

Extraction and identification of essential oil components from the leaves of *Syzygium malaccense* (L.) MERR. & L.M. PERRY, MYRTACEAE

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ABSTRACT

Popularly known as red jamb, the species *Syzygium malaccense* (L.) Merr. & L.M. Perry is an Asian tree from India, Malaysia, Vietnam and Thailand that has adapted to the climate and soil of the North, Northeast and Southeast of Brazil. The antioxidant and anti-inflammatory activities of this species have already been cited in the literature. This study aimed to extract and identify the essential oil constituents of the leaves of this species. The leaves were dried and ground; and the essential oil was extracted by Clevenger apparatus. The essential oil presented yield of 0.03%, containing monoterpenes and sesquiterpenes and the major compounds were identified as spatulenol, sesquisabinene transhydrate and (E)-Caryophyllene.

Keywords: Spatulenol; Sesquisabinene transhydrate; (E)-Caryophyllene

RESUMO

Popularmente conhecido como jambo vermelho, a espécie *Syzygium malaccense* (L.) Merr. & L.M. Perry é uma árvore asiática da Índia, Malásia, Vietnã e Tailândia que se adaptou ao clima e ao solo do norte, nordeste e sudeste do Brasil. As atividades antioxidantes e anti-inflamatórias dessa espécie já foram citadas na literatura. Este estudo teve como objetivo extrair e identificar os constituintes do óleo essencial das folhas desta espécie. As folhas estavam secas e moídas; e o óleo essencial foi extraído pelo aparelho de Clevenger. O óleo essencial apresentou rendimento de 0,03%, contendo monoterpenos e sesquiterpenos e os principais compostos foram identificados como espatulenol, Trans-hidrato de tesquisabineno e (E)-cariofileno.

Palavras-chave: Espatulenol; Trans-hidrato de Sesquisabineno; (E)-cariofileno

[F1]

[F1] Texto adicionado a pedido do editor

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1 INTRODUCTION

Myrtaceae is one of the most important Angiospermae families in Brazil (CRUZ; KAPLAN, 2004) and has wide economic use in food, ornamental, timber and as a source of energy (firewood) (LEUCENA *et al.*, 2014). Many species of the Myrtaceae family are present in the daily life of Brazilians such as guava, pitanga, araçá and jaboticaba, that are consumed fresh or jam, are rich in vitamins and minerals, are aromatic and serve as inspiration for fragrances and cosmetics (EMBRAPA, 2012; CASTILHOS *et al.*, 2017).

Syzygium malaccense is an Asian tree, located especially in the region of India, Malaysia, Vietnam and Thailand and has adapted to the climate and soil of the North, Northeast and Southeast of Brazil (REATGUI, 2015; FERNANDES; RODRIGUES, 2018). Studies report the antioxidant potential of edible parts of fruits, anti-inflammatory and antioxidant activities in hydroalcoholic and aqueous extracts of their leaves. Secondary metabolites such as catecholamines, quercetin, carotenoids and flavonoids were found in leaves (aqueous and hydroalcoholic extracts) (BATISTA *et al.*, 2017).

GC-MS analyzes of the essential oil of fresh leaves of *Syzygium malaccense* showed that it was composed mainly of monoterpenes characterized mainly by α -pinene, β -pinene, p-cymene and α -terpineol. The sesquiterpenes constituting the oil have β -caryophyllene as the main component (KARIOTI *et al.*, 2007).

This article aims to obtain the essential oil of *Syzygium malaccense* leaves, identify its components and compare it with compositions found in other parts of the world.

2. MATERIALS AND METHODS

The species *Syzygium malaccense* was collected between July and August 2018, at the Paraná Agronomic Institute, Morretes unit, Paraná coast, (Latitude 25°30'6"S; Longitude 48°48'5.22"O). The identification of the vegetal species was made by

comparison with the exsiccata registered at the MBM Herbarium of the Botanical Museum of the Curitiba City Hall, under the number MBM-379581.

