

Chemical profile of the twigs of *Ozoroa obovata* by HPLC-MS-ESI and antimicrobial activity

Perfil químico dos galhos de Ozoroa obovata por CLAE-EM-IES e atividade antimicrobiana

Domingos A. João^{1,7}; Tiara C. Silva¹; Diego G. Prado¹; Carlos H. G. Martins²; Mariana B. Santiago³; Luiz R. Goulart⁴; Mário M. Martins⁴; Francisco J. T. Aquino⁵; Alberto de Oliveira⁵; Raquel M. F. Sousa⁵; Luís C. S. Cunha⁶; Sérgio A. L. Morais⁵

¹Aluno do Núcleo de Pesquisa em Produtos Naturais, Universidade Federal de Uberlândia, Uberlândia, Minas Gerais, Brasil. E-mail: d.augustojao@gmail.com; diego_godina@yahoo.com.br; tiaracostak14@gmail.com;

²Professor do Laboratório de Pesquisa em Ensaios Antimicrobianos, Universidade Federal de Uberlândia, Uberlândia, Minas Gerais, Brasil. Orcid: <https://orcid.org/0000-0001-8634-6878>. E-mail: carlos.martins2@ufu.br

³Aluna do Laboratório de Pesquisa em Ensaios Antimicrobianos, Universidade Federal de Uberlândia, Uberlândia, Minas Gerais, Brasil. Orcid: <https://orcid.org/0000-0002-6060-379X>. E-mail: mari.brentini@hotmail.com

⁴Professor do Laboratório de Nanobiotecnologia, Universidade Federal de Uberlândia, Uberlândia, Minas Gerais, Brasil. E-mail: lrgoulart@ufu.br; mariomm1988@yahoo.com.br

⁵Professor do Núcleo de Pesquisa em Produtos Naturais, Universidade Federal de Uberlândia, Uberlândia, Minas Gerais, Brasil. E-mail: salemos@ufu.br, aquino@ufu.br, albertho.iq@gmail.com, rsousa@ufu.br

⁶Professor do Núcleo de Bioprospecção em Produtos Naturais, Instituto Federal do Triângulo Mineiro, Uberaba, Minas Gerais, Brasil. Orcid: <https://orcid.org/0000-0002-4796-2480>E-mail: luiscunha@iftm.edu.br

⁷Professor da Universidade Rovuma, Lichinga, Moçambique.

ABSTRACT: In Mozambique, a large part of the population depends on plants for the treatment of various diseases. However, some of them have been little studied in relation to chemical and biological aspects. Among these species, *Ozoroa obovata* is widely used in traditional medicine in Mozambique. The factors that influence the use of medicinal plants in developing countries are mainly cultural habits, the inefficiency of the health system and the high cost of medicines. This work aimed to study the chemical composition and evaluate the antimicrobial activity of the twigs of *O. obovata*. Through the analysis by liquid chromatography coupled to mass spectrometry with electrospray ionization (HPLC-MS-ESI), it was possible to propose that in the ethanolic extract of the twigs, most of the constituents belong to the classes of phenolic acids and flavonoid. The phenolic compounds were represented by quinic, gallic and protocatechuic acids and five anacardic acids, whereas flavonoids were represented by mangiferin, taxifolin and quercetin mono and dihexoside. The extract was evaluated against bacteria in the oral cavity and showed moderate activity against *Streptococcus mutans*, *S. mitis* and *Porphyromonas gingivalis* (minimum inhibitory concentration - MIC 400 µg mL⁻¹). In relation antifungal test, the extract showed activity against *Candida albicans* with MIC of 3000 µg mL⁻¹. The biological results indicated that the twigs of *O. obovata* have bioactive metabolites with antimicrobial potential.

Keywords: antimicrobial activity, phenolics, flavonoids, *Ozoroa obovata*.

