

## Chemical composition and *in vitro* antibacterial activity of essential oils from *Murraya paniculata* (L.) Jack (Rutaceae) ripe and unripe fruits against bacterial genera *Mycobacterium* and *Streptococcus*

Flávia Fernanda Alves da Silva<sup>1</sup>, Cassia Cristina Fernandes<sup>1</sup>,  
Mariana Brentini Santiago<sup>2</sup>, Carlos Henrique Gomes Martins<sup>2</sup>,  
Tatiana Manzini Vieira<sup>3</sup>, Antônio Eduardo Miller Crotti<sup>3</sup>,  
Mayker Lazaro Dantas Miranda<sup>4\*</sup>

<sup>1</sup>Instituto Federal de Educação, Ciência e Tecnologia Goiano, Campus Rio Verde, Rio Verde, GO, Brazil, <sup>2</sup>Centro de Pesquisa em Ciências Exatas e Tecnologia, Universidade de Franca, Franca, SP, Brazil, <sup>3</sup>Departamento de Química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil, <sup>4</sup>Instituto Federal de Educação, Ciência e Tecnologia do Triângulo Mineiro, Campus Uberlândia Centro, Uberlândia, MG, Brazil

This study aims to investigate chemical composition of essential oils from *Murraya paniculata* (L.) Jack (Rutaceae) ripe and unripe fruits and determine their *in vitro* antibacterial activity. Essential oils were extracted by hydrodistillation from *Murraya paniculata* (L.) Jack ripe and unripe fruits collected in the *Cerrado*, in Rio Verde, southwestern Goiás, Brazil. They were analyzed by gas chromatography with flame ionization detector (GC-FID) and by gas chromatography-mass spectrometry (GC-MS). Sesquiterpenes, which represent the most abundant class of compounds in oils, predominated in both ripe and unripe fruits. Major constituents of essential oils extracted from ripe fruits (RF-EO) were  $\beta$ -caryophyllene (21.3%),  $\alpha$ -ylangene (13.3%), germacrene-D (10.9%) and  $\alpha$ -zingiberene (9.7%) whereas the ones of unripe fruits (UF-EO) were sesquithujene (25.0%),  $\alpha$ -zingiberene (18.2%), germacrene-D (13.1%) and  $\alpha$ -copaene (12.7%). *In vitro* antibacterial activity of essential oils was evaluated in terms of its minimum inhibitory concentration (MIC) values by the broth microdilution method in 96-well microplates. Both essential oils under investigation showed moderate anti-streptococcal activity against the following bacteria: *Streptococcus mutans*, *S. mitis*, *S. sanguinis*, *S. sobrinus* and *S. salivarius*. MIC values ranged between 100 and 400  $\mu\text{g/mL}$ . Regarding the antimycobacterial activity, essential oils from *M. paniculata* (L.) Jack unripe and ripe fruits were active against *Mycobacterium kansasii* (MIC = 250  $\mu\text{g/mL}$ ), moderately active against *M. tuberculosis* (MIC = 500  $\mu\text{g/mL}$ ) and inactive against *M. avium* (MIC = 2000  $\mu\text{g/mL}$ ). This study was pioneer in revealing similar chemical profiles of both essential oils extracted from *Murraya paniculata* (L.) Jack unripe and ripe fruits, besides describing their *in vitro* anti-streptococcal and antimycobacterial activities.

**Keywords:** *Murraya paniculata* (L.) Jack. Rutaceae. Anti-streptococcal activity. *Mycobacterium kansasii*. Antibacterial agent. Antimycobacterial activity.

### INTRODUCTION

Chemical studies of essential oils have been widely carried out due to their several biological applications (Properzi *et al.*, 2013). Therefore, this study aims to evaluate antibacterial activity of essential oils from

\*Correspondence: M. L. D. Miranda, Instituto Federal de Educação, Ciência e Tecnologia do Triângulo Mineiro, Campus Uberlândia Centro, Rua Blanche Galassi, 150, Morada da Colina, 38411-104, Uberlândia, MG, Brazil. Phone: (35) 9 9720-8492. E-mail: maykermiranda@iftm.edu.br

*Murraya paniculata* (L.) Jack against both bacterial genera *Streptococcus* and *Mycobacterium*.

Tooth decay, which is a pathology that occurs in the hard tissues of the teeth, results from the accumulation of bacteria and their metabolism on tooth surfaces, a fact that leads to the development of the so-called biofilm (Soares *et al.*, 2012). Tooth decay has been considered an important public health issue since there are more than 700 species of bacteria in the oral cavity and some of them are responsible for this pathology and other periodontal diseases (Melo *et al.*, 2017).

