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organizadores

PESQUISAS AGRÁRIAS E AMBIENTAIS

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APRESENTAÇÃO

As áreas de Ciências Agrárias e Ciências Ambientais são importantes para a humanidade. De um lado, a produção de alimentos e do outro a conservação do meio ambiente. Ambas, devem ser aliadas e são imprescindíveis para a sustentabilidade do planeta. A obra, vem a materializar o anseio da Editora Pantanal na divulgação de resultados, que contribuem de modo direto no desenvolvimento humano.

O e-book "Pesquisas Agrárias e Ambientais Volume III" é a continuação dos e-books Volume I e II com trabalhos que visam otimizar a produção de alimentos, o meio ambiente e promoção de maior sustentabilidade nas técnicas aplicadas nos sistemas de produção das plantas. Ao longo dos capítulos são abordados os seguintes temas: desafios e estratégias da fitorremediação no meio ambiente, composição de óleo essencial das folhas de Qualea grandiflora e Qualea multiflora Mart. e antileishmanial, eventos extremos e o clima no semestre de janeiro a junho de 2020, comportamento reprodutivo e aspectos ecológicos das árvores de um remansescentes em Bandeirantes - PR, maximizando o retorno do investimento em projetos florestais no Norte de Minas Gerais, elementos conceituais da importância dos biofertilizantes líquidos para a agroecologia e análises de anéis etários em escamas e értebras do peixe Brycon falcatus. Portanto, esses conhecimentos irão agregar muito aos seus leitores que procuram promover melhorias quantitativas e qualitativas na produção de alimentos e do ambiente, ou melhorar a qualidade de vida da sociedade. Sempre em busca da sustentabilidade do planeta.

Aos autores dos capítulos, pela dedicação e esforços sem limites, que viabilizaram esta obra que retrata os recentes avanços científicos e tecnológicos na área de Ciência Agrárias e Ciências Ambientais Volume III, os agradecimentos dos Organizadores e da Pantanal Editora. Por fim, esperamos que este e-book possa colaborar e instigar mais estudantes e pesquisadores na constante busca de novas tecnologias e avanços para as áreas de Ciências Agrárias e Ciências Ambientais. Assim, garantir uma difusão de conhecimento fácil, rápido para a sociedade.

Alan Mario Zuffo
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SUMÁRIO

Capítulo II

Composition of the essential oil of leaves Qualea grandiflora and Qualea multiflora Mart. and antileishmanial activities

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INTRODUCTION

The Cerrado

The Cerrado occupies a large area of the country once it is the second largest biome in the country surpassed in area by Amazon. It covers the states of Goiás, the Federal District, Minas Gerais, Tocantins, Piauí, Mato Grosso, Mato Grosso do Sul and part of Paraná, Bahia, Ceará, Maranhão, Rondônia, Roraima, Amazonas, Pará and São Paulo. The Cerrado houses a flora in which many species have food and / or medicinal value (God, 2011; Pereira et al., 2012). Despite the ecological and economic importance, it has gradually been devastated mainly due to forms of occupation and the use of natural resources in a disorderly way, that leads to a process of degradation of its natural environment. If rational utilization measures are not taken, it may disappear altogether by 2030. Most optimistic visions suggest that this biome can shrink around 8%, a loss of approximately 160 square kilometers by 2050 (Machado et al., 2004; Sassine, 2009).

Moreover, the human factors change the environment, due to the increase of the population that requires more areas for food grain crops for export. There are also environmental factors of climate change, such as prolonged drought or excessive rainfall and even poorly distributed rainfall, warmer

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winters and drier that influence negatively the loss of the Cerrado typical vegetation. Thereby, so many plants which have medicinal and pharmacological potential cannot be tested and used as feedstock for the study and development of drugs for specialized industries. Many studies still have done concerning the medicinal plants of the cerrado biome. The possible production of pharmaceuticals from plant essential oils as well as their production and marketing can contribute to the knowledge and the possible introduction of new products in the market from Brazilian flora.

