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Antioxidant and antimicrobial capacity of *Maytenus boaria* leaves, recovery by infusion and solvent extraction

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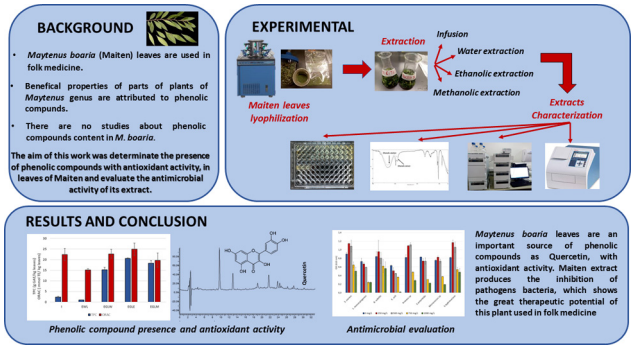
GRAPHICAL ABSTRACT



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ABSTRACT

Background: Infusions of Maiten (*Maytenus boaria*) leaves are used in folk medicine as an antipyretic and for skin allergic rash treatment. Antioxidant, antipyretic and anti-inflammatory properties of medicinal plants have been attributed to phenolic compounds presence. This work, the first in this type, reports the presence of total phenolic compounds (TPC), total flavonoids (TFC), and the antioxidant activity by DPPH, FRAP and ORAC methods of extracts of maiten leaves, including an infusion; also, antimicrobial activity on Gram + and Gram − bacteria were determined. In addition, the presence of Quercetin and catalase were determined, and the FTIR spectrum was done.
Results: The best values of TPC and TFC were 20.55 g_{GAE}/kg_{leaves}, and 23.69 $g_{QE}/kg_{leaves,}$ respectively, in an ethanolic extract. Best antioxidant activities were obtained in a methanolic extract for DPPH, aqueous extract for FRAP. No significant differences were observed in ORAC activity determination of aqueous, ethanolic, and methanolic extracts and a maiten infusion, with an average value of 22.41 $mmol_{TE}/kg_{leaves}$. In the case of antimicrobial activity, extract of maiten leaves can inhibit the growth of bacteria as *Escherichia coli* and *Listeria monocytogenes*, among others.
Conclusions: *M. boaria* leaves are an important source of phenolics compounds with antioxidant activity; these properties are observed in extracts and infusions. In addition, the extract of maiten leaves produces

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the inhibition of pathogens Gram+ and Gram− bacteria, showing the great therapeutic potential of this plant used in folk medicine.

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1. Introduction

The maiten (*Maytenus boaria mol.*) is an evergreen tree with a rounded, leafy crown and hanging branches; of greyish and cracked bark, reaching up to 15 m in height. It belongs to the family of the *Celastraceae*, which includes more than 200 species of the genus *Maytenus* [1,2].

This tree grows in countries such as Argentina, Peru, Brazil, and Chile, where it is found from sea level to 1800 m altitude, being very common in lowlands and slopes. It is a very adaptable species to diverse types of environments, and generally grows associated with other species in different soil and climate conditions. Although it is used mainly for ornamental purposes, as a source of wood and food for livestock, there are also some antecedents that indicate its use in folk medicine. Leaves, roots, stems, and bark of plants of *Maytenus* genus [2] such as *M. buxifolia* (leaves), *M. camariensis* (leaves), *M. emarginata* (fruit), *M. heterophyla* (leaves) are traditionally used due to their beneficial effects to health, such as antiulcer, diuretic, laxative, antitumor, anti-inflammatory, digestive, contraceptive, anti-diarrhea, antiallergic, antiasthmatic [3,4,5,6,7,8], among other things. These effects are attributed to the presence of bioactive compounds that have been identified in some *Maytenus* species, such as sesquiterpenes [3,9,10,11], triterpenes and agarofurans [1,12,13], and some flavonoids such as Quercetin, quercitrin (glycoside derived from Quercetin), kaempferol glucosides, quercetin glucosides as rutin and others [7,14,15,16]. Specifically, in the case of *M. boaria*, the seeds contain oil that has been recognized as a scarring agent; also, popular medicine recognizes the antipyretic properties, and the skin rashes healing properties of maiten leaves. The literature about antipyretic, anti-inflammatory and antioxidants properties of medicinal plant extract suggest that these properties are delivered by the presence of phenolic compounds [17,18,19]. However, regarding the identification or determination of the existence of specific compounds in the *M. boaria* leaves, the literature only shows the presence of some isoterpenes, derivatives of agarofurans, and a glycoside called boaroside and its derivatives [3].