RESUMO: Em Moçambique, grande parte da população depende das plantas para o tratamento de várias doenças. No entanto, algumas delas foram pouco estudadas com relação aos aspectos químicos e biológicos. Dentre essas espécies, *Ozoroa obovata* é amplamente utilizada na medicina tradicional de Moçambique. Os fatores que influenciam o uso de plantas medicinais nos países em desenvolvimento são principalmente os hábitos culturais, a ineficácia do sistema de saúde e o elevado custo dos medicamentos. Este trabalho teve como objetivo estudar a composição química e avaliar a atividade antimicrobiana dos galhos de *O. obovata*. A partir da análise por cromatografia líquida acoplada a espectrometria de massas com ionização por electrospray (CLAE-EM-IES) foi possível propor que no extrato etanólico dos galhos, a maioria dos constituintes pertence a classes dos ácidos fenólicos e flavonoides. Os compostos fenólicos foram representados pelos ácidos quínico, gálico, protocatecuico e cinco ácidos anacárdicos, enquanto que os flavonoides foram representados por mangiferina, taxifolina e quercetina mono e dihexosídeo. O extrato foi avaliado frente as bactérias da cavidade bucal e mostrou moderada atividade contra *Streptococcus mutans*, *S. mitis* e *Porphyromonas gingivalis* (concentração inibitória mínima - CIM 400 µg mL⁻¹). Com relação ao ensaio antifúngico, o extrato etanólico apresentou atividade contra *Candida albicans* com CIM de 3000 µg mL⁻¹. Os resultados biológicos indicaram que os galhos de *O. obovata* possuem metabólitos bioativos com potencial antimicrobiano.

Palavras chave: atividade antimicrobiana, fenólicos, flavonoides, *Ozoroa obovata*.

INTRODUCTION

Despite advances in the fields of science and medicine, diseases remain a serious threat to public health in developed and developing countries, urban and rural areas and all ethnic groups (PAN et al., 2014). It is estimated that in developing countries, a large part of the population depends on traditional practices and medicinal plants to meet basic health needs (SINGH, 2015). In countries where the population is most vulnerable to health problems, medicinal plants and herbal medicines play an important role in the treatment of diseases. For thousands of years, medicinal plants have been used in virtually all cultures as flavorings, preservatives in foods and as a source of medicines for the treatment and prevention of diseases (SINGH, 2015). Currently, plants are one of the main sources for the development of new drugs (NEWMAN; CRAGG, 2016, CALIXTO, 2019).

Particularly in Mozambique, trade and the use of various species of plants are strongly inserted in the daily life of the population. In Mozambique, bacterial and parasitic diseases contribute to a high rate of mortality and morbidity. Even with the intervention of antibiotics and antiparasitic drugs, the potential for treating some diseases has decreased due to the indiscriminate use of these drugs. As a result, the population has looked to medicinal plants for an alternative source for the treatment of diseases (SHARIFIFAR et al., 2016, BARBOSA et al., 2020).

In this context, *Ozoroa obovata* (Oliv.) R. & A. Fer. (Anacardiaceae), is marketed and traditionally used in Mozambique for the treatment of dysentery, inflammation of the chest, respiratory infections, low back pain, cough, chest pain, fever, diarrhea, and wounds (BANDEIRA; GASPAR; PAGULA, 2001, GRACE et al., 2003, YORK; DE WET; VAN VUUREN, 2011, YORK; VAN VUUREN; DE WET, 2012, WÜRGER; MCGAW; ELOFF, 2014, SHARIFIFAR et al., 2016, BARBOSA et al., 2020). The potential against microorganisms that cause respiratory infections (YORK; VAN VUUREN; DE WET, 2012) and the presence of phenolic compounds (WÜRGER; MCGAW; ELOFF, 2014) in the leaves of *O. obovata* have already been reported. *O. obovata* is generally found in several South African countries such as Mozambique, Zimbabwe, Malawi and Tanzania (BANDEIRA; GASPAR; PAGULA, 2001, GRACE et al., 2003). Part of the economy and livelihood of the population of many of these countries depends on the exploration and trade of medicinal plants (TIMMERMANN; SMITH-HALL, 2020). However, the rapid growth of urbanization (BARBOSA et al., 2020), the destruction of forests by fire (MLIGO, 2019), overexploitation of plant resources, unsustainable harvesting practices (BRUSCHI et al., 2014), has aroused concern with the availability of these resources in the future (SEN; SAMANTA, 2014).

In Maputo (Mozambique), an important center for the collection and trade of medicinal plants, of the different parts of the plants explored by the population, 75% are roots, 10% leaves, 7% stems, 4% fruits and 4% other parts (BARBOSA et al., 2020). When the root is collected, the vegetable is removed permanently from its habitat. Thus, this type of practice, if not done in a planned way, can contribute to a decline in local and planetary biodiversity (SEN; SAMANTA, 2014). Therefore, it is of fundamental importance to investigate parts of the plant that preserve its integrity.