After collection, the botanical material was separated into leaves and stem, the leaves were selected as object of study, cleaned with drinking water, dried in the shade and ground.

2.1 Obtaining the essential oil

The essential oil of *Syzygium malaccense* (L.) Merr. & L.M. Perry leaves was obtained by means of the Clevenger apparatus modified by Wasicky (1963). Extraction was performed by water hydrodistillation, based on the technique described in the Brazilian Pharmacopoeia 5th edition (BRASIL, 2010). In a round bottom flask was added 600 g of plant material (dried leaves, crushed and kept in tightly sealed plastic bags at a temperature below 8 ° C), adding sufficient distilled water to cover the material and allow extraction (4500 mL). The balloon was coupled to Clevenger apparatus and kept under heating blanket with approximate temperature of 100°C for 6 consecutive hours.

After cooling the extraction system at room temperature, the essential oil volume was read directly on the Clevenger apparatus separator tube scale, and then the milliliter (mL%) yield of essential oil per 100 g of the drug was calculated (Equation 1).

$$\text{Yield(\%)} = \frac{\text{Volume obtained of essential oil (mL)}}{\text{Mass of ground vegetable material (g)}} \times 100$$

The obtained oil was conditioned and kept in a freezer, protected from light, to avoid its volatilization and degradation.

2.2 Identification of Essential Oil Constituents - Chemical analysis

The characterization of the essential oil components of *Syzygium malaccense* leaves was performed by mass spectrum coupled gas chromatography (Laboratory of Chemical Ecology and Synthesis of Natural Products of the Department of Chemistry of UFPR).

Analysis was performed using a Shimadzu CGMS-QP2010 Plus system equipped with a quadrupole mass detector with a Rtx-5MS (Crossbond 5% diphenyl/95% dimethylpolysiloxane) low-bleeding column (30m × 0.25mm×0.25 µm), with helium as the carrier gas at a flow rate of 1.02mL/min. 1 µL of the sample was injected splitless at an initial oven temperature of 60 °C. The injector and detector temperatures were adjusted to 250 °C. The programmed oven temperature was 60–250 °C at 3 °C/min.; EIMS: electron energy, 70 eV; ion source temperature and connection parts at 180 °C (SILVA, *et al.*, 2019).

2.2.1. Peak identification

Individual components were identified by comparing retention indices (RIs) and mass spectra with those of authentic compounds given in Adams Libraries of mass spectral data and by a computer database using Wiley 275, NIST 21, NIST 10733 (ADAMS, 2005; NIST, 1998).

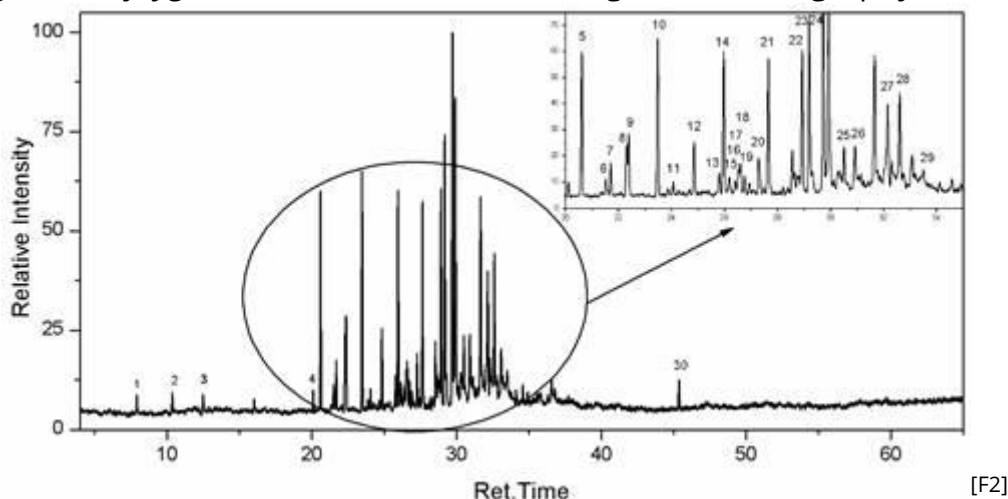
3 RESULTS AND DISCUSSION

3.1 Identification of essential oil constituents

Essential oils are widely used in the pharmaceutical and cosmetic industries because they have widely known medicinal properties (LAWAL *et al.*, 2014). Differences in the composition of essential oil alter its pharmacological properties, and chemical analysis of the composition of essential oils is important (REZENDE *et al.*, 2013).