According to our knowledge, there are no studies that have evaluated the presence of polyphenolic compounds with antioxidant and antimicrobial activity from *M. boaria* leaves. Then, the purpose of this work was to establish the presence of compounds with biological activity, especially phenolic compounds with antioxidant activity, in leaves of maiten (*M. boaria*), to establish if the use of infusions– as traditional way of consumption– allows to recover these compounds, in comparison with extraction processes using different solvents; and evaluate the antimicrobial activity of the maiten extract on Gram+ and Gram− bacteria.

2. Experimental

2.1. Material

Leaves of maiten (*M. boaria mol.*) were collected in the city of Viña del Mar (Chile; latitude: 33°1,4742′S, longitude 71°33,1098′W) in June 2018 (autumn). The climatic characteristics in the collection area for the 2018 year included monthly average temperatures between 10 and 17°C, with a mean value of 13°C in June; total rainfall of 345 mm per year, with 103 mm only for June; an average atmospheric pressure of 1018 for the month of June, with similar values for the other months of the year; and relative environmental humidity in the order of 85%. The leaves were washed with tap water, dry with paper and stored at −18°C. Then, they were lyophilised (Ilshin, Korea), and vacuum stored until its use.

2.2. Phenolic compounds extraction from maiten leaves

Several solvents were used to extract the maiten leaves. An infusion of maiten leaves (traditional way of consumption) was compared with extracts obtained from grounded leaves (≤0.0.5 mm particle size; mill Ika A10 basic, Brazil). Extraction conditions are summarised in Table 1. After the extraction time, the samples were filtered, the volume recovered was measured, and the liquid extracts were freeze stored (−18°C) until its analysis. Extractions were done by duplicate, and analyses were done by triplicate.

2.3. Extracts characterization

Extracts were characterized by total solids content by drying at 100°C; soluble solids as Brix number using a refractometer (Manual Refractometer, Atago, Japan); total phenolic compounds; total flavonoids content; antioxidant activity; and quercetin content by HPLC. FTIR spectrums were evaluated to compare possible variations in the presence of functional groups. In addition, catalase activity was determined in an aqueous extract as a first approximation to antioxidant enzyme presence determination.

2.4. Catalase activity determination

An aqueous extract of maiten leaves was characterized by its catalase activity. For this, the decrease of absorbance at 240 nm for 2 min was recorded, when 3 mL of a solution of hydrogen peroxide (H_2O_2) in sodium phosphate buffer (196 mmol/L of H_2O_2, concentration used to obtain an absorbance at time 0 higher than 0.3) was mixed with 0.1 mL of properly diluted sample. The catalase activity was determined as:

$$catalase\ activity = \frac{\Delta abs * \left(\frac{V_{total}}{V_{sample}}\right) * Fd}{2 * Coef * m}$$

Where:Δabs: (absorbance time cero – absorbance 2 min2: 2 min of reactionFd: factor dilution sample.Coef: extinction molar coefficient (40 abs M^{-1})m: solid concentration of the sample.V_{total}: total volume of the assay (3.1 mL)V_{sample}: volume of the sample (0.1 mL)

2.5. Total phenolic compounds (TPCs) determination

Folin-Ciocalteu method, according to the report by Soto et al. [20] was applied to quantify the content of TPCs. For this, 0.5 mL of sample (adequately diluted) were mixed with 3.75 mL of deionized water, 0.25 mL of Folin-Ciocalteu phenol reagent (diluted

Table 1
Processes of phenolic compounds extraction from maiten (*Maytenus boaria mol*) leaves.

Sample/extract	State of leaves	Solvent	Solid/Liquid ratio	Other conditions
Infusion (I)	Whole	Boiling tap water	1 g_{leaves}/200 mL	5 min
Extract of whole leaves (EWL)	Whole	Deionized water	1 g_{leaves}/20 mL	1 h, 50°C
Extract of Grounded Leaves with Water (EGLW)	Grounded	Deionized water	1 g_{leaves}/20 mL	1 h, 50°C
Extract of Grounded Leaves with Ethanol (EGLE)	Grounded	Ethanol 80%	1 g_{leaves}/20 mL	1 h, 50°C
Extract of Grounded Leaves with Methanol (EGLM)	Grounded	Methanol 80%, acidified (0.1% o-phosphoric acid)	1 g_{leaves}/20 mL	1 h, 50°C

twice) and 0.5 mL of 10% (w/v) sodium carbonate. The mix was maintained at room temperature for 1 h, and then, the absorbance at 765 nm was determined compared to a blank prepared with the extracted solvent instead of the sample. Gallic acid was used as standard.