The prevalence of antibiotic-resistant microorganisms represents a great threat to public health, justifying the development of new strategies for the prevention and treatment of infectious diseases (SHARIFIFAR et al., 2016, HOKKEN et al., 2019). According to the World Health Organization, bacteria and fungi have been developing new resistance mechanisms resulting in ineffective treatments and longer illnesses leading to the patient's

death (WHO, 2020). Microbial resistance is a natural phenomenon that occurs when microorganisms are treated with drugs, some susceptible are killed and the most resistant can survive and multiply making the drugs ineffective (PRESTINACI; PEZZOTTI; PANTOSTI, 2015). The microorganisms evaluated in the present work are related to diseases of the oral cavity and diseases caused by fungi. The oral bacteria tested promote diseases such as periodontitis, gingivitis and dental caries, in addition to systemic conditions that include cardiovascular and intestinal diseases, rheumatoid arthritis, brain abscesses, diabetes, among others (ZARCO; VESS; GINSBURG, 2011, CHIMENOS-KÜSTNER; GIOVANNONI; SCHEMEL-SUÁREZ, 2017). Fungi are involved with skin diseases and invasive infections such as fungemia, meningitis, pneumonia and pulmonary aspergillosis, bronchopulmonary allergies, asthma and obstructive pulmonary disease. (WHITE et al., 2014, PERLIN; RAUTEMAA-RICHARDSON; ALASTRUEY-IZQUIERDO, 2017, HOKKEN et al., 2019).

Within this work, the chemical composition by liquid chromatography coupled to mass spectrometry (HPLC-MS-ESI) and the antimicrobial activity of the twigs from *O. obovata* were investigated. Highlights here that the studies were focused on the twigs because the indications of popular use, chemical composition and biological properties are open in the literature.

MATERIAL E METHODS

Extract preparation and phytochemical screening

The twigs of *O. obovata* were collected in Maputo Province, Marracuene District, Mozambique. The species was identified by a specialist and a voucher specimen was deposited at the Herbarium of the Institute of Traditional and Alternative Medicine in Misau, Maputo-Mozambique under identification number 18. The samples were dried at room temperature for ten days and taken to Brazil at the Federal University of Uberlândia. The plant material was placed in a circulating air oven at 35°C and the moisture content was monitored in an infrared light balance (QUIMIS, model kett FD-600). The samples were removed from the oven when the moisture content was below 10%. The dry vegetable material was ground (61g) and then the ethanolic extract was prepared by maceration using ethanol 98% for 48 hours. This extraction procedure was repeated five times. The ethanolic extract (EE) was concentrated on a rotary evaporator (IKA, RV 10), lyophilized (TERRONI, LS3000) and finally stored in the freezer at - 5 °C.

Phytochemical screening was performed using Thin Layer Chromatography (CCD), with silica gel 60 plates and fluorescence indicator (UV₂₅₄). The extract was solubilized in ethanol and developed with the mobile phase consisting of hexane: ethyl acetate (4:1v/v). The following developing agents were used: iodochloroplatinate, dragendorff, KOH, NP/PEG, sulfuric vanillin, Liebermann Burchard and ceric sulfate (WAGNER; BLADT, 1996).

Analysis by High Performance Liquid Chromatography coupled to Mass Spectrometry (HPLC-MS)

Analysis by HPLC-MS of the ethanolic extract of *O. obovata*, was carried out on a liquid chromatograph (Agilent, model Infinity 1260), coupled to a high-resolution mass spectrometer QTOF (Quadrupole Time of Flight - Agilent, model 6520 B), with electrospray

ionization source (ESI). A volume of 1.0 μL of the sample was injected into the chromatograph using a Agilent Zorbax C18 column (2.1mm x 50 mm, 1.8 μm). The chromatographic conditions were ultrapure water with formic acid (0.1%, v/v) (mobile phase A) and methanol (mobile phase B). The gradient system was: 10% B (0 min), 98% B (0–10 min), remaining with 98% B (10 - 17 min) with a flow of 0.6 mL min^{-1} . The ionization parameters were: nebulizer pressure of 58 psi, drying gas at 8 L min^{-1} at a temperature of 220 $^{\circ}\text{C}$ and an energy of 4.5KV was applied to the capillary. The analysis was performed in the negative mode $[\text{M-H}]^{-}$ in high resolution (MS). The molecular formula was proposed for each compound according to a list suggested by the MassHunter® Software following the lowest difference between the experimental mass and the exact mass, error in ppm, unsaturation equivalence and nitrogen rule. The sequential mass spectrometry (MS^2) of the molecular ions was performed at different collision energies. The chemical composition of the extract was proposed comparing the mass spectra of fragments and the mass in high resolution obtained with other works in the literature and Metlin library.

