; RODRIGUES, J. D. **ESTAQUIA: Uma abordagem dos principais aspectos fisiológicos**. Curitiba: Universidade Federal do Paraná, 2001.