Trop J Nat Prod Res, September 2020; 4(9):578-585

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original research Article

Phytochemical, Antioxidant and Anti-Inflammatory Characterization of Leaves and Bark of *Mimusops coriacea* (A.DC) Miq from Ecuador

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ARTICLE INFO

Article history:
Received 26 August 2020
Revised 18 September 2020
Accepted 03 October 2020
Published online 03 October 2020

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ABSTRACT

Mimusops coriacea (A.DC) belonging to the family Sapotacea is a tree that grows widely in Ecuador. It is traditionally used as an analgesic and anti-inflammatory medicine. There is no phytochemical information on the leaves and bark of the plant, or pharmacological studies that justifies its traditional use. The objective of this study was to determine the chemical composition, antioxidant and anti-inflammatory activity of hydroalcoholic extracts of the leaves and bark of *M. coriacea*. Extracts were obtained by maceration from leaves and bark, using 80% hydroalcohol as solvent. Total phenols were quantified by Folin-Ciocalteu, total flavonoids by the colorimetric method of aluminum chloride and an analysis was performed by LC-MS. The antioxidant capacity by FRAP, DPPH and ABTS assays and anti-inflammatory activity using carrageenan-induced paw edema model in rats were evaluated. The results revealed a significant difference in the content of phenols and flavonoids, being higher for the leaf extract. Two glycosylated flavonoids (myricetin-3-*O*-α-L-rhamnoside and myricetin-3-*O*-glucoside) and two triterpenic saponins derived from protobasic acid were identified by LC-MS. The two extracts showed a high ferro-reducing capacity and antiradical activity, with the leaf extract being the most active (IC_{50} = 4.58 µg/mL, DPPH and 197.00 µg/mL, ABTS). Both the extract from the leaves and the bark showed an anti-inflammatory effect, somewhat higher than the extract from the leaves. The results provide the first findings on the chemical and biological study of the leaves and bark of *M. coriacea* that grow in Ecuador, which justifies the traditional use of the species as anti-inflammatory.

Keywords: Antioxidant activity, Anti-inflammatory activity, Barks, Leaves, *Mimusops coriacea,* Phytochemical.

Introduction

Medicinal plants have traditionally been used in almost all cultures as a relevant therapeutic resource.[1, 2] Many plant extracts contain a variety of secondary metabolites with important redox modulating properties that effectively regulate the inflammatory response, triggered by noxious stimuli and conditions such as infection and tissue injury.[3]
Several medicinal plants that have antioxidant activities also have anti-inflammatory activities, due to the presence of polyphenols, flavonoids, alkaloids, terpenoids, among other compounds. Reports suggest that phytochemicals improve cell longevity, prevent aging, and minimize predisposition to certain inflammatory disorders.[4]
Despite the wide use of medicinal plants, a large unexplored quantity prevails, an example of this is the Ecuadorian flora, which despite its great diversity, there is still much to study; there is a need for documentation and it is essential to expand research to acquire a better understanding of the therapeutic virtues of this natural product.[5]
Mimusops coriacea (A.DC) Miq (Sapotaceae) is one of the many species distributed in Ecuador, of restricted abundance in the coastal regions and in the Amazon, in areas with well-drained soils and rainy weather. It has been widely cultivated in the tropics for centuries, native only to Madagascar and the Comoros Islands.[6]
Traditionally, the species is used as a tonic, febrifuge, in the treatment of inflammation of the urethra,[7] cystitis, diarrhea and dysentery.[8] Particularly in Ecuador, it is used as an analgesic and anti-inflammatory.[9] This has motivated the study of leaves and bark of the species, for which there are no reports that support its bioactive potential that could justify its use in traditional Ecuadorian medicine. The objective of this work is to determine the chemical composition, antioxidant and anti-inflammatory activity of hydroalcoholic extracts of leaves and bark of *M. coriacea*.