Thus, the Cerrado has a very special feature for Brazil, for its vast expanse of land, a very rich flora and due to the threat of devastation, can represent a huge loss of a large load of promising pharmacological properties (Carvalho et al., 2007). The conversion of natural areas of the Cerrado biome in agricultural fields and the impounding of water have reduced a lot its area without the knowledge of existing and its chemical constituents, especially when it is compared to the diversity and the occupied area. A smaller number of information on the flora of the Brazilian savannah involves a great loss of knowledge about the use of plants for medicinal use since it is estimated that about 40% of the biome has been ravaged and the Cerrado has only about 1.5% of its area protected by law. Nowadays, the Cerrado is at greater risk of being extinct in the country (Neto et al., 2003). The species, the object of this study are examples of this biome and they should be preserved because there are a few literary studies, therefore it is important the conservation of these individuals as well as entire biome. A literature review indicated that many studies have not found related to the chemical composition of the essential oils and biological activities of species *Qualea multiflora* and *Qualea grandiflora*. Therefore, the development of this work is extremely valuable for new applications vegetal resources in the Cerrado.

About Leishmaniasis

Leishmaniasis is a group of parasitic diseases transmitted of global distribution by the bite of about 30 species of phlebotomine (popularly known as sandfly) infected with *Leishman* protozoa. It is estimated that two million new cases occur each year worldwide, of which 1.5 million are cases of cutaneous leishmaniasis, a type of this disease. Leishmaniasis is transmitted by the mosquito and infects dogs and human beings, especially children, elders and people with chronic diseases.

Leishmaniasis is a severe, chronic and fatal disease to human beings with high mortality if untreated. It is a chronic cutaneous or visceral manifestation caused by protozoa of the genus *Leishmania* and it is a common disease to man and the dog (WHO, 2007).

There are studies that use plant extracts to combat this disease. Many dogs are euthanized due to the effects of the disease, but there could be treatments to avoid this radical attitude.

Genre Qualea, Vochysiaceae Family

The genus *Qualea* belongs to the family of Vochysiaceae. This genus has two subfamilies, six genera and about 200 species of tropical trees, of which three of them are common in the Brazilian cerrado: *Q. grandiflora, Q. multiflora.* Locals use *Qualea grandiflora and multiflora* as folk medicine treatments for external ulcers, gastric diseases and inflammation (Santos, 2006). It was identified in the family of Vochysiaceae some organic compounds such as flavonoids, triterpenoids, steroids, tannins, benzoquinones, anthraquinones (Ayres et al., 2008).

It was done phytochemical studies with genre *Qualea* and it was identified fatty acids, polysaccharides, tannins, pyrogallic and catéchutaniccompounds, flavonoids, terpenoids and ellagic acid derivatives (Nasser et al., 2013).

Species Studied

The *Qualea grandiflora* of the genus *Qualea*, from the Vochysiaceae called pau-terra-largo is a tree found in vegetation type or habitat such as edge of gallery forest, edge of ciliated forest, cerradão, cerrado (strict sense), dirty field, field with murundus, Amazon savannah and carrasco. It is distributed through the following states: Pará, Amazonas, Acre, Maranhão, Piauí, Ceará, Bahia, Mato Grosso, Goiás, Minas Gerais, São Paulo and Paraná. We can note that has greater distribution in the Brazilian space compared with other species in the study (Ministry of Environment, 2011).

The *Qualea multiflora* is found in vegetation type or habitat called edge of gallery forest, cerradão (strict sense), vereda and field with murundus. It is distributed for nine states: Maranhão, Piauí, Bahia, Mato Grosso, Goiás, Minas Gerais, São Paulo, Rio de Janeiro, Paraná and the Federal District (Ministry of Environment, 2011).

The species *Q. multiflora* is a tree 4-6 meters with 15 to 25 cm in diameter, large distribution by Cerrado, with more occurring in several states. It is commonly known as pau-terra-liso, boizinho, pau-terra, bagre, cinzeiro. The species has a woody scale. It is used in folk medicine in the treatment of ulcers, gastritis, amebiasis, bloody diarrhea, intestinal cramps and inflammation (Lorenzi, 2002).

The *grandiflora* also known as Ariauá (PA), pau-terra-do-campo, pau-terra-do-cerrado, pau-terra-folha-grandeis a very ornamental tree, with branches usually thick, crooked trunks and rough bark. It is found from the Amazon to São Paulo, Minas Gerais, Goiás and Mato Grosso do Sul, reaching 20 meters in height with crooked trunk and thick bark. In folk medicine, the bark is used to heal wounds and inflammations. The leaves are used for diarrhea, cramps and fighting amebiasis (Lorenzi, 2002).