Tuberculosis is an infectious disease caused by mycobacteria of the genus *Mycobacterium*, even though *Mycobacterium tuberculosis* is the species which has led to the highest morbidity and mortality rates (Fernandes, Chin, Santos, 2017). In 2015, the World Health Organization reported that there were 9,6 million new cases worldwide and 1,5 million deaths caused by the disease (Fernandes, Chin, Santos, 2017). Besides, there has been significant increase in the number of nontuberculous mycobacteria, such as *Mycobacterium kansasii* and *M. avium*, which also affect the lungs, lymph, skin and joints. Resulting diseases may produce severe after-effects if they are not properly treated (Alves *et al.*, 2015).

The importance of evaluating the antibacterial potential of essential oils has been attributed to their hydrophobicity, since it enables them to interact with lipids of the cell membrane and mitochondria of bacteria. It disturbs cell structures, increases membrane permeability, leads to leakage of substances that are essential to survival and causes cell death (Miranda *et al.*, 2016).

*Murraya paniculata* (L.) Jack, which has been known as murta de cheiro in Brazil, belongs to Rutaceae, a botanic family that has about 150 genera and 1,600 species distributed in tropical, subtropical and temperate regions all over the world, even though it is more abundant in tropical America, southern Africa and Australia (Campelo *et al.*, 2013). In Brazil, the family Rutaceae is represented by about 29 genera and 182 species. Some have medicinal, ecological and economic importance (Campelo *et al.*, 2013).

The genus *Murraya*, which comprises about 35 species of flowering plants, is native to southeastern Asia, even though it is also widely distributed all over the world (Martín *et al.*, 2011). Examples of species that belong to this genus are *Murraya euchrestifolia*, *M. koenigii* (L.) Spreng, *M. paniculata* (L.) Jack, *M.*

*sumatrana*, *M. amoena*, *M. omphalocarpa* Hayata, *M. alata* Drake, *M. caloxylon* Ridl, *M. crenulata* (Turcz.) Oliv, *M. brevifolia*, *M. burmanni*, *M. alternans* and *M. siamensis* (Martín *et al.*, 2011).

The species *M. paniculata* (L.) Jack, which is a tree native to India, was brought to Brazil to be used for afforestation and garden ornamentation (Mesquita *et al.*, 2008). In tropical and subtropical regions in Asia, besides China and Indonesia, this species is considered medicinal; thus, it has been used for treating intestine disorders, rheumatism and cough (Mesquita *et al.*, 2008).

Many other pharmacological activities shown by *M. paniculata* (L.) Jack extracts, such as their application to treatments for diarrhea, asthma and hypertension, have been described by the literature (Saqib *et al.*, 2015). Their antifungal, analgesic, cytotoxic and antioxidant potential is also well-known; they are even used for mitigating toothache (Souza *et al.*, 2008; Sharkar *et al.*, 2009; Faisal *et al.*, 2014; Zhu, Lei, Luo, 2015; Kusuma, Irma, Yuliasih, 2017). However, few studies of chemical composition and biological activity of essential oils extracted from *M. paniculata* can be found in the literature (Neta *et al.*, 2017). The ones carried out in Nigeria, Bangladesh, Nepal and Cuba should be highlighted (Olawore *et al.*, 2005; Chowdhury, Bhuiyan, Yusuf, 2008; Rodríguez *et al.*, 2012; Dosoky *et al.*, 2016).

Regarding *M. paniculata* (L.) Jack in Brazil, a recent study has described the chemical composition of the essential oil from its leaves collected in Espírito Santo state, Brazil, besides its cytotoxic, antifungal and antibacterial properties (Neta *et al.*, 2017). However, it should be mentioned that this study aimed at pioneering the description of chemical profiles and *in vitro* antimycobacterial and anti-streptococcal activities of essential oils from *M. paniculata* (L.) Jack unripe and ripe fruits collected in the Cerrado in Goiás state, Brazil.

## MATERIAL AND METHOD

### Plant material

*Murraya paniculata* (L.) Jack unripe (UF-EO) and ripe fruits (RF-EO) were collected in the Cerrado in Rio Verde, Goiás, Brazil, in October 2017. The plant was identified by botanist Erika Amaral and a sample was deposited at the Herbarium Jataiense Professor Germano Guarim Neto (exsiccate number HJ 28760/MP).

## Extraction of essential oils

Samples of *M. paniculata* (L.) Jack unripe and ripe fruits were subjected to hydrodistillation for 2 hours by a Clevenger-type apparatus. In order to carry out the analysis, 300 g plant material was divided into three 100-g samples and 500 mL distilled water was added to each sample. After manual collection of the essential oil samples, traces of remaining water in the oils were removed by anhydrous sodium sulfate. Filtration was then carried out. The extraction procedure was done in triplicate. The isolated oil was stored under refrigeration up to the analysis and test. Yield (w/w) calculations were based on unripe and ripe fruit weight and expressed as the average of the triplicate analyses.