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Citation: Bustamante-Pesantes KE, Gutiérrez-Gaitén YI, Pesantes Domínguez OG, Miranda-Martínez M. Phytochemical, Antioxidant and Anti-Inflammatory Characterization of Leaves and Bark of *Mimusops coriacea* (A.DC) Miq from Ecuador. Trop J Nat Prod Res. 2020; 4(9):578-585. doi.org/10.26538/tjnpr/v4i9.14

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Materials and Methods

Plant material

The species were collected in May 2019 at the "Botanical Garden," a protected natural vegetative area located in the North zone of "Las Orquídeas" area, next to the Francisco de Orellana Avenue, in the hills of "Cerro Colorado" of Guayaquil city, Guayas province, Ecuador (coordinates 02°12′13.6800″S 079°53′50.6400″W). The area is located in an altitudinal belt between 50 and 200 m. a. s. l. in a tropical dry forest climate, with alluvial and sedimentary soils, cumulative rainfall of 1,150 mm/year, with monthly average temperatures of 31.1°C in

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ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

winter and 22.6°C in summer, mean relative humidity of 72% and total evaporation of 1,638.7 mm/year.[10]

Adult plants approximately 3 m tall were used, with flowers and fruits. One specimen was identified at the herbarium GUAY of Natural Science Faculty from Guayaquil University and deposited under the voucher specimen of 13111. The genetic characterization of the species was also carried out.[11] The leaves and back were obtained and washed with water. The samples were dried in an oven Mettler Toledo model with controlled temperature, at 40°C ± 2°C, over 7 days until reaching constant weight and subsequently fragmented in a knife mill and were stored in amber jars for analysis.

Preparation of extracts

Extracts were prepared from the leaves and barks, at the rate of 20 g of drug/100 mL of solvent, by the maceration method with sporadic agitation over a period of seven days at a temperature of 30°C ± 2°C, using 80% hydro alcohol as the solvent.[12] For the anti-inflammatory activity extracts were concentrated on a rotatory evaporator (Buchi RE 120) under vacuum at 40°C and re-suspended in the carboxymethyl cellulose (Sigma Aldrich) solution at 0.5%.

Phytochemical analysis

Determination of total phenols and total flavonoids

Total phenols were determined by the Folin-Ciocalteu method.[13] The hydroalcoholic extracts of leaves and bark of *M. coriacea* and gallic acid (reference substance) at concentrations of 10, 20, 30, 40 and 50 mg/mL were used. The reaction mixture was composed of 200 µL of the extracts or reference substance, 10 mL of Folin-Ciocalteu reagent 1:10, 1.8 mL of distilled water and 8 mL of sodium carbonate 7.5%, after 2 h, the absorbances were read at 765 nm on a spectrophotometer (Rayleigh UV-1601, China). The total phenolic content was expressed in terms of gallic acid equivalent (mg/mL).

The content of total flavonoids was carried out by the colorimetric method using aluminum chloride.[14,15] The hydroalcoholic extracts and quercetin (reference substance) at concentrations of 5, 20, 50, 60, 80 µg/mL were used. The test sample contained 500 µL of the extracts or reference substance, 1.5 mL of ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The mixture was allowed to stand for 30 min and the absorbance was measured at 415 nm with a spectrophotometer (Rayleigh UV-1601, China). The concentration of flavonoids was expressed in terms of quercetin equivalent (mg/mL).

LC-MS analysis

The samples were prepared by dissolving 10 mg of dry extract (from leaves and bark) in 1 mL of HPLC grade methanol in an ultrasonic bath and subsequently filtered (filter Titan 3 of 0.20 µm). The analysis by LC-DAD-MS was performed with a UPLC" (Thermo Scientific) system equipped with a two quaternary pump (Dionex ultimate 3000 RS LC), an autosampler (Dionex ultimate 3000 RS), a diode array detector (Dionex ultimate 3000 RS) and linear ion trap mass spectrometer LTQ XL and operated under Xcalibur 3.1 version software. The separation was performed using an Accucore RP-MS column (100 mm x 2.1 mm, 2.6 µm, Thermo scientific) protected by a prepacked column (4 mm x 2 mm). The mobile phase consisted of acetonitrile (A) and 0.1% formic acid (B). The gradient used was 0-2 min, 10-90% B; 2-6 min 27-73% B; 6-9 min, 30-70% B; 9-12 min, 33-67% B; 12-18 min, 95-5% B; 18-21 min. 10-90% B. The solvent flow rate was 0.4 mL/min and the injection volume was 2 µL. Ultraviolet spectra were monitored from 200-600 nm.

Positive and negative ionization modes were used for the mass spectra with an "ESI" interface. Mass spectra data were recorded on a full scan mode for a mass range of m/z 100-1800. Other mass spectrometer conditions were as follows: capillary temperature 225°C; capillary voltage -50 V; spray voltage 5 Kv; gas flow 34 (nitrogen gas, arbitrary units); auxiliary gas flow 5 and scanning gas 3. The tuning file was optimized with a direct infusion of quercetin (10 µg/mL concentration). Compounds were assigned by comparing the ultraviolet and mass spectra obtained with those of the equipment library and the literature.