2.6. Flavonoids determination

Total flavonoids content (TFC) were determined using $AlCl_3$ method [21]. Briefly, 0.5 mL of sample was mixed with 1.5 mL of ethanol (95%). Then, 0.1 mL of 100 g/L $AlCl_3$ and 0.1 mL 1 mol/L CH_3COOK, and 2.8 mL of distilled water were incorporated. The samples were maintained at room temperature for 30 min, and the absorbance was measured at 415 nm. Quercetin was used as standard.

2.7. Antioxidant capacity determination

The antioxidant activity of the samples was determined by three methods: DPPH radical scavenging method, FRAP method and ORAC method. (a) The DPPH (2,2diphenyl1picrylhydrazyl = 2,2 diphenyl1(2,4,6trinitrophenyl) hydrazyl) radical scavenging capacity was measured according to Laroze et al. [22]. Briefly, 0.2 mL of 3.6 10^{-5} mol/L of methanolic solution of DPPH (Sigma) was added to 0.005 mL of the sample in a 96 wells plate. The absorbance at 515 nm was determined at time zero (0) and 16 min using a multi-wells plate reader spectrometer. The antioxidant capability was expressed as the percentage of discoloration of the DPPH solution (free radical scavenging); (b) the FRAP method, which employs a redox-linked colorimetric assay, was conducted according to Soto et al. [20]. Briefly, 3 mL of FRAP solution (10:1:1 of 300 mmol/L acetate buffer pH 3.6, 10 mmol/L of 2,4,6-tripyridyl-s-triazine (TPTZ) prepared in 40 mM HCl, and 20 mmol/L $FeCl_36H_2O$, respectively) was mixed with 0.1 mL of the phenolic extract. The absorbance at 593 nm was recorded after 6 min, Trolox was used as the standard. Results were expressed as g Trolox equivalents (TE) per kg of dry maiten leaves; (c) The ORAC method was done according to Soto et al. [20], using 0.2 mL of fluorescein (108 nmol/L in PBS buffer pH 7.4) and 0.02 mL of phenolic extract. The samples were incubated at 37°C for 10 min, and then, 0.075 mL of 2,2-azobis (2-amidinopropane) dihydrochloride (AAPH) (79.7 mmol/L in PBS buffer pH 7.4) was added. Fluorescence was recorded during 60 min using 485/538 nm excitation/emission wavelengths. Trolox was used as the standard. Results were expressed as mmol TE per kg of dry maiten leaves.

2.8. Quercetin determination by HPLC

As a first determination of the presence of phenolic compounds present in the extracts of maiten leaves, HPLC with a diode array detector was used. For this purpose, acetonitrile was used as mobile phase A, and water/acetic acid/phosphoric acid (89/10/1) as mobile phase B, in a process comprising 5 min of equilibrium with 98% B, a gradient of 28 min until reaching 80% of phase B, and then a gradient of 5 min to reach 60% of B; the flow was 1 mL/min. The column used was a C18 (250 × 4.6 mm, 5 μm) maintained at 40°C. The detection of the compounds present in the system was carried out at 520 and 280 nm. Specifically, for the Quercetin case, a calibration curve of 2–20 mg/L was made.

2.9. FTIR spectroscopy

Liquid samples (extracts) were analyzed with an ATR-FTIR (FT/ir 4600, Jasco, EEUU). The IR spectra were recorded at room temperature in the range of 4000–600 cm^{-1} (mid infrared spectra). The spectrums were displayed in terms of transmittance (%), using 60 scans for a simple spectrum. The samples were compared with the spectrum of the extraction solvents used in the processes.

2.10. Antimicrobial activity

Eight bacteria were used to evaluate the antimicrobial activity of maiten extracts. *Salmonella typhimurium* (ATCC 140285), *Proteus* sp. wild type, *Escherichia coli* wild type, *Salmonella enteritidis* (SARB16), *Staphylococcus aureus* (VQSA68), *Listeria monocytogenes*, *Micrococcus* sp. wild type and *Bacillus subtilis* were cultured in a Mueller Hinton broth at 37°C until reaching a cell density of 10^8 cells/mL. For the antioxidant extract, the organic solvent was removed and subsequently lyophilised until a powdery product was obtained. The maiten extract was resuspended in a sterile Mueller Hinton medium and filtered in a sterile way. For the assays, the extract was used at concentrations of 0 (growth control in Mueller Hinton broth), 250, 500, 750 and 1000 ppm. The microorganisms were cultured in 96-well plate at 37°C for 24 h. Additionally, the culture medium was incubated without and with the extracts, without the presence of the microorganisms. The OD of the samples was recorded at 610 nm.