The plant material was subjected to hydrodistillation in Clevenger apparatus. The extracted oil had a golden-yellow color with a mild odor and the yield was 0.03%. In previous studies the yield presented was 0.15% (KARIOTI *et al.*, 2007) and 0.0297% (ISMAIL *et al.*, 2010), both using fresh leaves of *Syzygium malaccense*.

The resulting analysis chromatogram is shown in figure 1.

Figure 1 - *Syzygium malaccense* essential oil gas chromatography

The chemical composition of the essential oil, its respective times and retention rates, classification and percentage are described in Table 1.

Table 1 - *Syzygium malaccense* essential oil composition

Number ^[F3]	Retention Time	AI*	AI Theoretical	Compound	[]%	Rating
1	7.915	1026	1024	Limonene	0.47	Monoterpene
2	10.370	1097	1095	Linalool	0.51	Oxygenated monoterpene
3	12.495	1151	1148	Menthone	0.64	Monoterpene
4	20.110	1335	1335	Elemene	0.61	Sesquiterpene
5	20.610	1347	1348	α -Cubebene	5.78	Sesquiterpene
6	21.515	1369	1373	α -Ylangene	0.60	Sesquiterpene
7	21.700	1373	1374	α -Copaene	1.27	Sesquiterpene
8	22.295	1388	1400	Sibirene	1.88	Sesquiterpene
9	22.375	1390	1389	β -Elemene	2.33	Sesquiterpene
10	23.475	1417	1417	E - Caryophyllene	6.56	Sesquiterpene
11	24.065	1431	1434	gamma - Elemene	0.54	Sesquiterpene
12	24.855	1451	1452	α -Humulene	2.04	Sesquiterpene
13	25.805	1474	1478	gamma - Muurolene	0.92	Sesquiterpene
14	25.960	1478	1430	β -Copaene	5.51	Sesquiterpene
15	26.170	1483	1458	allo - Aromadendrene	0.61	Sesquiterpene
16	26.390	1489	1513	gamma - Cadinene	0.45	Sesquiterpene
17	26.510	1492	1502	gamma - Patchoulene	0.71	Sesquiterpene
18	26.595	1494	1474	10 - epi - β - Acoradiene	1.05	Sesquiterpene