Antioxidant activities

Ferric reducing antioxidant power (FRAP) assay

The reducing capacity of hydroalcoholic extracts of the leaves and bark of *M. coriacea* was measured.[16] A UV spectrophotometer (Rayleigh UV-1601, Shanghai, China) was used at an absorbance of 593 nm. All reagents used were from Merck (2,4,6-tripyridyl-s-triazine (TPTZ), Sodium Acetate Anhydrous, Acetic Acid (99.7%), Hydrochloric Acid (37%), FeCl3) and reference substances ascorbic acid (99% purity) and FeSO4 x 7 H_2O.

The extracts were evaluated at concentrations of 0.75, 2, 6, 10 and 12.5 µg/mL. The results were expressed as µmol equivalents of ascorbic acid and as µmol equivalents of $FeSO_4$, from the calculation by interpolating the optical density (OD) of the samples in the calibration curves of both reference substances at concentrations of 100, 200, 400, 800 and 1000 µM. The readings were made in triplicate.

2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals scavenging activity

For the quantitative determination the DPPH free radical method was used.[17,18] A UV spectrophotometer was used, and the determinations were measured at 517 nm after 30 min. The extracts of the leaves and bark and reference substances Vitamin C (99% pur) and trolox were tested at concentrations of 2, 6, 10, 15 and 20 µg/mL. The percentage of inhibition of DPPH radical was calculated according to the following formula:

$$\%\,inhibition\ DPPH = \frac{Absorbance\ control - Absorbance\ sample}{Absorbance\ control} \times 100$$

The mean inhibitory concentration (IC_{50}) was determined with the help of the GraphPad prism 5.0 statistical program.

2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging activity

The assay was based on the ability of different substances to sequester the cationic radical $ABTS^{•+}$.[19,20] A UV-visible spectrophotometer was used, and absorbance was taken at 734 nm. Reagents used were of analytical grade, and they include [ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline) -6-sulfonic acid), potassium persulfate, ethanol 96%]. The extracts and reference substances Vitamin C (99%) and trolox were tested at concentrations of 100, 200, 300, 500 and 600 µg/mL. The percentage of inhibition of the ABTS radical was calculated as:

$$\%\,Absorbance\ ABTS = \frac{Absorbance\ ABTS - Absorbance\ antioxidants}{Absorbance\ ABTS} \times 100$$

The mean inhibitory concentration (IC_{50}) was determined using the GraphPad prism 5.0 statistical program.

Anti-inflammatory activity

The animal model used in this test were female albino rats of the Wistar line, from the CENPALAB (National Center for the Production of Laboratory Animals) with their corresponding quality certificates that guaranteed their health, being suitable to carry out this type of test. Anti-inflammatory activity of the extracts was evaluated using carrageenan-induced paw edema model in rats.[21,22] Adult female albino Wistar rats (180-200 g) were divided into four groups (n = 6). Group 1 (control): 0.5 mL/kg of 0.9% sodium chloride solution of body weight (b.w), Group 2 (positive control):10 mg/kg (b.w) of Indomethacin (Sigma Aldrich) by oral route, Group 3: hydroalcoholic extract of leaves at 200 mg/kg (b.w) by oral route and Group 4: hydroalcoholic extract of bark at 200 mg/kg (b.w) by oral route. The 200 mg dose was considered taking into account anti-inflammatory studies in other species of the same genus.

Animals were housed under standard environmental conditions at temperature (20 ± 3°C), RH = 30-70%, light /dark (12/12 h). Water and food were administrated *ad libitum*. Food was withdrawn from the rats 24 h before the test and they were only allowed access to water.

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 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

All treatments were carried out using an intragastic cannula and after 30 min, 0.1 mL of 3% aqueous carrageenan solution (inflammation inducing agent) was administered in the right plantar aponeurosis of all animals. One hour after the carrageenan administration, the inflammation was measured in mL at time 1, 2, 3 and 5 h, using a digital plethysmometer (Panlab, Spain). The percent inhibition of edema was calculated in comparison to the control animals and was calculated using the following formula:[23]

% inhibition =

$$\frac{Paw\ volume\ (control) - paw\ volume\ (treated)}{paw\ volume\ (control)} \times 100$$

The trial was approved by the quality committee of the CIEB-IFAL, Cuba, complying with all the procedures established for the treatment of the animals, which, once the experiment was completed, were sacrificed in a chamber saturated with ether, complying with the procedures of Refinement to avoid pain and suffering of the animals. The Bioethics and Biosafety Standards established by The World Medical Association[24] were also considered.