2.11. Statistical analyses

Extractions and analyses were performed in triplicate. The data are presented as the mean value ± SD. GraphPad Prism 5 software was used for statistical analyses. *P* values >0.05 means no-significant differences between the groups compared.

Table 2
Effect of extracting solvent in solids recovery from maiten (*Maytenus boaria mol*) leaves.

Sample	Soluble solids (°Brix $_{ref.}$ or g solid/100 g)	Total Solids (g_{solids}/L)	Extraction Yield (g_{solids}/kg $_{leaves}$)
I	Nd	1.08 ± 0.22	194.77 ± 10.95
EWL	Nd	3.93 ± 0.51	67.10 ± 12.75
EGLW	2.1 ± 0.36	18.15 ± 0.81[a]	324.25 ± 14.53
EGLE	20.33 ± 0.58	15.35 ± 0.21	266.19 ± 8.47
EGLM	6.90 ± 0.10	17.30 ± 0.66[a]	300.90 ± 2.39

nd: not detected.
Brix $_{ref}$: not corrected for interferences.
The same letter in the column means: non-significant differences ($P > 0.05$).

3. Results and discussion

3.1. Extraction yield

Results of solid extractions from maiten leaves are showed in Table 2. As it is observed, a low concentration of solids is obtained with samples produced with whole maiten leaves, probably due to the smaller contact surface between the raw material and the solvent; however, it is possible to see that the solids extraction yield from the infusion (I) is much greater than the obtained in the process of extracting whole leaves (EWL), this given by the high temperature of the water used in the infusion, which increases the solubility of different molecules. With respect to the effect of the solvent on the solids's recovery during the extraction, no significant differences are observed between extract of grounded leaves with water (EGLW) and extract of grounded leaves with methanol (EGLM); while samples obtained with ethanol (80%) (Extract of grounded leaves with ethanol – EGLE) shows a value slightly smaller. Because dielectric constants of water, methanol and ethanol are 82, 33 and 24 respectively, the results obtained in this work suggest that the compounds extractables from maiten leaves are highly polar.

On the other hand, the values of extraction yields are higher those reported for others *Maytenus* species leaves, such as *Maytenus royleanus* with 122 $g_{extract}/kg_{leaves}$ [23], and between 10 and 348.6 $g_{extract}/kg_{leaves}$ for *Maytenus imbricata* [24], values that depends of the solvent used in the extraction.

3.2. Catalase activity

About catalase activity, 2,280,850 $U/kg_{extract}$ (equivalent to 900,940 U/kg_{leaves}) was detected. Catalase is an enzyme with oxidoreductase activity with the ability to degrade reactive oxygen species (ROS), which is why it is recognized as an antioxidant enzyme; also, it is used in the food industry [25]. There are few reports about catalase activity in medicinal plants and none about plant parts of *Maytenus* species. The result obtained is lower than those reported by Gaamoune et al. [26], which indicated that leaves of *S. nigrum* and *W. somnifera*, among others, have a catalase activity over 1000 U/mg_{leaves}.

3.3. Total phenolic compounds and total flavonoids

As it is observed in Table 3, best results of TPC was obtained with ethanolic extracting solvent with 20.56 g_{GAE}/kg_{leaves}. This result is agreeing to those reported by Cansian et al. [27], with values of 22. g_{GAE}/kg_{leaves} for *Maytenus dasyclada* and 24 g_{GAE}/kg_{leaves} for *Maytenus aquifolium*. While, aqueous extract of *M. ilicifolia* have 50 g_{GAE}/kg_{leaves} [27] and an extract from *M. imbricata* leaves [24] produced with ethyl acetate have 659.27 g_{GAE}/kg_{leaves}, but non polyphenols were detected when other solvents were used in the extraction in the last work. In the same way, it is possible to observe that methanolic extracts of *M. royleanus* leaves possess about 9.27 g_{GAE}/kg_{leaves} [23]. On the other hand, the TPC content of *Maytenus macrocarpa* bark [28] obtained by ultrasound-assisted extraction was reported as from 4.30 to 29.60 g_{GAE}/kg_{leaves} depending of the extraction conditions. It is important to consider that the results obtained for the leaves of *M. boaria* show that on the contrary as it happens with the total solids, more phenolic compounds are recovered when the solvent with lower dielectric constant was used (ethanol), which suggests that the highly concentrated extracts have a large amount of other soluble macromolecules, such as sugars, salts and even proteins.