The *Q. grandiflora* usually has much larger leaves and fruits than the species *Q. multiflora* and has yellow flowers. However, the flowers of the species *Qualea multiflora* are yellow and white with red spots, so they are *multiflora*, according to the Digital Herbarium Pirenópolis (Herbário, 2014).

MATERIALS AND METHODS

Data Collection

The collection was made at random and from different individuals, close to each other. The leaves *Qualea grandiflora* and *Qualea multiflora* were collected close to the highway 230 MG (19° 13' 39.06" S, 46° 12' 30.95" W), in Rio Paranaíba-MG, situated approximately 300 km away from Uberlândia - MG and transported to the Laboratory of Natural Products, Federal University of Uberlândia (UFU) - Campus Santa Monica, for then the vouchers are prepared, identified by an expert (Professor Glein Monteiro de Araújo Institute Science Biology UFU), and deposited with the proper registration in Uberlandense Herbarium of the Federal University of Uberlândia (HUFU) under the following numbers: 66,895 HUFU *Qualea grandiflora* and *Qualea multiflora* HUFU 66,896.

Preparation and obtaining essential oil from leaves

The fresh leaves of *Qualea multiflora* and *Qualea grandiflora* were cut and submitted to distillation by steam drag using the modified Clevenger for a period of 4 hours device (Morais et al., 2009).

For the essential oil extraction, the extract was used with dichloromethane and submitted to liquid-liquid partition separating funnel, performing three washes of the extract with three portions of dichloromethane. The organic fractions were combined, dried and the solvent evaporated at room temperature. The oil was collected and conditioned in a flask and kept under refrigeration at low temperature, approximately -18.0 ± 5 ° C until the biological assay.

After evaporation of the dichloromethane, dried at a temperature of 35 ° C, the mass obtained from the essential oil obtained was weighed on an analytical balance and the corresponding percentage of essential oil extracted from leaves was calculated on the mass of the sample used initially, disregarding moisture content of green leaves not to be considered as mass of essential oil.

Separation, identification and analysis of the essential oil constituents by chromatography GC/MS

The separation and identification of volatile constituents by chromatography coupled to mass spectrometry (GC / MS) mass were performed in gas in an apparatus of Shimadzu GC17A/QP5010. The temperature program used in a DB-5 column (Supelco SPB-5) of 30 meters in length, 25 mm in inner diameter and 0.25 mm thick film, the heating ramp was 60-246 ° C (3 ° C min^{-1}); injector in split mode 1:20 to 220 ° C; Helium carrier gas flow of 1.0 mL min^{-1}. The interface temperature, ion source and detector were 220-240° C. The volume of 1.0 μl of sample (diluted in dichloromethane essential oil at a concentration of 10 mg/mL) was injected, and the identification of compounds was performed by means of mass spectra libraries Wiley (7, 139 and SHIM2205/Adams). Arithmetic Index (AI) was used and compared with the reference Arithmetic Index calculated. The mass detector was operated with impact energy of 70 eV and fragments 40-650 Da were obtained (Adams, 2007).

Test Activity Antileishmanial Sample preparation

The assays of activity against *Leishmania*in the analysis of cytotoxicity and analysis of cytotoxic activity are from the Institute of Biomedical Sciences, Laboratory of Trypanosomatids the Federal University of Uberlândia with the support of Prof. Dr. Claudio Vieira da Silva, Institute of Biomedical Sciences, UFU (Federal University of Uberlândia), and helps the student initiation Paulla Vieira Rodrigues.

For the test with *Leishmania amazonensis* samples are dissolved in methanol and diluted with BHI, until it forms a stock solution of 640 mg L^{-1}. The final methanol concentration of the mother liquor does not exceed 3%.

Cell viability test

The cell viability test is performed by microdilution in a 96 well plate, from the mother solution, and the dilutions are done with a BHI culture for *L. amazonensis,*by obtaining the concentrations to be tested. The final volume of each well will be 100 μL, 20 μL the measure of the inoculum (1,108 parasites in a solution containing 2 mL) at concentrations of 80 μL of simple A structure of a 96 well plate.

The plate *Leishmania amazonensis* is incubated for 48 hours at 25 ° C. 2 μL. Then it is added to each well of a developing solution of 3m Mresazurin in PBS and incubated again under the same conditions. Then, the absorbance at 594 nm in a microplate spectrophotometer is performed.