Statistical analysis

The experiments were carried out in triplicates and the results were expressed as mean/standard deviation (SD). One-way analysis of variance (ANOVA) was used to determine the differences between multiple groups, followed by Dunnett's post hoc test with $p \leq 0.05$. A Student-t test was used to evaluate the differences in the quantitative determination of phenols and flavonoids and the FRAP assay between two extracts. All data were processed by the statistical program SPSS for Windows version 8.0.

Results and Discussion

Phytochemical analysis

M. coriacea has a few studies and there is no information on the chemical composition of the leaves and barks of the plant. In the present investigation, the phytochemical characterization of hydroalcoholic extracts of the said plant part was carried out. Total phenols and flavonoids were quantified as they are widely distributed metabolites in the genus *Mimusops* and the Sapotacea family.[25]

Phenolic compounds are formed as intermediate or final products of the secondary metabolism of the plant; they play a role in growth and reproduction, as well as in adaptation and survival in stressful or adverse environmental conditions. Like other types of metabolites, the content and type can vary according to the plant species, the different parts of the plant and the ecosystem.[1, 26]

There were significant differences in the content of phenols and flavonoids in the extracts evaluated, the concentration being higher for the leaf extract (Table 1). These differences could be related to the biological functions of the vegetative parts studied.

The concentration of phenolic compounds in a plant part depends upon the phenolic synthesis in those organs. The variation in the content of these compounds is due to the morphological and anatomical differences between the structures, as well as to the numerous physiological processes that occur in each plant parts. Flavonoids belong to the largest group of phenolic compounds and can be found in all plant parts, particularly in the cells of the photosynthetic apparatus and accordingly in leaves.[27, 28]

LC-MS analysis

The LC-MS was performed on the hydroalcoholic extracts of the leaves and barks. Figure 1 shows the TIC chromatograms of both extracts. The main signals eluted before 10 min and the ones that showed the greatest intensity were observed between 7 and 10 min. It was also possible to observe two chromatographic peaks with a shorter retention time at 3.43 and 4.02 min, only in the leaf extract. The mass spectra of the two compounds and the proposed fragmentation scheme are illustrated in Figure 2.

In the range of 7-10 minutes, two weak signals were detected (9.30 and 9.49 min in the extract of the leaves; 9.34 and 9.50 min in the extract of the barks), being those of less intensity those of the extract of leaves.

The LC-MS study allowed the identification of two glycosylated flavonoids, assigned only to the leaf extract. Analysis of the peaks with RT 3.43 and 4.02 min (Figure 1), together with the UV spectrum, suggested the presence of compounds with aromatic chromophores. Pseudo molecular ions were seen in the mass spectrum at m/z 481 and 465 (+), respectively. Therefore, nominal masses 480 and 464 Da were derived for these compounds. Both compounds originate the same m/z 319 (+) fragment and their nominal masses differ by 16 Da. These arguments suggest that both compounds have a structural relationship and that they differ by the presence of an oxygenated function (Figure 2).

The lower mass compound (464 Da, Rt = 4.02 min) showed a MS/MS spectrum equal to that reported for myricetin-3-*O*-α-L-rhamnoside.[29] The pseudo molecular ion $[M+H]^+$ corresponds to m/z 465 and the fragment ion at m/z 319 is associated with $[M+H-146]^+$, caused by the loss of the rhamnose residue. A similar behavior can be justified for the compound with greater mass (480 Da, RT = 3.43 min), where the same fragment ion is seen at m/z 319 but with a pseudo molecular ion at m/z 481; in this case the base peak was justified by the ion $[M+H-162]^+$ (Figure 2). The loss of 162 Da can be caused by a glucose residue, thus justifying the difference of 16 Da between both compounds and allowing the suggestion of myricetin-3-*O*-glucoside.[30] the identified compounds despite being known in nature, constitute a new report for the species and genus *Mimusops*.