In comparison to other medicinal plants, the results observed in this work from *M. boaria* leaves are much better those reported by Rajurkar and Hande [29] for 11 Indian medicinal plants, which possess up to 15.88 g_{GAE}/kg_{leaves} as it is the case of *Acacia nilotica* bark; while in the case of leaves of medicinal plants such as *Centella asiatica, Gymnena sylvestre* and *Vitex negundo*, the TPC content only reach values of 0.75, 1.14, and 3.07 g_{GAE}/kg_{leaves} respectively. On the other hand, if it is compared methanolic extracts of medicinal plants with those obtained from the leaves of *M. boaria* in this work (EGLM, corresponding to 60.50 $g_{GAE}/kg_{extract}$), it is possible to observe lower values, e.g., for extracts of *A. pancicii* and *A. sylvestrus*; while *A. grandifolia*, *H. officinalis*, and *A. crithmifolia* produce extracts with a higher content of phenolic compounds than those reported in this work, even reaching values of 170 $g_{GAE}/kg_{extract}$ [30]. In the same way, Li et al. [31] reports for traditional medicinal plants from China extracted with methanol (800 mL/L), with TPC values in the range of 1.15 and 52.35 $g_{GAE}/kg_{plant\ part}$, confirming the variability of polyphenols content on worldwide medicinal plants. When comparing the ethanolic extract of maiten leaves with some medicinal plants from Egypt [32] and from the Peruvian Amazon [33], it is possible to observe that *V. sinaiticum* has a lower content of TPC (22.2 $g_{GAE}/kg_{extract}$), whereas leaves of *C. hirsuta*, *D. angustifolia*, *J. abyssimium* and *O. lamiifolium,* and *A. montana, B. excelsa, I. edulis, M. dubia, T. cacao* and *T. grandiforium* produce extract richer in polyphenolic compounds than the one obtained in this work (EGLE, corresponding to 77.23 $g_{GAE}/kg_{extract}$); but, the EGLE TPC value is higher than the content of TPC in extracts of other parts of the plants such as stems, fruits, seeds among other [33].

Respect to the TFC value, Shabbir et al. [23] reports 7.75 g_{rutin}/kg_{leaves} of *M. royleanus* obtained using methanol (950 mL/L) as solvent. In this work, total flavonoids recovered depends of solvent used in the extraction; the best result was obtained with the ethanolic extraction (EGLE) with 23.89 g_{QE}/kg_{leaves} followed by aqueous extraction and then by methanolic extraction with a result in the same magnitude order (6.70 g_{QE}/kg_{leaves}) those above mentioned by Shabbir et al. [23]. It is important to consider that the assays performed in this work and those reported by Shabbir et al. [23] used different patterns (standard flavonoid) of equivalence; but, rutin is a derivative of Quercetin (glycosylated Quercetin). Medicinal plant leaves such as *C. asiatica, G. sylvestre* and *V. negundo* have 7.49, 5.55 and 14.13 g_{QE}/kg_{leaves} of TFC respectively [29], and obtained with a methanolic solution; these values are

Table 3
Total phenolic content (TPC), Total Flavonoid content (TFC) and antioxidant activity of maiten (*Maytenus boaria mol*) leaves obtained by different methods.

Sample	TPC (g_{GAE}/kg_{leaves})	TFC (g_{QE}/kg_{leaves})	DPPH (% inhibition/ IC_{50})	FRAP ($mmol_{TE}/kg_{leaves}$)	ORAC ($mmol_{TE}/kg_{leaves}$)
I	2.36 ± 0.36	nd	5.35% (nd)	25.27 ± 2.17	$22.37 \pm 2.92^{a,b,c}$
EWL	1.01 ± 0.04	nd	5.28% (nd)	17.71 ± 1.04	15.10 ± 0.71
EGLW	15.24 ± 1.07	10.48 ± 0.42	89.45% (8.74 g/L)	194.56 ± 12.50	$22.70 \pm 2.12^{a,d,e}$
EGLE	20.55 ± 0.29	23.89 ± 1.26	90.34% (5.43 g/L)	157.20 ± 19.64	$24.88 \pm 2.96^{b,e}$
EGLM	18.20 ± 1.29	6.70 ± 1.09	94.14% (2.34 g/L)	126.00 ± 5.74	$19.70 \pm 3.39^{c,d}$

IC_{50}: Sample concentration required to decrease DPPH absorbance in a 50%.
nd: non detected.
The same letter in the column means: non-significant differences ($p > 0.05$).

comparable or even higher those obtained from *M. boaria* leaves using similar solvent composition in this work; but, are lower those obtained with ethanolic solution (EGLE sample).