All assays were performed in triplicate, and each assay and each concentration tested in triplicate. From the results of absorbance, cell viability is calculated as the positive control, through these ones it will be constructed a graph of dose-response for the nonlinear regression for the calculation of EC_{50} (concentration in which 50% of the parasites were inhibited) (Chibale et al., 2007).

Cell Culture

The culture of ATCC CCL 81 Vero cells (kidney fibroblasts of African green monkey) is maintained in DMEM, at 37° C in humidified atmosphere and 5% CO_2.

Statistical Analysis

All determinations were made in triplicate and the results were an arithmetic mean with standard deviation. The means were analyzed statistically by the Tukeytest for the results of *Leishmania* and cytotoxicity assay in 5% of probability. P values ≤ 0.05 were considered to denote a statistically significant difference.

Analysis of the compounds of the essential oils from leaves of Qualeamultiflora and Qualea grandiflora

It is low the yield of essential oils from leaves of *Qualea grandiflora* and *Qualea multiflora*. It was analyzed the chemical composition of essential oils and it was observed the presence of the following percentages of compounds for *Qualea multiflora* species: oxygenated monoterpenes (29.3%) also highlighted the presence of alcohols (19.5%), aldehydes (12.2%) and ketones (7.3%). For *Qualea grandiflora* species, the most numerous compounds are oxygenated sesquiterpenes (36.6%), oxygenated monoterpenes (12.2%) and aldehydes and carboxylic acids that have 4.9% each.

Table 1. Percentage of compounds per organic function.

Functional groups	***Q.grandiflora* (%)**	***Q.multiflora*(%)**
Alcohol	2,4	19,5
Aldehyde	4,9	12,2
Carboxylic Acid	4,9	-
Ketone	2,4	7,3
Oxygenated diterpene	4,9	2,4
Ester	2,4	2,4
Cyclicether	2,4	-
Sesquiterpene	2,4	-
Sesquiterpene oxygenated	36,6	4,9
Oxygenated monoterpene	12,2	29.3
Monoterpene	2,4	-
Nitrile	-	2,4
N.I.	22	19,5
Total	99,99	99,99

The Table 2 below cites the compounds in a higher percentage of peak area of essential oil from leaves of both species.

Table 2. The table below shows the compounds identified in the essential oil from leaves of *Qualea multiflora* and *grandilfora*. NI = Not identified; TIC = Total ion chromatogram; AI = arithmetic index; RT = retention time in min. [a] NIST Chemistry Web Book: http://webbook.nist.gov/chemistry/ [b] Adams.

Compound	RT		AI reference		AI calculated		Composition % TIC	
	Q.m.	***Q.g.***	***Q.m.***	***Q.g.***	***Q.m.***	***Q.g.***	***Q.m.***	***Q.g.***
(*Z*)-Hex-3-en-1-ol	4,34	4,32	850[b]	850[b]	855	854	13,16	1,86
Phenylacetaldehde	9,66	9,63	1042[a]	1042[a]	1046	1045	16,85	7,32
Linalool	11,81	11,79	1102[a]	1102[a]	1103	1103	10,31	1,04
Cis-linalool oxide	10,81	10,73	1077[a]	1076[a]	1077	1075	3,15	1,44
Alpha-terpineol	15,75	15,73	1191[a]	1186[b]	1197	1196	3,76	4,7
Vitispirane	19,53	19,51	1272[a]	1272[a]	1282	1282	3,46	4,77
(*E*)-nerolidol	31,54	31,55	1566[a]	1569[a]	1568	1568	2,08	13,18
Benzoate-3*Z*-hexenyl	-	31,99	-	1568[a]	-	1577	-	3,37
(*E*)-2-decen-al	18,71		1263[a]	-	1264	-	3,89	-
(2*E, 6E*)-farnesol	-	37,71	-	b 1742	-	1730	-	4,79
N-hexadecanoic	**-**	46,06	-	1971[a]	-	1970	-	3,34

RESULTS AND DISCUSSION

Results Antileishmaniose Activity

The Table 3 below presents the results of Vero cell cytotoxicity assay using antiprotozoal the parasite *Leishmania amazonensis* are presented samples of essential oils leaves *Qualea multiflora* and *Qualea grandiflora* were used.