Antibacterial activity

The evaluation of antibacterial activity of ethanol extract from *O. obovata* twigs was performed using the broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI, 2012a, CLSI, 2012b). The microorganisms used in the tests of the antibacterial activity and their respective references from the American Type Culture Collection (ATCC, RockvilleMD, USA) were *Streptococcus sanguinis* (ATCC10556), *Streptococcus mitis* (ATCC49456), *Streptococcus mutans* (ATCC25175), *Agreggatibacter actinomycetemcomitans* (ATCC43717), *Fusobacterium nucleatum* (ATCC25586), *Porphyromonas gingivalis* (ATCC33277), *Actinomyces naeslundii*, (ATCC19039) and *Bacteroides fragilis* (ATCC25285). The tested concentrations varied between 400 to 25 $\mu\text{g mL}^{-1}$. The minimum inhibitory concentration (MIC) was correlated to the lowest concentration of extract capable of inhibiting the growth of the microorganisms. The details of the methodology, as well as the controls used to validate the results are described in the work by Rocha et al., 2018.

Antifungal activity

The evaluation of antifungal activity of ethanol extract from *O. obovata* twigs was performed using the broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI, 2008). The following microorganisms from the American Type Culture Collection were used: *Candida albicans* (ATCC 28366), *Candida tropicalis* (ATCC 13803) and *Candida glabrata* (ATCC 15126). The tested concentrations varied between 3,000 to 1.46 $\mu\text{g mL}^{-1}$. The entire procedure and controls used in the tests are available in Rocha et al., 2018.

RESULTS AND DISCUSSION

Extract yield and phytochemical screening

The EE of the twigs of *O. obovata* was prepared using the maceration technique, a process that occurs at room temperature allowing the extraction of the substances present

in the extract without degradation (SIMOES et al., 2017). The yield of *O. obovata* ethanolic extract was 3.0 g in relation to the dry mass of the twigs (4.9% m/m).

Preliminary phytochemical screening (**Table 1**) showed the presence of flavonoids and phenolic compounds. Alkaloids, anthraquinones, anthrones and coumarins were not found in the EE of *O. obovata*.

Table 1. Phytochemical screening of *O. obovata* extract

Developers	Class of Compounds	Ethanol Extract
NP/PEG	Flavonoids	++
Iodocloroplatinate Dragendorff	Alkaloids	–
KOH	Anthraquinones, anthrones and coumarins	–
Liebermann Buchard	Triterpenes and steroids	++
Ceric sulfate	Terpenes and flavonoids	++
Sulfuric vanillin	Phenylpropanoids, tannins and flavonoids	++

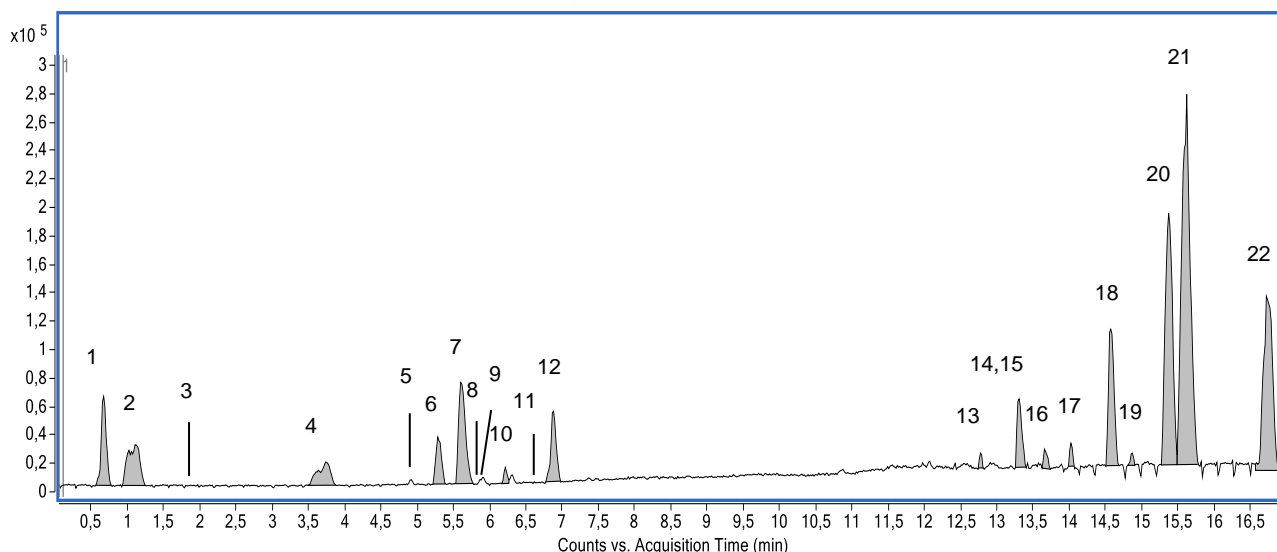
In literature, no studies using *O. Obovata* such as in this paper were found. However, other species of the genus have already been investigated. The phytochemical analysis of *Ozoroa pulcherrima* showed the presence of compounds such as phenols, flavonoids, terpenes, condensed and hydrolyzable tannins, triterpenes and saponins. (JATSA et al., 2019). *Ozoroa paniculosa* and *Ozoroa Mucronata* presented phenolic compounds, proanthocyanidins, galotanins and flavonoids (AHMED et al., 2014). *Ozoroa insignis* extracts showed the presence of hydrolyzable tannins, flavonoids, saponins, steroids and alkaloids (NYABERI et al., 2010).