3.4. Antioxidant capacity of extracts from Maiten leaves

For antioxidant capacity determination, three methods were assessed: DPPH, FRAP and ORAC (Table 3). It is interesting to consider the fact that DPPH and ORAC methods are useful for the determination of antioxidant activity of both, hydrophilic and hydrophobic compounds [34]. The results obtained show that extract produced with acidified methanolic solution (EGLM) has the best DPPH scavenging activity with an IC_{50} value of 2.34 g_{solids}/L; while, when FRAP method was evaluated, the aqueous extract produced the best results, and for ORAC method, the ethanolic and aqueous solvents produce similar values. This behaviour can be explained for the type of reaction that evaluate every method: DPPH and FRAP techniques are related to an electron transfer mechanism in antioxidant capacity, but ORAC method is related to the hydrogen atom transfer mechanism [35]. This suggests that extracts of maiten leaves obtained with different solvents have different polyphenols profiles. On the other hand, the results observed with the extractive processes (1 g_{leaves}, 20 $mL_{solvent}$, 1 h, 50°C), show a positive correlation between TPC and FRAP (r^2: 0.7986), and TPC and ORAC (r^2: 0.7792), what does not happen with TFC, which generates much lower correlations, which would indicate that the antioxidant activity is given by phenolic compounds, but not necessarily flavonoids.

Specifically, in the case of antioxidant activity according to DPPH method, Negri et al. [36] report for *M. ilicifolia* an IC_{50} value between 4 and 7 mg/L, a much better result those obtained in this work. There is interesting that Cansian et al. [27] indicated that IC_{50} value for aqueous extracts of *M. ilicifolia* is 30 mg/L, which suggest that the origin (geographical source) or a previous treatment can affect the antioxidant activity. Other species of *Maytenus* as *M. aquifolium* and *M. dasyclada* shows IC_{50} values of 584 and 990 mg/L, respectively [27]. Also, it is possible to observe that antioxidant capacity (determined by DPPH method) depends on the extracting solvent. Silva et al. [24] indicate that extracts of *M. imbricata* leaves produced with several solvents (ethanol, chloroform, and ethyl acetate) inhibit DPPH free radical achieved up to 68.15% when a solution of 300 mg/L in ethyl acetate was used. With solvents such as ethanol and chloroform, the authors observed a lower inhibition of DPPH, even close to 17% for the last-mentioned solvent. Similar behavior was reported by Magalhães et al. [37] for extracts obtained from *Maytenus salicifolia* leaves using hexane, ethyl acetate, butanol and methanol with a 90% of DPPH inhibition when 100 mg/L of extract in ethyl acetate was used; while extracts obtained with the other solvents, at the same concentration, just produce until a 20% of inhibition.

In the case of FRAP method it is possible to observe for *M. macrocarpa* bark extracts values from 32.00 to 94.80 mmol $_{TE}$/kg bark [28], which are lower those observed for *M. boaria* leaves in this work; while the only one report about FRAP method determination in extracts from *Maytenus* leaves was done by Kgopa et al. [38] indicating that methanolic extracts from *Maytenus heterophylla* leaves produces a value of 11.12 g_{Fe}/kg. Due to this fact, an alternative is to compare with other medicinal plants; in this aspect, *M. boaria* leaves have a higher FRAP activity than *C. asiatica, G. sylvestre* and *V. negundo* plants, which report 2.92, 4.00 and 10.76 $mmol_{TE}$/kg respectively [29].

To our knowledge, there is not any report about antioxidant activity of *Maytenus* leaves (any species) determined by ORAC methods. As is above mentioned, the best ORAC results was obtained with EGLE, with 24.88 $mmol_{TE}$/kg $_{leaves}$ (equivalent to 147.64 g_{TE}/$kg_{extract}$); but non-significant differences are observed between ORAC activity – per leaves extracted– of samples I, EGLW, EGLE, and EGLM. These results are lower than those observed for *Maytenus krukovii* bark (4.50 $mmol_{TE}$/$g_{extract}$) [34] and those reported by several medicinal plants [32,33,39]. This difference with the results obtained by other authors may be due mainly to the development of more exhaustive processes of raw materials extraction, where in general, it is observed that a double extraction process is carried out at least. However, since there are no previous records concerning the type of raw material used in this work, it is impossible to make an accurate comparison, nor to determine if the result obtained with maiten leaves is affected by factors such as previous treatment or the geographical origin.