As the behavior was similar for the leaves and bark extracts, only the mass spectra (negative ionization mode) corresponding to the leaf extract are presented (Figure 3). In both mass spectra, the ion m/z 1207.8 was identified as the base peak, related to the loss of a rhamnose residue previously reported in the isolated saponins of the genus.[31, 32]

Other fragment ions (Figure 3) detected in both compounds and that confirm the presence of triterpenic glycosides derived from protobassic acid are: m/z 1221.7 $[M-H-132]^-$, m/z 1075.7 $[MH-132-146]^-$, m/z 665.7, m/z 503 (corresponding to $[M-H]^-$ of protobassic acid), among others. The fragment ion m/z 797.6 that was detected only in the compound that elutes with RT 9.49 min, could be defining in the differentiation of both compounds with the same molecular mass.

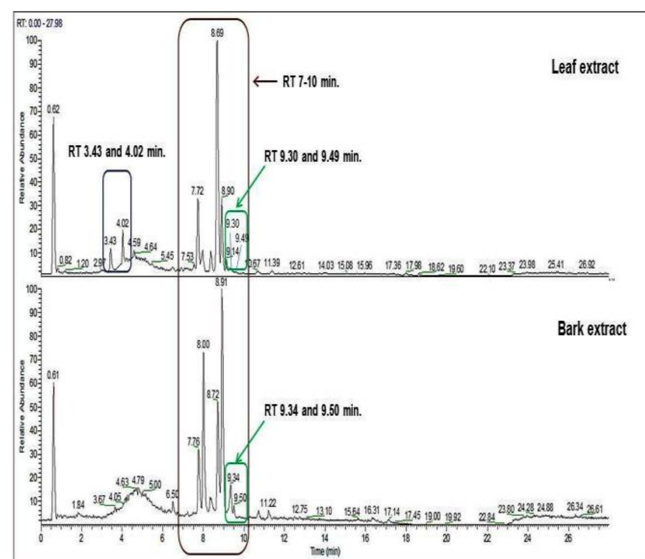

Figure 1: TIC (-) chromatograms of the hydroalcoholic extracts of leaves and barks of *M. coriacea* in the mass range of 100-1800 Da

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 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

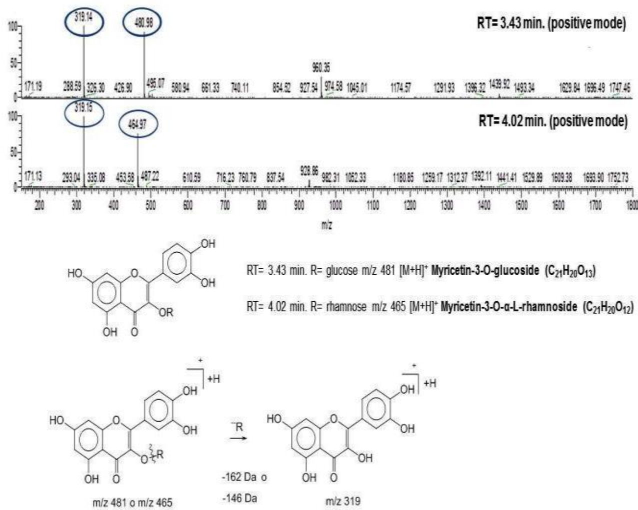
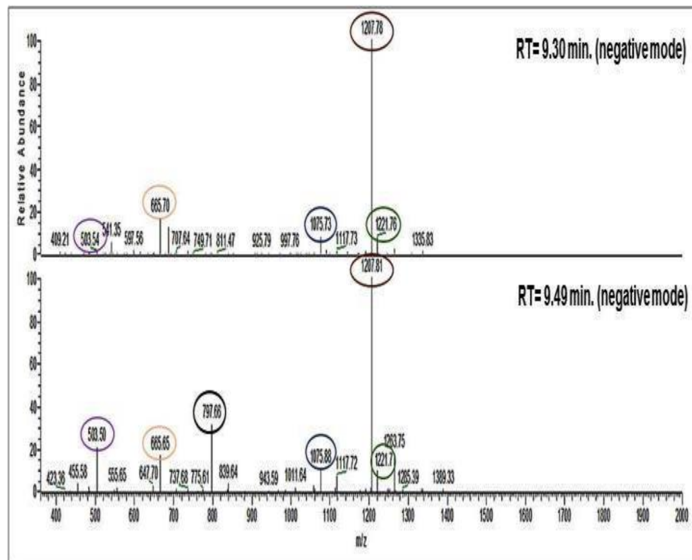