Identification of the compounds present in the ethanolic extract of the twigs of *O. obovata* by HPLC-MS-ESI.

The proposal to identify the compounds present in the *O. obovata* extract was carried out from the analysis by HPLC-MS-ESI in the negative mode. The chromatogram represented in **Figure 1** shows the chemical profile of the ethanolic extract. It was possible to propose the identification of 15 compounds (**Table 2**), through the mass of molecular ions in high resolution, error (ppm) and analysis of the fragmentation profile of the compounds, comparing with data from the literature and Metlin library. The proposed chemical structures are shown in **Figure 2**.

In the first 5.2 minutes of the chromatogram (**Figure 1**), it was possible to identify phenolic acids in which (1, 2 and 3) corresponding to the compounds quinic, galic and protocatechuic, respectively, as well as compound 6, an ester of gallic acid. Between 5.5 and 7.0 minutes, the following compounds were identified: aglycone flavonoid as taxifolin (8) and hexosides flavonoids corresponding to the compounds mangiferin (7), quercetin-dihexoside I (10), quercetin-hexoside (11) and quercetin-dihexoside II (12). At 13.7 minutes (compound 16), was identified as palmitic acid. Finally, after 14.0 minutes, a series of phenolic acid derivatives with long chain were identified, these compounds correspond to anarcadic acids (18, 19, 20, 21, and 22).

Figure 1. Chromatogram of the twigs of *O. obovata* obtained in the negative mode



Chromatographic conditions: C18 column; gradient: methanol/water acidified with 0.1%v/v formic acid. 10-98% methanol (0-10 min), 98% methanol (10-17 min).

Through the identification proposal it was possible to confirm the presence of flavonoids, phenolic compounds and derivatives that were evidenced in phytochemical screening. The chemical composition of other species of *Ozoroa* corroborate with some classes of compounds identified in *O. obovata*. From the roots of *O. pulcherrima*, a derivative of phenolic acid has been identified (JATSA et al., 2019) and two anacardic acids have already been isolated (CHRISTELLE et al., 2011). A flavonoid and several anacardic acids were isolated from the roots of *O. insignis* (LIU; ABREU, 2006, NG'ANG'A et al., 2009). These classes of metabolites have also been reported in the leaves of *O. mucronata* and *O. paniculosa* (AHMED et al., 2014).

Table 2. Proposal for identification of the compounds in the ethanolic extract of the twigs of *O. obovate* (To be continued)