[F2] Figura revisada a pedido do editor, com numeração e maior resolução

[F3] Essa coluna foi inserida para esclarecer a figura 1

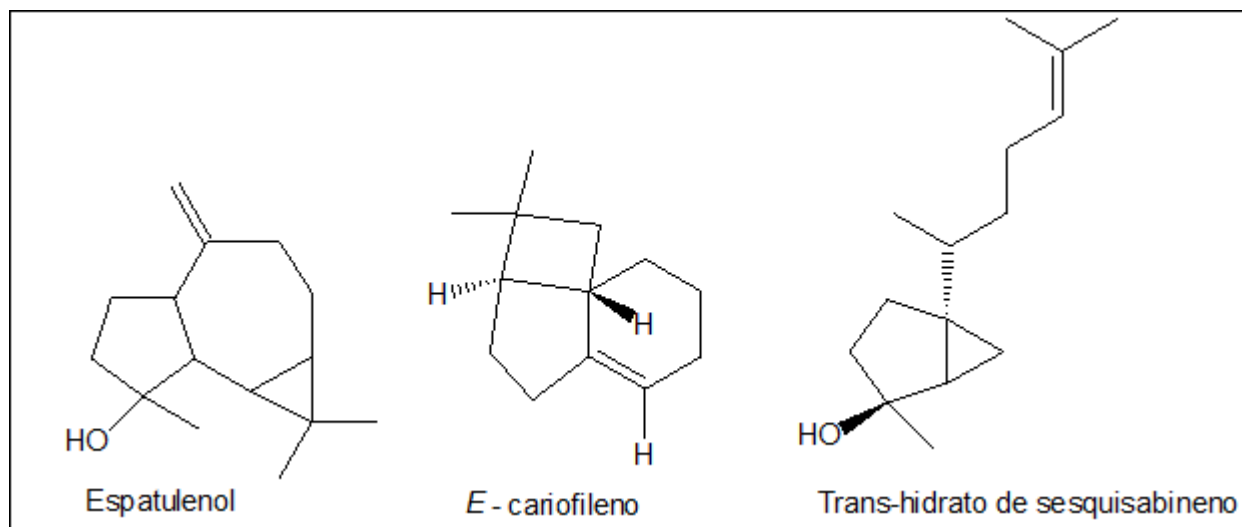
19	26.745	1498	1500	α - Muurolene	0.57	Sesquiterpene
20	27.280	1512	1513	gamma - Cadinene	1.73	Sesquiterpene
21	27.650	1521	1522	delta - Cadinene	5.27	Sesquiterpene
22	28.930	1555	1509	α - Bulnesene	5.98	Sesquiterpene
23	29.190	1562	1577	Trans-sesquisabinene hydrate	7.00	Sesquiterpene
24	29.720	1576	1577	Spathulenol	10.25	Oxygenated sesquiterpene
25	30.500	1596	1600	Guaiol	1.22	Oxygenated sesquiterpene
26	30.925	1607	1582	Caryophyllene oxide	2.14	Oxygenated sesquiterpene
27	32.155	1641	1638	epi - α - Cadinol	4.92	Oxygenated sesquiterpene
28	32.610	1653	1652	α -Cadinol	3.84	Oxygenated sesquiterpene
29	33.505	1678	1676	Mustakone	0.58	Sesquiterpene
30	45.370	2034	2042	Kaurene	0.83	Sesquiterpene
Total compounds identified					76.81%	

* Concentrations above 0.4% have been identified.

The GC/MS analysis of the essential oil of *Syzygium malaccense* leaves identified 37 components, 30 terpenes (monoterpenes and sesquiterpenes) corresponding to 76.81% of the components present in the analyzed essential oil. Of the identified terpenes, two were monoterpenes (1.44%), one oxygenated monoterpene (0.66%), 21 sesquiterpenes (68.76%) and 5 oxygenated sesquiterpenes (29.12%).

The major compounds found were oxygenated sesquiterpene spatulenol (13.34%), sesquiterpenes sesquisabinene transhydrate (9.11%), (*E*) -caryophyllene (8.54%). The molecular formula of these compounds is shown in FIGURE 2.

Figure 2 - Chemical structures of major constituents of essential oil of leaves of *Syzygium malaccense*.



In the study by Santos (2016) with fresh leaves of *Syzygium malaccense* 25 substances were identified, the sesquiterpenes were 16.95% of the extracted compounds and the major compounds were β -selinene (3.85%) and α -humulene (3.63%). The chemical pattern was similar to the study presented by Ismail *et al.* (2010), but differing in the identity of the major compounds.

Unlike the results found in the present study, Karioti *et al.* (2007), described that the essential oil extracted from the fresh leaves of a species cultivated in Ile-Ife (Nigeria) presented 38 substances, being the monoterpenes the majority compounds (61.1%), represented mainly by para-cymene (13.5 %), β -pinene (8.0%), α -terpineol (7.5%) and α -pinene (7.3%). Sesquiterpenes made up 30.8% of this essential oil, whose main component was β -caryophyllene (9.0%). A chemical pattern very similar to the previous one was reported by Lawal *et al.* (2014) who also analyzed fresh leaves grown in Nigeria (Ikotun region), the essential oil obtained was rich in monoterpenes (79.9%), but differed from Karioti *et al.* (2007) in the identity of the majority, represented by limonene (48.8%) and α -terpinene (26.2%).