Figure 2: Peak MS/MS spectra with TR 3.43 and 4.02 min and fragmentation scheme



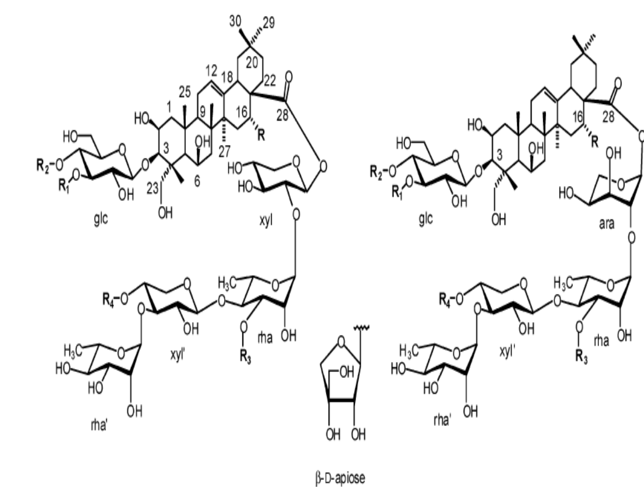


Figure 3: MS/MS spectra with TR 9.30 and 9.49 min.

Table 1: Total phenols content and total flavonoids content of the hydroalcoholic extracts of leaves and barks of *M. coriacea*

Extracts	Total phenols (mg/mL) X̄/SD	Total flavonoids (mg/mL) X̄/SD
Leaves	6.00 / 0.03[a]	1.52 / 0.01[c]
Barks	5.44 / 0.05[b]	0.54 / 0.02[d]

Legend: X̄/SD: Average value of determinations (n = 3)/standard deviation. Different letters in a column show significant differences ($p < 0.05$) according to t-Student test

The compounds detected showed the same molecular mass 1353.8 $[M-H]^-$, corresponding to a molecular formula of $C_{63}H_{102}O_{31}$ and MS/MS spectra very similar to two saponins isolated from the methanol extract of *M. laurifolia* identified as: 3-*O*- (*β*-D-glucopyranosyl)-28-*O*- (α – L- rhamnopyranosyl- (1→3) - [β -D-xylopyranosyl- (1→4)] - *β* -D-xylopyranosyl- (1→4) -α -L-rhamnopyranosyl- (1→2) -α -L-arabinopyranosyl) protobassic acid (RT = 9.30 min) and 3-*O*- (*β*-D-apiofuranosyl) - (1→4) - (*β*-D-glucopyranosyl) -28-*O*- (α – L-rhamnopyranosyl- (1→3) - [*β* -D-xylopyranosyl- (1→4)] - α-L-rhamnopyranosyl- (1→2) -α -L-arabinopyranosyl) protobassic acid (9.49 min).[31]

Other unidentified compounds, with peaks of greater intensity, eluted in the range of 7-10 min (Figure 1) with RT 8.36, 9.0 and 9.65 min. Characteristic fragment ions of triterpenic saponins derived from protobassic acid were observed in all MS/MS spectra, although it is necessary to confirm the structures by NMR experiments, among others.

Although the triterpenic saponins derived from protobassic acid are distinctive features in the *Minusops* genus, their identification in *M. coriacea* also constitutes a new report and confirms the chemotaxonomic relationship with other species of the same genus.

Antioxidant activities

The antioxidant activity of hydroalcoholic extracts of leaves and bark were assessed *in vitro* by FRAP, DPPH and ABTS assays. In the FRAP test the results were expressed as µM equivalent of ascorbic acid and µM equivalent of $FeSO_4$. The DPPH and ABTS assays measured the antiradical ability of the extracts, together with vitamin C and trolox used as reference substances. Antioxidant activity was observed for the tested extracts. The results are shown in Table 2 (2A FRAP assay, 2B DPPH assay and 2C ABTS assay).

Plant extracts contain numerous biologically active compounds with antioxidant activity that act through different reaction mechanisms.[33-36]

For these reasons in the present investigation, the antioxidant capacity of the extracts was evaluated by three *in vitro* methods (FRAP, DPPH and ABTS).

In the FRAP test (Table 2A) the two extracts were able to reduce ferric (Fe^{3+}) to ferrous iron (Fe^{2+}), which was reflected in the high values of µM equivalents of ascorbic acid and µM equivalents of $FeSO_4$ (reference substances). A concentration-dependent activity was shown, and at the maximum concentration tested (12.5 µg/mL) the extracts had a similar behavior.