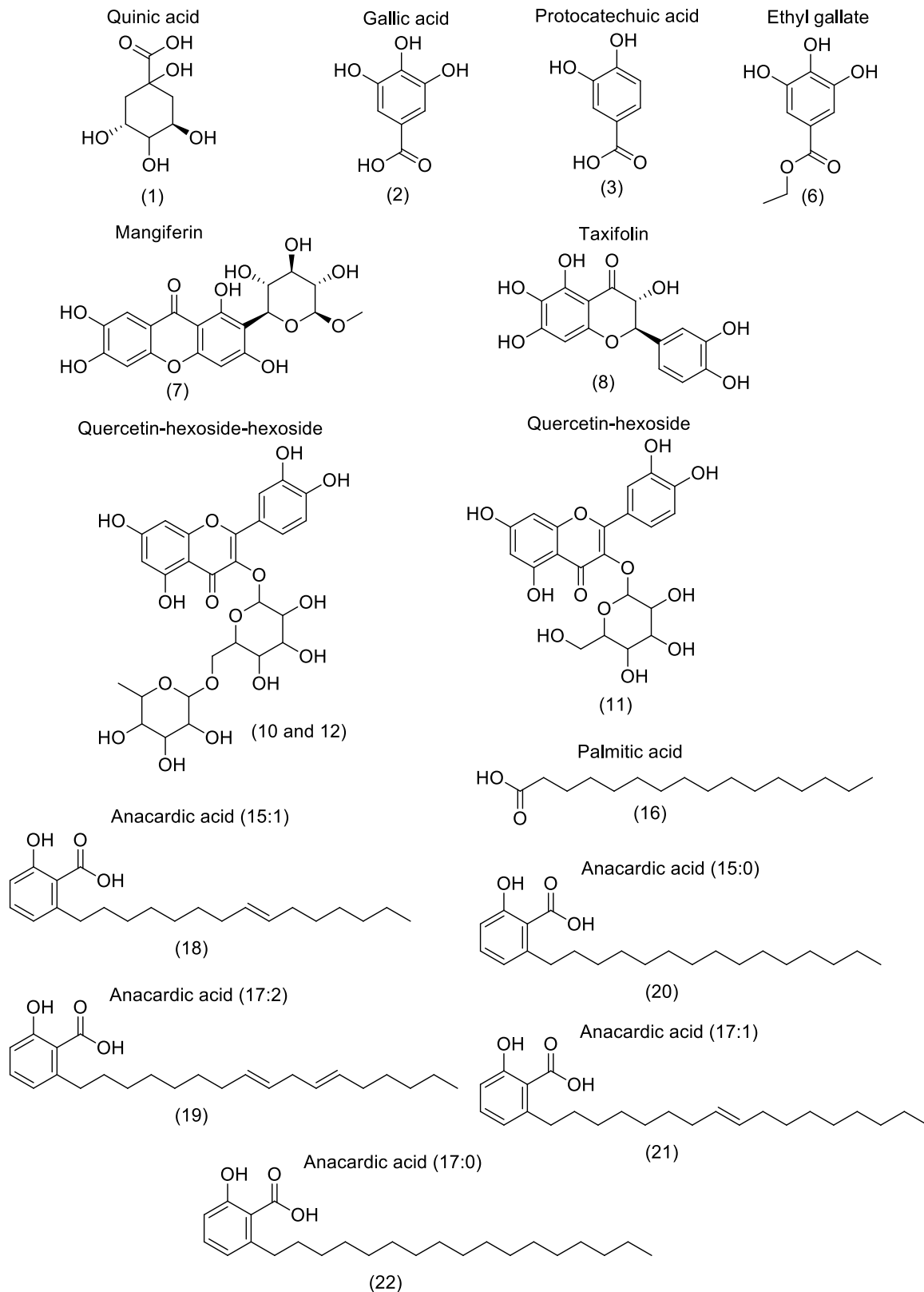
Num.	Rt	[M – H] ⁻	Exact mass	Error (ppm)	Fragments MS ² (-)	Molecular formula	Tentative identity	Reference
1	0.77	191.0558	191.0561	- 1.57	20 eV: 173, 109	C ₇ H ₁₂ O ₆	Quinic acid	Abu-Reidah et al., 2015
2	0.93	169.0141	169.0142	- 0.59	20 eV: 125	C ₇ H ₆ O ₅	Gallic acid	Wyrepkowski et al., 2014; Erşan et al., 2016; Metlin
3	1.92	153.0191	153.0193	- 1.31	15 eV: 109 , 108	C ₇ H ₆ O ₄	Protocatechuic acid	SUN et al., 2007, COSTA SILVA et al., 2019
4	3.73	407.0984	–	–	10 eV: 317, 287 , 271, 245, 145, 193, 161, 125	–	NI	–
5	4.91	421.1131	–	–	10 eV: 301, 258, 207 , 192, 179	–	NI	–
6	5.23	197.0455	197.0455	0.0	20 eV: 169; 125, 124	C ₉ H ₁₀ O ₅	Ethyl gallate	Sun et al., 2007
7	5.66	421.0776	421.0776	0.0	20 eV: 331, 301 , 272, 271, 259, 258, 243, 215, 109	C ₁₉ H ₁₈ O ₁₁	Mangiferin	Dorta et al., 2014; Lasano et al., 2019
8	5.91	303.0512	303.0510	0.33	20 eV: 285, 257, 217, 200, 199, 175, 151, 125 , 107	C ₁₅ H ₁₂ O ₇	Taxifolin	Sun et al., 2007; Ye et al., 2012; Metlin
9	5.93	435.0937	–	–	–	–	–	–
10	6.30	609.1463	609.1461	0.32	10 eV: 301, 300, 271, 255, 243, 179, 151	C ₂₇ H ₃₀ O ₁₆	Quercetin-hexoside-hexoside I	Sun et al., 2007; Said et al., 2017