To the presence of phenolic compounds, it was of interest of this work to establish the presence of Quercetin. Samples I and EWL have such a low concentration of phenolic compounds, which are potentially below the detection limit of the equipment. On the other hand, regarding the presence of Quercetin, it can be established that the samples extracted with the methanolic solution have the best results with 0.031 g/L, followed by the ethanolic sample with 0.018 g/L, which corresponds to 0.5310 and 0.32 $g_{quercetin}$/kg of dried *M. boaria* leaves, respectively. These results are lower those reported by Soobrattee et al. [40], which evaluate the presence of different phenolic compounds in Mauritian endemic plants, observing 0.9 $g_{quercetin}$/g of fresh leaves of *Maytenus pyria*; however, the later result was obtained after that the extract was hydrolyzed, which could release the quercetin present in the extracts in its glycosylated form.

3.5. FTIR spectra of maiten leaves extracts

Finally, the infrared (IR) spectrum of the different samples studied was evaluated, as well as the extraction solvents used as a baseline. However, under the study conditions, only the ethanolic extract presented differences with respect to the pure solvent. As shown in Fig. 1, where the spectrums of ethanol (80%) and the EGLE sample are presented, there are small variations in bands at 2977, 2927, 2901, 1646, 1453, 1410, 1380, 1273, 1085, 1066 and 876 cm^{-1}, probably due to the presence of different compounds in the extract. The bands at 2977 cm^{-1}, 2927 cm^{-1} and 2901 cm^{-1} can be associated to CH stretching vibration, which can represent aliphatic groups or the presence of amino acids (CH_2); the band at 1646 cm^{-1} indicate C=O stretch, which may indicate the presence of flavonoids (amides); in the same way, happens with the band at 1410 cm^{-1} which would indicate a stretch vibration of C-N group in primary amide. Bands at 1380 cm^{-1} can represent t-butyl group or a CH3 deformation, while the bands at 1085 cm^{-1} and 1066 cm^{-1} probably represent groups CH_2 (amine) or CH –O–H in alcohols [41,42]. When Preto et al. [43] evaluate leaves of *Maytenus ilicifolia*, observe characteristic bands in 653, 1.030; 1.234; 1.608; 2.355; 2924; 3313 and 3724 cm^{-1}; while, extracts of *M. royleanus* [42] shows characteristic bands in 3310, 2938, 1608, 1458 and 1046 cm^{-1}. In this case, it is possible to observe that in the samples of *M. ilicifolia*, *M. royleanus* and *M. boaria* (this work) have similarity in bands such as those present in the vicinity of 2900 cm^{-1}, 1610 cm^{-1}, and 1030–1060 cm^{-1}.

3.6. Antimicrobial activity of maiten extract

As it is possible to observe in Fig. 2, the incorporation of maiten's extract in the culture medium of different Gram + and Gram – bacteria produces an inhibition of growth in comparison to the culture without the extract. Specifically, it is appreciated that for bacteria such as *L. monocytogenes*, *E. coli*, *S. enteritidis* and *Bacillus subtilis* the inhibition is generated from the lowest concentration used (250 mg/L). While in the case of *Proteus* sp., *S. typhimurium*, *S. aureus* and *Micrococcus* sp. at the low concentrations there is a