In the DPPH assay, a reduction of the radical is monitored by the decrease in absorbance at a characteristic wavelength (517 nm). Consequently, the disappearance of DPPH provides an index to estimate the ability of the test compound to trap radicals.[37]

From a qualitative point of view, a change in color from purple to yellow was observed in the two extracts as the concentration increased, showing antiradical activity, although the leaf extract was

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 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

the one that showed the highest percentage of inhibition of the DPPH radical at all the concentrations evaluated, with significant differences with respect to the two reference substances (Table 2B), as well as the lowest IC_{50} value (4.58 µg/mL).

During the development of the $ABTS^{\bullet+}$ method, a discoloration of the cationic radical was observed at all the concentrations evaluated, due to the ability of the samples to neutralize the radical, this was reflected in a decrease in absorbance. At a concentration of 600 µg/mL the two extracts had radical inhibition percentages like the reference substances, although the highest antioxidant activity was assigned to vitamin C and the leaf extract with IC_{50} values of 196.30 µg/mL and 197.00 µg/mL, respectively.

The antioxidant activity has been reported for some species of the genus *Mimusops* such as *M. elengi*[38] *M. hexandra*[39] and *M. zapota*,[40] evaluating extracts of different polarities, mainly of leaves and bark by various *in vitro* methods. An analysis of the research results with those of the previous species, allowed us to suggest a good antioxidant power of the evaluated extracts. For example, in the DPPH test, when comparing the IC_{50} of the two extracts of *M. coriacea* (4. 58 µg/mL, leaves and 5.77 µg/mL, bark) with *M. elengi* (31.80 µg/mL, leaves), *M. hexendra* (46.62 µg/mL, bark) and *M. zapota* (42.5 µg/mL, aqueous extract of leaves), it is noted that the values obtained are lower, which denotes the good anti-radical power of the species studied.

Table 2: Antioxidant activity of the hydroalcoholic extracts of leaves and bark of *M. coriacea*

A. Ferric reducing power (FRAP) assay				
Concentrations (µg/mL)	**µM equivalents of Vitamin C/SD**		**µM equivalents of $FeSO_4$ / SD**	
	Leaves extract	**Bark extract**	**Leaves extract**	**Bark extract**
0.75	345.48 /16.88[a]	339.61 / 9.62[a]	302.89 / 15.09[h]	288.71 / 8.59[h]
2	634.84 / 19.09[b]	549,92 / 36.96[c]	552.54 / 17.06[i]	476.65 / 33.03[j]
6	726.91 / 19.09[d]	719.76 / 46.55[d]	634.81 / 17.06[k]	607.15 / 12.09[k]
10	784.84 / 15.85[e]	753.89 / 10.99[f]	686.58 / 14.16[l]	634.81 / 17.06[m]
12.5	976.91 / 13.11[g]	910.24 / 60.90[g]	858.21 / 11.71[n]	798.64 / 54.42[n]
B. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals scavenging activity				
Concentrations (µg/mL)	**% inhibition of the DPPH / SD**			
	Leaves extract	**Bark extract**	**Vitamin C**	**Trolox**
2	69.73 / 0.16[a]	55.60 / 1.18[b]	48.45 / 0.22[c]	60.29 / 0.38[d]
6	82.33 / 0.27[e]	72.12 / 0.34[f]	64.85 / 0.16[g]	69.37 / 0.21[h]
10	83.35 / 0.82[i]	75.56 / 0.49[j]	74.26 / 0.39[k]	75.82 / 0.33[j]
15	84.03 / 0.21[l]	78.96 / 0.28[m]	76.03 / 0.23[n]	80.19 / 0.27[o]
20	86.26 / 0.45[p]	83.31 / 0.51[q]	78.78 / 0.54[r]	84.21 / 0.49[q]
IC_{50} (µg/mL)				
	4.58	5.77	5.77	7.54
C. 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging activity				
Concentrations (µg/mL)	**% inhibition of the ABTS•+ / SD**			
	Leaves extract	**Bark extract**	**Vitamin C**	**Trolox**
100	48.46 / 1.19[a]	43.94 / 1.68[b]	47.15 / 0.42[a]	42.68 / 1.45[b]
200	73.13 / 1.30[c]	73.41 / 1.33[c]	73.18 / 1.48[c]	55.81 / 0.71[d]
300	91.10 / 0.84[e]	75.65 / 0.71[f]	93.38 / 0.53[g]	64.14 / 0.66[h]
500	93.94 / 0.28[ij]	94.03 / 0.59[ij]	94.36 / 0.12[j]	93.14 / 0.14[i]
600	95.38 / 0.77[k]	96.03 / 0.28[k]	96.04 / 0.79[k]	95. 25 / 0.25[k]
IC50 (µg/mL)				
	197.00	213.50	196.30	301.60