In the study by Ismail *et al.* (2010) the analyzed species was cultivated in Malaysia, 180 substances were identified in the oil obtained, the major compounds were identified as hexanoic acid (12.16%), methyl salicylate (8.27%), 3-hexen-1-ol (7.81%), 1-octen-3-ol (5.89%), n-hexadecanoic acid (5.07%), 2-hexenal (4.89%) and 3-buten-2-one (3, 68%).

Morais *et al.* (1996) also explains that the composition of the essential oil may be different due to chemotypes, where the chemical constitution developed by a given plant occurs due to its necessity to adapt to the environmental factors of the ecosystem where it is found, such as climate, soil composition, altitude, water scarcity, light, as well as variations that occur due to the time of harvest and the method of extraction of the essential oil. These variables influence the relative concentration of each component in the oil (MAGINA *et al.*, 2009).

Lopes (2008) demonstrated in his work that secondary metabolites vary during the seasons, as well as genetic differences (CERQUEIRA *et al.*, 2009) and climatic factors such as temperature, water availability, soil nutrients, altitude, radiation, among others, which could explain the variations found in the essential oils obtained from the leaves.

3.2 Major constituents of *Syzygium malaccense* essential oil

Caryophyllene is a natural bicyclic sesquiterpene that is a constituent of many essential oils, including *Syzygium aromaticum* (clove). It is a sweet-tasting compound that can be found in foods such as allspice, fig and marjoram, which makes β -caryophyllene a potential biomarker for these products (NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION, 2019).

Positive results have already been found in the literature to evaluate the anti-mutagenicity of natural sesquiterpene β -caryophyllene and its metabolite β -caryophyllene oxide (NGUYEN *et al.*, 2017; ZHANG *et al.*, 2017; GIACOMO *et al.*, 2018).

In other studies treatment with β -caryophyllene caused hypolipemic effect, improved hepatic oxidative damage, reduced cardiac lipid content, was able to improve antioxidant / oxidant status in cardiac tissue of hypercholesterolemic animals; showing antioxidant properties, preventing lipid oxidative damage and improving the activity of GPx, an important enzyme linked to the prevention of atherosclerosis (BALDISSERA *et al.*, 2017).

The effect of β -caryophyllene on hyperglycemia-mediated oxidative and inflammatory stress was observed in streptozotocin-induced diabetic rats. Oral

administration of β -caryophyllene rescued pancreatic β cells, mitigating hyperglycemia by increasing insulin release and also prevented oxidative / inflammatory stress in pancreatic tissue of diabetic rats, compared with a standard antidiabetic such as glibenclamide (BASHA; SANKARANARAYANAN, 2016).

Transsesquisabinene hydrate is involved in studies of antibacterial activity of essential oils, and its relevance to antimicrobial action against Gram-positive strains such as *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis* and *Enterococcus faecalis*, *Candida albicans* is not yet clear (BENOMARI *et al.*, 2016).

Spatulenol has antibacterial and anti-inflammatory properties when in complex essential oil compositions (MALTI *et al.*, 2019). The antioxidant, anti-inflammatory, antiproliferative and antimicobacterial properties of spatulenol have already been described in the literature (NASCIMENTO *et al.*, 2018).

4. FINAL CONSIDERATIONS

From the literature review on the species *Syzygium malaccense* it was known that it has wide popular use, mainly in Indian countries (native region of the plant). According to the literature, catechin, mearnsitrin, myricitrin and quercitrin are the main contributors to the antioxidant, anti-inflammatory and antiglycemic properties of *Syzygium malaccense* leaf extracts. The essential oil, obtained from the leaves of *Syzygium malaccense*, was extracted by water vapor drag hydrodistillation and yielded 0.03%.

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