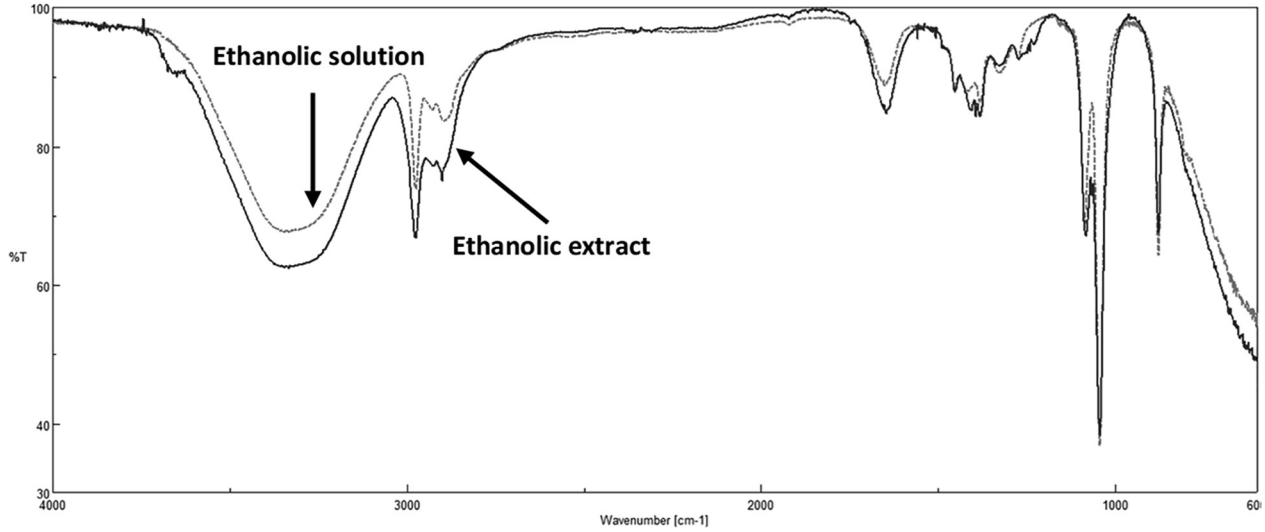

Fig. 1. IR spectra of ethanolic extract (black line) of *Maytenus boaria* leaves, compared to ethanolic solution (grey line).

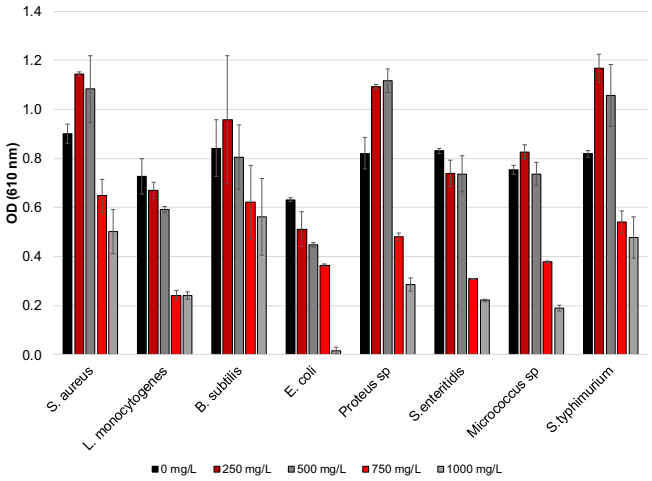

Fig. 2. Effect of maiten extract concentration in the growth of different pathogens bacteria, cultured in Mueller Hinton broth (37°C during 24 h).

slight increase in growth, but at higher concentrations of extract (from 750 mg/L) there is inhibition of cell proliferation, which potentially it is due to the presence of phenolic compounds with antioxidant activity present in the extract. Although the mechanisms by which these compounds are not well elucidated, it has been suggested that phenolic compounds can interact with some components of the membrane and the cell wall of some bacteria, and/or inhibit some microbial enzymes, generating the inhibition of growth or even cell death [44]. Particularly in the case of compounds obtained from some *Maytenus* species, the results on their antimicrobial effect are variable. For example, it has been reported that extracts of *M. ilicifolia* are not capable of inhibiting the proliferation of periodontopathogens [45]; while in the case of root-bark extracts of *M. senegalensis*, specifically of the maytenonic acid, the ability to inhibit the growth of bacteria such as *B. subtilis*, *E. coli*, *K. pneumonia* and *S. aureus* was observed [46]. A similar result was obtained for the case of *S. aureus* with extracts of *M. rigida* and *M. buchananii* [6,47]; however, it is also reported that extracts of *M. rigida* is not capable of inhibiting the growth of different strains of *Salmonella* sp. [47].

4. Conclusion

This is the first study developed in relation to the presence of phenolic compounds and antioxidant activity from leaves of *M. boaria*. The results presented show that *M. boaria* leaves are an important source of phenolic compounds (up to 20.55 g_{GAE}/kg_{leaves}), some of which correspond to flavonoids as well as Quercetin. The extraction processes were carried out to provide a first approximation of the antioxidant activity of the maiten leaves. The results depend on the solvents used and the quantification methods. Specifically, in the case of ORAC method, over 15 $mmol_{TE}/kg_{leaves}$ were detected. The results of the ethanolic extract and the infusion are similar; those suggest the good characteristics of maiten leaves on health at its traditional way of consumption. Moreover, maiten extract produces the inhibition of pathogens Gram+ and Gram− bacteria, which shows the great therapeutic potential of this plant used in folk medicine.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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