## Identification of the chemical composition of essential oils

Gas chromatography (GC) analyses were performed by a Shimadzu GC2010 Plus gas chromatograph equipped with an AOC-20s autosampler and fitted with FID and a data-handling processor. An Rtx-5 (Restek Co., Bellefonte, PA, USA) fused silica capillary column (30-m x 0.25-mm i.d.; 0.25- $\mu$ m film thickness) was employed. Operation conditions were as follows: column temperature programmed to rise from 60 to 240 °C at 3 °C/min and, then, to hold at 240 °C for 5 min; carrier gas = He (99.999%), at 1.0 mL/min; injection mode; injection volume, 0.1  $\mu$ L (split ratio of 1:10); and injector and detector temperatures = 240 and 280 °C, respectively. Relative concentrations of components were obtained by peak area normalization (%). Relative areas were the average of triplicate GC-FID analyses.

GC-MS analyses were carried out by a Shimadzu QP2010 Plus (Shimadzu Corporation, Kyoto, Japan) system equipped with an AOC-20i autosampler. The column was a RTX-5MS (Restek Co., Bellefonte, PA, USA) fused silica capillary column (30 m x 0.25 mm i.d. x 0.25  $\mu$ m film thickness). Electron ionization mode occurred at 70 eV. Helium (99.999%) was employed as the carrier gas at a constant flow of 1.0 mL/min. Injection volume was 0.1  $\mu$ L (split ratio of 1:10). Injector and ion-source temperatures were set at 240 and 280 °C, respectively. The oven temperature program was the same as the one used for GC. Mass spectra were taken at a scan interval of 0.5 s, in the mass range from 40 to 600 Da.

Identification of volatile components of *M. paniculata* (L.) Jack unripe and ripe fruits (Table I) was

based on their retention indices on an Rtx-5MS capillary column under the same operating conditions as the ones in the case of GC relative to a homologous series of n-alkanes (C8-C20). Structures were computer-matched with the Wiley 7, NIST 08 and FFNSC 1.2 spectra libraries and their fragmentation patterns were compared with literature data (Adams, 2007).

## Bacterial strains and antimicrobial assays

*In vitro* anti-streptococcal activity of RF-EO and UF-EO were determined by minimum inhibitory concentration (MIC) assays which were based on the broth microdilution method (CLSI, 2009). *Streptococcus salivarius* (ATCC 25975), *Streptococcus sobrinus* (ATCC 33478), *Streptococcus mutans* (ATCC 25175), *Streptococcus mitis* (ATCC 49456) and *Streptococcus sanguinis* (ATCC 10556) were the standard strains in the assays. Initially, bacteria were transferred to blood agar (Difco Labs, Detroit, MI, USA), and individual 24-h colonies were suspended in 10.0 mL tryptic soy broth (Difco). A spectrophotometer (Femto, São Paulo, SP, Brazil) at a wavelength ( $\lambda$ ) of 625 nm was used for standardizing the suspensions of each microorganism so as to match the transmittance of 81, equivalent to 0.5 in the McFarland scale ( $1.5 \times 10^8$  CFU/mL). Dilution of the standardized suspension generated the final concentration of  $5 \times 10^5$  CFU/mL. Essential oils (RF-EO and UF-EO) were dissolved in DMSO (Merck, Darmstadt, Germany) at 16.0 mg/mL. Concentrations ranging from 400 to 3.9  $\mu$ g/mL were achieved after dilution of essential oils in tryptic soy broth (Difco). After the dilutions, DMSO concentrations were between 4% and 0.0039% (v/v). Negative controls, three inoculated wells with DMSO at concentrations ranging from 4% to 1% and one non-inoculated well, free of any antimicrobial agent, were included. An inoculated well helped to test whether the broth was adequate for microorganisms to grow. The positive control was chlorhexidine dihydrochloride (CHD) (Sigma-Aldrich, St. Louis, MO, USA) at concentrations ranging from 5.9 to 0.115  $\mu$ g/mL, diluted in tryptic soy broth (Difco). Ninety-six-well microplates were sealed with parafilm and incubated at 37 °C for 24 h. After that, 30 mL of an aqueous solution with 0.02% resazurin (Sigma-Aldrich, St. Louis, MO, USA) was added to each microplate well to indicate the viability of the microorganism (Palomino *et al.*, 2002). The lowest concentration of the sample that inhibited microorganism growth (MIC value) was

determined as the lowest concentrations of RF-EO and UF-EO that were able to prevent the resazurin solution from changing its color (Sarker *et al.*, 2007). All assays were conducted in triplicate.