Average value of determinations (n = 3)/standard deviation (SD). Different letters in a row show significant differences ($p < 0.05$) according to Dunnett's test (DPPH and ABTS) and *t-Student* test (FRAP)

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ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 3: Volumes of plantar edema and percentage inhibition at different times of *Wistar albino* rats treated with studied products on the inflammation induced by carrageenan

Groups	Volumes of edema (mL)/SD [Percentage inhibition of edema (%)]			
	1h	**2h**	**3h**	**5h**
Control (NaCl 0.9%)	0.78/0.02[a]	0.80/0.01[e]	0.85/0.02[i]	0.96/0.04[l]
Indomethacin mg/kg (b.w)	0.49/0.02[b] [37.17]	0.42/0.02[f] [47.50]	0.37/0.04[j] [56.47]	0.30/0.07[m] [68.75]
Leaves extract 200 mg/kg (b.w)	0.58/0.05[cd] [25.64]	0.48/0.07[g] [40.00]	0.41/0.06[j] [51.76]	0.37/0.07[m] [61.45]
Bark extract 200 mg/kg (b.w)	0.61/0.02[d] [21.79]	0.54/0.04[h] [32.50]	0.50/0.06[k] [41.17]	0.46/0.03[n] [52.08]

Average value of determinations (n = 6)/standard deviation (SD). Different letters in a column show significant differences ($p < 0.05$) according to Dunnett's test

Anti-inflammatory activity

Anti-inflammatory activity was evaluated by the carrageenan-induced plantar edema model in the rat paw. This method allows quantifying in a reproducible and simple way two of the most characteristic parameters of inflammation, such as edema and plasma extravasation, by inducing an acute inflammation located in the animal's leg after the administration of carrageenan.[41]

Hydroalcoholic extracts of leaves and bark of *M. coriacea* were evaluated on the first signs that appear during acute inflammation, mainly, edema during the first 5 h (Table 3). A tendency to increase the volume of edema was observed in the control group. On the contrary, in the groups treated with indomethacin and the extracts, a significant decrease in the volume of edema was observed after 3 h, where the leaf extract had a behaviour comparable to indomethacin. The leaf extract and indomethacin showed the highest anti-inflammatory effect, with inhibition percentages of 61.45% and 68.75%, respectively (Table 3).

The anti-inflammatory activity has also been evaluated in some species of *Mimusops*, among them is *M. elengi.* The bark extract at the dose of 200 mg/kg showed a percentage of inhibition of inflammation of 37% after 5 h of testing,[42] lower than those obtained for the extracts of the leaves and bark of *M. coriacea* (61.45% and 52.08%, respectively), which suggests a good anti-inflammatory effect of the studied species.

The antioxidant and anti-inflammatory action can be caused by active principles of a very varied chemical nature. According to the phytochemical results, myricetin glycosides and triterpenic saponins derived from protobasic acid were identified, which could contribute to their synergistic effect of the pharmacological activities demonstrated.

For example, flavonoids, including myricetin and its glycosides, have been shown to possess antioxidant[43] and anti-inflammatory properties due to their ability to inhibit enzymes involved in inflammation, especially the metabolic pathway of arachidonic acid and synthesis of prostaglandins.[44, 45] Triterpenic saponins also exhibit antioxidant and anti-inflammatory properties.[46, 47]

A greater antioxidant and anti-inflammatory activity were observed for the leaf extract, which presented the highest concentration of phenols and flavonoids. It has been shown that these metabolites can contribute directly to the activities previously described.[27]

Conclusion

The result of this research shows that there is a quantitative variation in metabolites according to plant part, which is related to the antioxidant and anti-inflammatory activities demonstrated, with the leaf extract being the most prominent in terms of quantity of metabolites and greater pharmacological activity. A valuable contribution has been made to the study of chemical composition and biological properties of the leaves and bark of *M. coriacea*, especially to the justification of its use in traditional Ecuadorian medicine.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

We are grateful to the Center for Research and Biological Evaluations, Institute of Pharmacy and Food, University of Havana, Cuba for the performance of the biological test.

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