Table 3. Results of Cytotoxicity and antileishmanial activity in essential oil of leaves

Sample	CI_{50} (μgmL^{-1}) Vero Cell	CI_{50} (μgmL^{-1}) *Leishmania amazonenses*
Q. grandiflora	>512	88 ± 8
Q. multiflora	>512	69 ± 4
amphotericin B	-	0,288±0,006

These results indicate that, for the essential oils from leaves 69 μgmL^{-1} of *Qualea multiflora* oil and 88 μgmL^{-1} of *Qualea grandiflora* oil are the needed concentrations to kill or inhibit the action of 50% of the parasite.

If the IC_{50} values are lower, the antileishmanial activity of the sample will be better, the is to say, sample of essential oil of leaves *Qualea multiflora* has a better response against the parasite than for samples of essential oil of leaves *Qualea grandiflora*, compared to the control drug, the result is not so promising.

When the leaves ethanol extracts of both species are analyzed, the ability to inhibit the parasite does not have as effectively as the essential oils from leaves.

Nevertheless, for the test on Vero cells and once it is related with the cytotoxicity, if the IC_{50} value is higher, the result will be better, by indicating that these samples have low cytotoxicity towards Vero cells.

The results of this study, with low cytotoxicity to Vero cells, contradict other work that quotes about the toxicity of materials derived from plants *Qualea grandiflora* and *Qualea multiflora*.

It was tested cytotoxic activity and antileishmanial activity against *Leishmania amazonensis*, by using essential oils from leaves of *Vernonialeopoldii* and the IC_{50} values found were 378 μmL^{-1} and 210 μmL^{-1}. Martins (2012) indicating promising results for the essential oils of *Vernonialeopoldii*, but worse than the two kinds of study of this work. For the cytotoxicity assay, the higher the IC_{50} value is, the better are the result, indicating that these samples have low cytotoxicity against Vero cells, but the essential oils of *Qualeas* have better results, especially *Qualea multiflora*.

Chemical compounds of benzenoids family were found in essential oils from leaves *Licaria canella*. Essential oil from leaves of this species was more active against strains of *Leishmaniaamazonensis* (Santos et al., 2012).

Essential oils from aerial parts of *Mitracarpus frigidus* showed significant antileishmanial activity against the forms *Leishmania major* and *Leishmania amozonensis* (Fabri et al., 2012).

Traditional medicines to treat leishmaniasis are toxic and have side effects, and derived from natural products can be a better alternative because they have low toxicity to Vero cells (Costa, 1998).

The antiparasitic activity of some types of essences oils is cited in the literature and according to the chemical composition of essential oil, this can have a greater or lesser power activity against *Leishmania*. Insulation can be made in the future in order to discover which chemicals compounds of essential oils of leaves have antileishmanial activity.

CONCLUSION

The essential oils of the plants studied showed good results in testing of antileishmanial activity, once the oil of species *Q. multiflora* showed better results for antileishmanial activity against *Leishmania amazonensis,* with lower cytotoxicity for Vero cells than oils *Q. grandilfora*.

The yield of essential oils from leaves of *Qulaea grandiflora* and *Qualea multiflora*is low and it was analyzed the chemical composition of essential oils and it was observed the presence of the following percentages of the major compounds by functional groups for *Qualea multiflora* species: oxygenated monoterpenes (29.3%) also it was highlighted the presence of alcohols (19.5%), aldehydes (12.2%) and ketones (7.3%). For *Qualea grandilfora* species compounds are most numerous oxygenated sesquiterpenes (36.6%), oxygenated monoterpenes (12.2%), the aldehydes (4.9%) and carboxylic acids (4.9%) each.

The major compounds identified in the oils were greater in area *Q. multiflora*: (*Z*)-Hex-3-en-1-ol, phenylacetaldehyde, linalool, alpha-terpineol and (*E*)-2-decen-al. For *Q. grandiflora*: phenylacetaldehyde, vitispirano, (*E*)-nerolidol, benzoate 3-Z-hexenyl.

These compounds present in the oil can be tested separately for the verification of biological activities in future work. The compounds can be synthesized or used as precursors of more effective drugs to combat diseases.

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ÍNDICE REMISSIVO

SOBRE OS ORGANIZADORES

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