Mycobacteria *Mycobacterium tuberculosis* H37Rv (ATCC 27294), *M. kansasii* (ATCC 12478) and *M. avium* (ATCC 25291) were obtained from the American Type Collection (ATCC) and maintained at -80 °C. Antimycobacterial activity of the essential oils from *M. paniculata* unripe and ripe fruits was evaluated by the MIC broth microdilution method conducted on microplates. Final concentration of *Mycobacterium* was equal to the standardized *Streptococcus* (5 x 10<sup>5</sup> CFU/mL). Resazurin was employed to reveal mycobacterial growth by the Resazurin Microtiter Assay (REMA) method (Palomino *et al.*, 2002). Essential oils were serially diluted (two-fold) with Middlebrook 7H9 broth (Difco™, Detroit, MI, USA). The *Mycobacterium inoculum* was then added to essential oil solutions to obtain concentrations ranging from 250 to 2000 µg/mL. The inoculated plates were then incubated for 42 days at 37°C (1st reading after 28 days, 2nd reading after 42 days) and the percentage of inhibition was determined (Gupta *et al.*, 2010). Isoniazid was used as positive control at concentrations ranging from 0.06 to 1.0 µg/mL whereas Middlebrook 7H9 broth and the inoculum were used as solvent and negative control, respectively.

## RESULTS AND DISCUSSION

Extraction of essential oils from *M. paniculata* (L.) Jack unripe and ripe fruits yielded 0.5% and 0.6%, respectively. CG-EM identified 28 chemical constituents in essential oils from ripe fruits (total: 95.9%) and 15 ones in oils from unripe fruits (total: 96.1%). Retention times, compounds, retention indexes and relative percentage (%) are shown in Table I. Major components of essential oils extracted from ripe fruits (RF-EO) were: β-caryophyllene (21.3%), α-ylangene (13.3%), germacrene-D (10.9%) and α-zingiberene (9.7%) whereas the ones from unripe fruits (UF-EO) were: sesquithujene (25.0%), α-zingiberene (18.2%), germacrene-D (13.1%) and α-copaene (12.7%).

**TABLE I** - Chemical composition of essential oils from *M. paniculata* (L.) Jack ripe fruits (RF-EO) and unripe fruits (UF-EO)

RT (min)	Compounds	RI <sub>exp</sub>	RI <sub>lit</sub>	%RA	
				RF-EO	UF-EO
27.30	Bicycloelemene	1334	1336	0.3	1.4
27.75	Elemene isomer	1341	1344	2.7	
28.28	α-Cubebene	1351	1352	0.9	6.1
29.51	α-Copaene	1379	1377	6.1	<b>12.7</b>
30.21	α-Ylangene	1405	1406	13.3	1.1
30.81	Sesquithujene	1415	1417	0.4	<b>25.0</b>
30.94	α-Gurjunene	1419	1419	0.1	
31.59	β-Caryophyllene	1425	1423	21.3	1.1
31.77	Isogermacrene D	1437	1439	0.8	
31.91	β-Gurjunene	1439	1440	0.7	
32.36	γ-Muurolene	1448	1449	0.4	6.4
32.81	α-Humulene	1456	1455	5.3	1.0
33.06	Aromadendrene	1465	1463	1.1	
34.02	Germacrene D	1480	1480	10.9	<b>13.1</b>
34.50	α-Zingiberene	1499	1496	9.7	<b>18.2</b>
34.61	Bicyclogermacrene	1503	1501	5.3	0.5
34.89	β-Bisabolene	1508	1506	0.8	1.7
35.58	β-Cadinene	1528	1527	5.5	5.6
35.92	Cadina-1.4-diene	1534	1533	0.3	
37.62	Germacrene-D-4-ol	1576	1574	1.0	1.0
37.94	Caryophyllene oxide	1590	1589	1.2	1.1
38.72	Lauryl acetate	1608	1606	0.2	
39.57	Octil 2-methylbutanoate	1624	1623	0.2	

(continuing)



**TABLE I** - Chemical composition of essential oils from *M. paniculata* (L.) Jack ripe fruits (RF-EO) and unripe fruits (UF-EO)

RT (min)	Compounds	RI <sub>exp</sub>	RI <sub>lit</sub>	%RA	
				RF-EO	UF-EO
39.71	Isovaleric acid. decyl ester	1657	1659	0.1	
40.12	τ-Muurolol	1659	1660	0.5	
40.59	α-Cadinol	1662	1663	0.3	
42.80	Decyl senecioate	1720	1719	4.0	
46.73	Isovaleric acid. dodecyl ester	1844	1845	2.5	
Hydrocarbon sesquiterpenes				85.9	94.0
Oxygenated sesquiterpenes				3.0	2.1
Others				7.0	
<b>Total</b>				<b>95.9</b>	<b>96.1</b>

RI<sub>exp</sub>: Retention index determined in relation to *n*-alkanes (C<sub>8</sub>-C<sub>20</sub>) in the Rtx-5MS column; RI<sub>lit</sub>: Retention index in the literature