Num.	Rt	[M – H] ⁻	Exact mass	Error (ppm)	Fragments MS ² (-)	Molecular formula	Tentative identity	Reference
11	6.78	463.0881	463.0882	- 0.21	20 eV: 301, 300, 271, 255, 243 179, 151	C ₂₁ H ₂₀ O ₁₂	Quercetin- hexoside	Han et al., 2008; Oliveira et al., 2018; Costa Silva et al., 2019
12	6.94	609.1461	609.1461	0.00	10 eV: 301, 300, 271, 255, 243, 179, 151	C ₂₇ H ₃₀ O ₁₆	Quercetin-hexoside-hexoside II	Sun et al., 2007; Said et al., 2017
13	12.77	377.2333	–	–	20 eV: 303, 259, 231 , 204, 163, 150	–	NI	–
14	13.30	405.2644	–	–	20 eV: 343, 331, 273, 259, 203, 150, 119, 106	–	NI	–
15	13.30	389.2693	–	–	25 eV: 343, 327, 287	–	NI	–
16	13.70	255.2327	255.2330	- 1.18	10 eV: 231, 213, 176, 148, 115	C ₁₆ H ₃₂ O ₂	Palmitic acid	Gómez-Romero et al., 2010
17	14.02	503.3735	–	–	20 eV: 469, 384, 344, 167	–	NI	–
18	14.60	345.2435	345.2435	0.0	20 eV: 301 , 119, 106	C ₂₂ H ₃₄ O ₃	Anacardic acid (15:1)	Erşan et al., 2016
19	14.82	371.2590	371.2592	- 0.54	20 eV: 327 , 133, 161, 119, 106	C ₂₄ H ₃₆ O ₃	Anacardic acid (17:2)	Erşan et al., 2016
20	15.35	347.2592	347.2592	0.0	20 eV: 303 , 119, 106	C ₂₂ H ₃₆ O ₃	Anacardic acid (15:0)	Erşan et al., 2016
21	15.67	373.2748	373.2748	0.0	20 eV: 329 , 133, 119, 106	C ₂₄ H ₃₈ O ₃	Anacardic acid (17:1)	Erşan et al., 2016
22	16.67	375.2905	375.2905	- 0.26	20 eV: 331 , 119, 106	C ₂₄ H ₄₀ O ₃	Anacardic acid (17:0)	Erşan et al., 2016

Rt: Retention time (minutes); NI: not identified; --: not obtained;

Metlin - Online library available at: https://metlin.scripps.edu/landing_page.php?pgcontent=mainPage

Figure 2. Structure of the compounds identified in the EE of the twigs of *O. obovata*



Antimicrobial activity

The antimicrobial activity of the ethanolic extract of the twigs of *O. obovata* was carried out using the broth microdilution method. The minimum inhibitory concentration (MIC) values for aerobic and anaerobic bacteria and yeast are shown in **Table 3**.

The MIC value is used as a parameter in the literature by several authors to classify the activity potential expressed by plant extracts. According to Kuete et al. (2010), MIC values below 100 $\mu\text{g mL}^{-1}$, the activity is considered significant; between 100 to 625 $\mu\text{g mL}^{-1}$, moderate and MIC values above 625 $\mu\text{g mL}^{-1}$, weak. Considering these parameters, *O. obovata* extract showed moderate activity for *S. mutans*, *S. mitis* and *P. gingivalis* (MIC of 400 $\mu\text{g mL}^{-1}$). Regarding anti-*Candida* activity, the *O. obovata* extract showed a weak effect against *C. albicans* (MIC of 3000 $\mu\text{g mL}^{-1}$) and was inactive against *C. tropicalis* and *C. glabrata* within the range of the tested concentrations (MIC > 3000 $\mu\text{g mL}^{-1}$).