Previous reports of essential oil from leaves of other *M. paniculata* (L.) Jack specimens indicated that terpenes predominate in the oil and that the chemical composition of the essential oil varied significantly, depending on the origin of the plant. For example, the seven major constituents of the essential oil from leaves cultivated in Bangladesh were caryophyllene oxide, β-caryophyllene, spathulenol, β-elemene, germacrene D, cyclooctene and 4-methylene-6-(1-propenylidene) (Chowdhury, Bhuiyan, Yusuf, 2008), whereas the ones of essential oil collected in Nepal were methyl palmitate, isospathulenol, (*E,E*)-geranyl linalool, benzyl benzoate, selin-6-en-4-ol, β-caryophyllene, germacrene B, germacrene D and γ-elemene and the major constituent of essential oils from leaves found in mountains in Central Cuba was β-caryophyllene (Rodríguez *et al.*, 2012; Dosoky *et al.*, 2016). On the other hand, in Nigeria, essential oil from leaves showed the following seven major constituents: β-cyclocitral, methyl salicylate,

*trans*-nerolidol, α-cubebene, (-)-cubenol, β-cubebene and isogermacrene (Olawore *et al.*, 2005).

Specifically, Olawore and collaborators (2005) reported that the major components found in the essential oils from *M. paniculata* (L.) Jack fruits were β-caryophyllene (43.4%), (-)-zingiberene (18.9%), germacrene D (8.3%), α-copaene (5.5%) and α-humulene (5.1%), even though they did not mention the maturation conditions of the fruits. Therefore, the study described by the authors in this paper pioneers the analysis of chemical profiles of essential oils extracted from *M. paniculata* (L.) Jack unripe and ripe fruits collected in the Cerrado in Goiás state, Brazil. By comparison with the previously reported chemical composition of *M. paniculata* (L.) Jack fruits, compositions of RF-EO and UF-EO found by this study were similar, since their major constituents were also α-copaene, β-caryophyllene, germacrene D, α-zingiberene and α-humulene, even though concentrations were different (Olawore *et al.*, 2005). It should be highlighted that α-ylangene (13.3%) and sesquithujene (25.0%), the other major constituents of RF-EO and UF-EO, were not found in the essential oil from fruits collected in Nigeria (Olawore *et al.*, 2005).

Anti-streptococcal activity of essential oils from *M. paniculata* (L.) Jack unripe and ripe fruits was determined so as to evaluate it against some oral pathogens (Table II).

**TABLE II** - Determination of minimum inhibitory concentration (MIC = µg/mL) of essential oils from *M. paniculata* (L.) Jack unripe and ripe fruits against bacteria of the genus *Streptococcus*

Bacteria	MIC	MIC	MIC
	Unripe fruit	Ripe fruit	CHD*
<i>Streptococcus mutans</i>	250	250	0.922
<i>S. mitis</i>	200	100	1.844
<i>S. sanguinis</i>	200	200	0.922
<i>S. sobrinus</i>	400	400	0.922
<i>S. salivarius</i>	200	250	0.922

MIC: minimum inhibitory concentration (µg/mL); CHD\*: chlorhexidine dihydrochloride (positive control).

Essential oils from *M. paniculata* (L.) Jack unripe and ripe fruits had moderate inhibitory activity against all bacteria under investigation. The literature reports that samples whose MIC values were below 100 µg/mL had good antibacterial activity; from 100 to 500 µg/mL, it was considered moderate; from 500 to 1000 µg/mL, it was weak; and above 1000 µg/mL, it was inactive (Carneiro *et al.*, 2017).

*Streptococcus mitis*, *S. mutans*, *S. sanguinis*, *S. salivarius* and *S. sobrinus* are bacteria whose pathogenicity affects tooth enamel and gingival tissue; thus, they are closely related to tooth decay and periodontal diseases (Estevam *et al.*, 2016). The bacterial plaque has been defined as a biofilm of microorganisms that are in an organic matrix composed of substances of both saliva and the host's diet, besides bacterial polymers (Estevam *et al.*, 2016).

The chemical composition of essential oils from *M. paniculata* (L.) Jack unripe and ripe fruits had some constituents whose antimicrobial activities have already been well-described by the literature. They are β-caryophyllene, germacrene D, α-humulene, α-copaene and α-zingiberene (Moreira *et al.*, 2014; Martins *et al.*, 2015; Dias *et al.*, 2017). The last was also the major constituent of essential oil from *Guarea kunthiana*, a fact that may explain the moderate antibacterial activity shown by the oils under study (Pandini *et al.*, 2018). In short, plants keep being promising targets of studies in the search for compounds that have anti-decay activity so as to enable the production of new agents for oral hygiene (Mohieldin *et al.*, 2017).

Antibacterial activity of essential oils has been associated with the lipophilicity of their chemical constituents, mainly monoterpenes and sesquiterpenes, which are often the main chemicals thereof (Crevelin *et al.*, 2015). Lipophilicity allows essential oils to diffuse across cell membranes easily and then kill microorganisms by affecting their metabolic pathways or organelles (Raut, Karuppaiyil, 2014).

Regarding antimycobacterial activity, since few studies mention the application of essential oils to inhibit mycobacteria (Alvarenga *et al.*, 2014), the authors of this study also believe it is relevant to carry out this biological activity *in vitro*. Therefore, the antimycobacterial activity of essential oils from *M. paniculata* (L.) Jack unripe and ripe fruits against *Mycobacterium tuberculosis*, *M. kansasii* and *M. avium* was investigated by determining minimum inhibitory concentrations (MICs, Table III).

**TABLE III** - Determination of minimum inhibitory concentration (MIC = µg/mL) of essential oils from *M. paniculata* (L.) Jack unripe and ripe fruits against bacteria of the genus *Mycobacterium*

	<i>M. tuberculosis</i>	<i>M. kansasii</i>	<i>M. avium</i>
EO-Unripe fruit	500	250	2000
EO-Ripe fruit	500	250	2000
Isoniazid*	0.06	1	> 1

EO - Essential oil; \*Positive control

The literature reports that essential oils with MIC values of 500 µg/mL and 250 µg/mL are considered moderately active and active, respectively, whereas values from 1000 to 2000 µg/mL are poorly active against mycobacteria under evaluation (Alves *et al.*, 2015).

Differences in antibacterial activity between MIC results of essential oils found by this study and others reported by the literature may be due to differences in qualitative and quantitative composition of the EOs, diluent agent, reference culture and cell concentration. In addition, other factors, such as geographical location, environment, incubation conditions and stage of maturity, can affect these constituents and influence MIC (Swamy *et al.*, 2016; Van de Vel, Sampers, Raes, 2017).

Plants have stood out as potential sources of new antimycobacterial agents (Leitão *et al.*, 2013). In this context, essential oils from *M. paniculata* (L.) Jack unripe and ripe fruits are worth mentioning since they are active against *M. kansasii* and moderately active against *M. tuberculosis* but inactive against *M. avium* (Table III). Taking into account the resistance developed by bacteria against antibiotics and conventional drugs used for treating diseases, such as tuberculosis, results found by this study are important (Gómez-Cansino *et al.*, 2017).

Antimycobacterial activity of essential oils from *M. paniculata* (L.) Jack unripe and ripe fruits against *M. kansasii* and *M. tuberculosis* may be related to some chemical constituents found in oil, such as α-cubebene, α-copaene, α-ylangene, α-gurjunene, β-caryophyllene, γ-humulene, bicyclogermacrene and caryophyllene oxide. They had already been identified in essential oils from *Pterodon emarginatus* fruits which showed

promising activity against bacteria of the genus *Mycobacterium* (Alves *et al.*, 2013; Machado *et al.*, 2015). Furthermore, studies have shown the synergistic effect of two or more constituents of essential oils on various human pathogens (Raut, Karuppayil, 2014).

## CONCLUSION

Results of this study showed that the chemical composition of essential oils from *M. paniculata* (L.) Jack unripe and ripe fruits had high concentration of sesquiterpenes. Major constituents were  $\beta$ -caryophyllene,  $\alpha$ -ylangene, germacrene-D,  $\alpha$ -zingiberene, sesquithujene and  $\alpha$ -copaene. Besides, when essential oils were biologically tested, RF-EO and UF-EO showed moderate antibacterial activity against all bacteria of the genus *Streptococcus* under evaluation. RF-EO and UF-EO were also active against *Mycobacterium kansasii* and moderately active against *Mycobacterium tuberculosis*. Anti-streptococcal and antimycobacterial activities of RF-EO and UF-EO were also described for the first time. Results suggest that bioactive molecules found in essential oils from *M. paniculata* (L.) Jack unripe and ripe fruits may be used as prototypes for the development of new pharmaceuticals and/or sources of pharmaceutical raw materials with antibacterial activity.

## ACKNOWLEDGMENTS

The authors would like to thank FAPEG, CNPq, IFGOIANO – Campus Rio Verde and CAPES for the financial support.