Table 3. Minimum Inhibitory Concentrations (MIC, $\mu\text{g mL}^{-1}$) of the *O. obovata* extract

	Microorganisms	EE	Positive Control
Aerobic bacteria	<i>S. mutans</i>	400	0.92 ^a
	<i>S. mitis</i>	400	3.68 ^a
	<i>S. sanguinis</i>	>400	3.68 ^a
	<i>A. actinomycetemcomitans</i>	>400	1.84 ^a
Anaerobic bacteria	<i>P. gingivalis</i>	400	3.68 ^a
	<i>F. nucleatum</i>	>400	1.84 ^a
	<i>A. naeslundii</i>	>400	1.84 ^a
Yeasts	<i>C. albicans</i>	3000	0.25 ^b
	<i>C. tropicalis</i>	>3000	0.25 ^b
	<i>C. glabrata</i>	>3000	0.12 ^b

^aChlorhexidine dihydrochloride, ^bAmphotericin B. Control yeasts for validation of the method by the protocol M27-A3 CLSI (2008): *Candida krusei* – CIM 1 $\mu\text{g mL}^{-1}$; *Candida parapsilosis* – CIM 0.25 $\mu\text{g mL}^{-1}$.

Several species of plants from Africa have already been evaluated in the literature regarding their antimicrobial potential. This is due to the wide use of medicinal plants by the population for the treatment of fevers, infectious diseases, wound healing, among others. (VAN VUUREN, 2008, NCUBE; FINNIE; VAN STADEN, 2012, VAN VUUREN; HOLL, 2017). Akhalwaya et al. (2018) evaluated the antimicrobial activity of 31 species used in folk medicine to treat oral infections. In this study, the authors considered the antimicrobial activity notorious when the leaves of *Clematis brachiata* exhibited MIC values below 1000 $\mu\text{g mL}^{-1}$ for *Candida* species, and when the stems of *Englerophytum magalismonatanum* inhibited the growth of *S. mutans* and *S. sanguinis* with concentrations of 830 $\mu\text{g mL}^{-1}$ and 670 $\mu\text{g mL}^{-1}$, respectively. The twigs of *O. obovata* inhibited the growth of *S. mutans*, an important etiologic agent of dental caries, with MIC value below what was considered

notorious by these authors ($400 \mu\text{g mL}^{-1}$). Thus, the antibacterial activity observed for EE of *O. obovata* twigs is relevant, particularly against cariogenic bacteria.

Regarding the *Ozoroa* species, *O. mucronata* and *O. paniculosa* were evaluated against various bacteria and fungi, and presented MIC values between 19–2500 and 19–1250 $\mu\text{g mL}^{-1}$, respectively (AHMED et al., 2014). The leaves, bark and roots of *O. reticulata* showed inhibition against Gram-positive and Gram-negative bacteria with MIC values between 3.9-1000 $\mu\text{g mL}^{-1}$ (MAREGESI et al., 2008). *O. engleri* showed a MIC value of 750 $\mu\text{g mL}^{-1}$ against *C. albicans* (NAIDOO et al., 2013). These results show that the *Ozoroa* genus has great potential as antimicrobial agents.

The phenolic compounds identified in the EE of the twigs of *O. obovata* are well known in the literature for presenting antimicrobial activity (ZACCHINO et al., 2017, TOCCI et al., 2018, BOUARAB-CHIBANE et al., 2019, LIMA et al., 2019). Anacardic acids have also been described as having antibacterial activity against *Staphylococcus aureus* (MAMIDYALA et al., 2013, ANJUM et al., 2019), *Streptococcus mutans* (GREEN et al., 2008) and other microorganisms (KUBO et al., 1993). Although the EE of *O. obovata* showed moderate activity against the tested microorganisms, the presence of bioactive compounds in the twigs of this species was evidenced. Future studies of fractionation of the ethanolic extract are desired, since the concentration of bioactive compounds in fraction can increase the antimicrobial potential and better express the biological properties of the species.

In addition, investigating the presence of active metabolites in parts of plants that are not related to destructive collection, is relevant to the preservation of biodiversity and maintenance of the livelihood of people living in areas dependent on local flora.

CONCLUSION

The phytochemical screening of EE of *O. obovata* twigs showed the presence of phenolic acids, flavonoids, and long chain phenolic derivatives (anacardic acids) that were confirmed by HPLC-MS-ESI. These classes have already been reported in the genus and have recognized antimicrobial properties. The evaluation of antimicrobial activity showed that EE has moderate activity for oral bacteria *S. mutans*, *S. mitis* and *P. gingivalis*. The biological activity results and MS analysis are relevant because indicated the presence of active compounds justifying for advanced of antimicrobial studies with this plant. The chemical composition of *O. obovata* twigs and their antimicrobial evaluation against oral bacteria and *Candida* spp. are being reported here for the first time.

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