## REFERENCES

- Adams RP. In identification of essential oil components by gas chromatography/quadrupole mass spectroscopy. 4th ed. Allured Publishing Corporation: Carol Stream, 2007; 804 p.
- Alvarenga RFR, Wan B, Inui T, Franzblau SG, Pauli GF, Jaki BU. Airborne antituberculosis activity of *Eucalyptus citriodora* essential oil. *J Nat Prod*. 2014;77(3):603-610.
- Alves JA, Mantovani ALL, Martins MHG, Abrao F, Lucarini R, Crotti AEM, Martins CHG. Antimycobacterial activity of some commercially available plant-derived essential oils. *Chem Nat Compd*. 2015;51(2):353-355.
- Alves SF, Borges LL, Paula JAM, Vieira RF, Ferri PH, Couto RO, Paula JR, Bara MTF. Chemical variability of the essential oils from fruits of *Pterodon emarginatus* in the Brazilian cerrado. *Rev Bras Farmacogn*. 2013;23(2):224-229.
- Campelo LML, Sá CG, Feitosa CM, Sousa GF, Freitas RM. Constituintes químicos e estudos toxicológicos do óleo essencial extraído das folhas de *Citrus limon* Burn (Rutaceae). *Rev Bras Pl Med*. 2013;15(4):708-716.
- CLSI. Susceptibility testing of aerobic bacteria. Approved standard, 8th ed. CLSI document M7-A8". Wayne, PA: NCCLS; 2009, 15p.
- Carneiro NS, Alves CCF, Alves JM, Egea MB, Martins CHG, Silva TS, et al. Chemical composition, antioxidant and antibacterial activities of essential oils from leaves and flowers of *Eugenia klotzschiana* Berg (Myrtaceae). *An Acad Bras Cienc*. 2017;89(3):1907-1915.
- Chowdhury JU, Bhuiyan MNI, Yusuf M. Chemical composition of the leaf essential oils of *Murraya koenigii* (L.) Spreng and *Murraya paniculata* (L.) Jack. *Bangladesh J Pharmacol*. 2008;3(2):59-63.
- Crevelin EJ, Caixeta SC, Dias HJ, Groppo M, Cunha WR, Martins CHG, Crotti AEM. Antimicrobial activity of the essential oil of *Plectranthus neochilus* against cariogenic bacteria. *Evid Based Complement Altern Med*. 2015;2015:102317.
- Dias HJ, Vieira TM, Carvalho CE, Aguiar GP, Wakabayashi KAL, Turatti ICC, Willrich GB, Groppo M, Cunha WR, Martins CHG, Crotti AEM. Screening of selected plant-derived extracts for their antimicrobial activity against oral pathogens. *Int J Complement Alt Med*. 2017;6:00188.
- Dosoky NS, Satyal P, Gautam TP, Setzer WN. Composition and biological activities of *Murraya paniculata* (L.) Jack essential oil from Nepal. *Medicines*. 2016;3(1):1-10.
- Estevam EBB, Miranda MLD, Alves JM, Egea MB, Pereira PS, Martins CHG, et al. Composição química e atividades biológicas dos óleos essenciais das folhas frescas de *Citrus limonia* Osbeck e *Citrus latifolia* Tanaka (Rutaceae). *Rev Virtual Quim*. 2016;8(6):1842-1854.
- Faisal M, Sarker MM, Rahman A, Hossain AI, Rahman S, Bashir ABMA, Jahan R, Rahmatullah M. *Murraya paniculata* (L.) Jack: a potential plant for treatment of toothache. *J Dent Oral Disord Ther*. 2014;2:1-3.
- Fernandes GFS, Chin CM, Santos JL. Potenciais alvos moleculares para o desenvolvimento de novos fármacos antituberculose. *Quim Nova*. 2017;40(5):572-585.
- Gómez-Cansino R, Guzmán-Gutiérrez SL, Campos-Lara MG, Espitia-Pinzón CI, Reyes-Chilpa R. Natural compounds

- from Mexican plants as potential drug leads for anti-tuberculosis drugs. *An Acad Bras Ciênc.* 2017;89(1):31-43.
- Gupta R, Thakur B, Singh P, Sharma VD, Katoch VM, Chauhan SVS. Anti-tuberculosis activity of selected medicinal plants against multi-drug resistant *Mycobacterium tuberculosis* isolates. *Indian J Med Res.* 2010;131(6):809-813.
- Kusuma SAF, Irma EH, Yuliasih N. *In vitro* antifungal activity of the orange jasmine (*Murraya paniculata* [L.] Jack.) leaves ethanol extract from Indonesia against *Candida albicans*. *Inter J Scient Eng Applied Sci.* 2017;3(5):273-278.
- Leitão F, Leitão SG, Almeida MZ, Santos J, Coelho T, Silva PEA. Medicinal plants from open-air markets in the state of Rio de Janeiro, Brazil as a potential source of new antimycobacterial agents. *J Ethnopharmacol.* 2013;149(2):513-521.
- Machado RRP, Dutra RC, Raposo NRB, Lesche B, Gomes MS, Duarte RS, Soares GLG, Kaplan MAC. Interferometry as a tool for evaluating effects of antimicrobial doses on *Mycobacterium bovis* growth. *Tuberculosis.* 2015;95(6):829-838.
- Martins CM, Nascimento EA, Morais SAL, Oliveira A, Chang R, Cunha LCS, et al. Chemical constituents and evaluation of antimicrobial and cytotoxic activities of *Kielmeyera coriacea* Mart. & Zucc. Essential oils. *Evid Based Complementary Altern Med.* 2015;2015:842047.
- Martín CMC, Gaitén YIG, Amado ER. Acercamiento al género *Murraya* (Rutaceae) y a la especie *Murraya paniculata* (L.) Jack. *Rev Cubana Pl Med.* 2011;16(4):408-418.
- Melo DC, Miranda MLD, Júnior WGF, Andrade PM, Alcoba AET, Silva TS, Casal CM, Martins CHG. Anticariogenic and antimycobacterial activities of the essential oil of *Siparuna guianensis* Aublet (Siparunaceae). *Orbital Electron J Chem.* 2017;9(1):55-60.
- Mesquita SG, Martinez MF, Romoff P, Fávero OA, Lieber SR, Lago JHG. Constituintes químicos das folhas de *Murraya paniculata* (Rutaceae). *Rev Bras Farmacogn.* 2008;18(4):563-568.
- Miranda CASF, Cardoso MG, Batista LR, Rodrigues LMA, Figueiredo ACS. Óleos essenciais de folhas de diversas espécies: propriedades antioxidantes e antibacterianas no crescimento espécies patogênicas. *Rev Ciênc Agron.* 2016;47(1):213-220.
- Mohieldin EAM, Muddathir AM, Yamauchi K, Mitsunaga T. Anti-caries activity of selected Sudanese medicinal plants with emphasis on *Terminalia laxiflora*. *Rev Bras Farmacogn.* 2017;27(5):611-618.
- Moreira RRD, Martins GZ, Botelho VT, Santos LE, Cavaleiro C, Salgueiro L, Andrade G, Martins CHG. Composition and activity against oral pathogens of the essential oil of *Melampodium divaricatum* (Rich.) DC. *Chem Biodivers.* 2014;11(3):438-444.
- Neta MCS, Vittorazzi C, Guimarães AC, Martins JDL, Fronza M, Endringer DC, Scherer R. Effects of  $\beta$ -caryophyllene and *Murraya paniculata* essential oil in the murine hepatoma cells and in the bacteria and fungi 24-h time-kill curve studies. *Pharm Biol.* 2017;55(1):190-197.
- Olawore NO, Ogunwande IA, Ekundayo O, Adeleke KA. Chemical composition of the leaf and fruit essential oils of *Murraya paniculata* (L.) Jack. (Syn. *Murraya exotica* Linn.). *Flavour Fragr J.* 2005;20(1):54-56.
- Palomino JC, Martin A, Camacho M, Guerra H, Swings J, Portaels F. Resazurin microtiter assay plate: simple and inexpensive method for detection of drug resistance in *Mycobacterium tuberculosis*. *Chemotherapy.* 2002;46(8):2720-2722.
- Pandini JA, Pinto FGS, Scur MC, Santana CB, Costa WF, Temponi LG. Chemical composition, antimicrobial and antioxidant potential of the essential oil of *Guarea kunthiana* A. Juss. *Braz J Biol.* 2018;78(1):53-60.
- Properzi A, Angelini P, Bertuzzi G, Venanzoni R. Some biological activities of essential oils. *Med Aromat Plants.* 2013;2(5):1000136.
- Raut JS, Karuppayil SM. A status review on the medicinal properties of essential oils. *Ind Crops Prod.* 2014;62:250-264.
- Rodríguez EJ, Ramos GR, Heyden YV, Alfonso EFS, García MJL, Hernández YS, Monteagudo U, Morales Y, Holgado B, Martínez JMH. Chemical composition, antioxidant properties and antimicrobial activity of the essential oil of *Murraya paniculata* leaves from the mountains of central Cuba. *Nat Prod Commun.* 2012;7(11):1527-1530.
- Saqib F, Ahmed MG, Janbaz KH, Dewanjee S, Jaafar HZE, Haq MZU. Validation of ethnopharmacological uses of *Murraya paniculata* in disorders of diarrhea, asthma and hypertension. *Complement Altern Med.* 2015;15:319-326.
- Sarker SD, Nahar L, Kumarasamy Y. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals. *Methods.* 2007;42(4):321-324.
- Sharker SM, Shahid IJ, Hasanuzzaman M. Antinociceptive and bioactivity of leaves of *Murraya paniculata* (L.) Jack, Rutaceae. *Rev Bras Farmacogn.* 2009;19(3):746-748.



Soares GG, Souza PR, Purger FPC, Vasconcellos AB, Ribeiro AA. Métodos de detecção de cárie. Rev Bras Odontol. 2012;69(1):84-89.

Souza ADL, Filho ER, Souza AQL, Silva FH, Pereira JO. A new guaiane mannoside from a *Eutpa*-like fungus isolated from *Murraya paniculata* in Brazil. J Braz Chem Soc. 2008;19(7):1321-1325.

Swamy MK, Akhtar MS, Sinniah UR. Antimicrobial properties of plant essential oils against human pathogens

and their mode of action: an updated review. Evid Based Complement Altern Med. 2016;2016:3012462.

Van de Vel E, Sampers I, Raes K. A review on influencing factors on the minimum inhibitory concentration of essential oils. Crit Rev Food Sci Nutr. 2017;30(1):1-22.

Zhu C, Lei Z, Luo Y. Studies on antioxidative activities of methanol extract from *Murraya paniculata*. Food Sci Human Wellness. 2015;4(3):108-114.

Received for publication on 11<sup>th</sup> May 2018  
Accepted for publication on 02<sup>nd</sup> January 2019