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Original Research Article

Chemical Composition, Acute Oral Toxicity and Analgesic Activity of Hydroalcoholic Extracts of *Mimusops coriacea* (A.DC) Miq (Sapotaceae)

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ABSTRACT

Mimusops coriacea is used traditionally as a tonic, febrifuge, and in the treatment of cystitis, diarrhea and dysentery. In Ecuador the plant is used as an analgesic and anti-inflammatory agent, however, there is limited scientific evidence to support this claim. The study therefore is aimed at determining the chemical composition, acute oral toxicity and analgesic activity of the hydroalcoholic extracts of the leaves, bark and fruits of *Mimusops coriacea*.

The 80% hydroalcoholic extracts of the leaves, bark, and fruits (green and ripe), were obtained by maceration. The extracts were subjected to Gas Chromatography-Mass Spectrometric (GC-MS) analysis. An oral acute toxicity test was performed in rats according to the OECD draft guideline 423. The analgesic activity of the extracts was evaluated by the acetic acid-induced abdominal contortion model in mice.

The GC-MS analysis of the extracts shows variations in the type and relative abundance of some metabolites in the different plant parts. The predominant metabolites identified are fatty acids with palmitic and stearic acids as the major compounds in the leaves, bark and green fruits extracts, while benzoic acid was majorly in the ripe fruits extract. Other compounds identified are terpenoids, sugars, and amino acid. The extracts showed no sign of toxicity after acute oral administration. The leaves and bark extracts had higher analgesic effect compared to the fruits extract. The study therefore lends credence to the traditional use of *M. coriacea* as an analgesic agent..

Keywords: *Mimusops coriacea*, GC-MS, Hydroalcoholic extracts, Toxicity, Analgesic activity

Introduction

Pain involves a complex set of biochemical processes such as the activation of enzymes, the release of inflammatory mediators and extravasation of cellular fluid, migration, damage, and tissue repair.[1,2] It is a protective mechanism that helps a living organism gets rid of unpleasant stimuli. It transforms from a short-term warning sign to a devastating chronic condition.[3,4]

Many analgesic and anti-inflammatory agents present a significant risk of toxicity after acute and chronic use; adverse reactions include gastrointestinal upset, gastric ulcer, bleeding, and liver damage. Many studies are focused on the investigation of medicinal plants with analgesic and anti-inflammatory properties, because of their numerous biologically active constituents, fewer side effects, low cost and accessibility.[2, 5-8]

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For the *Mimusops* genus, different pharmacological properties have been reported including antioxidant,[9] anti-inflammatory,[10] antimicrobial,[11] and hypoglycaemic activities.[12]

Decoction of *M. coriacea* stems is used traditionally as a tonic, febrifuge, and in the treatment of urethratis,[13] cystitis, diarrhea and dysentery.[14] In Ecuador, the plant is used as an analgesic and anti-inflammatory agent.[15] Scientific studies have reported the antioxidant and anti-inflammatory activities of the fruits,[16] leaves and bark of the plant species.[17]

The present study therefore determined the chemical composition, acute oral toxicity and analgesic activity of the hydroalcoholic extracts of the leaves, bark and fruits of *Mimusops coriacea*.

Materials and Methods

Plant materials

The plant materials was collected in August 2019 from the Botanical Garden, located in the North area of the citadel "Las Orquídeas" Av. Francisco de Orellana, in the summits of Cerro Colorado, City of Guayaquil, Guayas province - Ecuador, with the coordinates 02°12′13.6800″S 079°53′50.6400″W.

Mature plants of approximately 30 m in height, with flowers and fruits, were used. It was identified at the herbarium GUAY of Natural Science Faculty, Guayaquil University and deposited with the voucher specimen number 13111. The genetic characterization of the species was also carried out.[18]

From the collections, the leaves, bark of matured branches and green and mature complete fruits were used. All the materials were washed

with potable water and dried in an oven (Mettler Toledo) at 40°C, until constant weight. The dried samples were ground on a Pulvex blade mill to 2 mm particle size and stored in amber glass jars.

Preparation of extracts
The leaves, bark, green and ripe fruits were extracted separately with ethanol (Sigma-Aldrich) (80% in distilled water) by maceration for seven days at room temperature, at the rate of 20 g sample/100 mL of solvent. The extracts were concentrated in a Heildoph Laboratory model 4001 efficient HB digital rotary evaporator at reduced pressure, 50 rpm and a temperature of 50°C.

GC-MS analysis
The hydroalcoholic extracts (from leaves, bark, green and ripe fruits) were dried, mixed with N-Trimethylsilyl-N-methyl trifluoroacetamide (MSTFA) and heated in a water bath to 80°C for 2 h to permit the silylation of metabolites.[19] Gas Chromatography-Mass Spectrometry (GC-MS) analysis was performed an Agilent Technologies GC-MS equipment (7890A GC system and 5975C inert XL MSD with triple axis detector). A capillary column DB-5MS (30 m × 0.25 mm) with phenyl dimethylpolysiloxane was used as stationary phase (0.25-micron film thickness) and helium as the carrier gas (1.2 mL/min). The injection of 1 µL of derivatized sample was performed at 250°C with splitless mode. The oven temperature was started at 70°C for 2 minutes, then it was increased to 300°C at 5°C/min, and it was maintained at 300°C for 6 minutes. The compounds identification was done by comparison of mass spectra data in the NIST 2011 MS Library (Wiley, 9th version). An electron ionization of 70 eV at 230°C was used as the ion source and the compound data were collected with the full scan mode (40-600 amu) in the quadrupole mass analyzer.[20]

Acute oral toxicity test
The test was conducted according to the OECD draft guideline number 423.[21] The assay lasted 19 days (five days for acclimatization and 14 days for testing).
Female Wistar rats (190 - 205 g) from the Center for Laboratory Animal Production (Cuba) were used. Animals were maintained at constant room temperature (20 ± 3°C), relative humidity of about 30 – 50% ± 5% and subjected to 12 h light/dark cycle with free access to food and water.
The animals were divided into eight groups of three animals each (n=3). Groups I and II received the leaves extract, groups III and IV received the bark extract, groups V and VI received the green fruits extract, while groups VII and VIII received the ripe fruits extract. All the extracts were administered orally at a single dose of 2000 mg/kg body weight.
Clinical and behavioral signs of toxicity were evaluated, various organs (lungs, heart, spleen, kidney and stomach) were examined and the body weight of the animals was recorded, at the beginning of the experiment, at day 7 and day 14.

Analgesic Activity evaluation
Acetic acid Writhing test
The study was carried out using the acetic acid-induced abdominal writhing reflex pain model.[22-24] Mice of the line OF1 (25-30 g) from the Center for Laboratory Animal Production (Cuba) were used. They were maintained at constant room temperature (20 ± 3°C), relative humidity of about 30 – 50% ± 5% and 12 h light/dark cycle with free access to food and water.
The male mice were divided into six groups of six animals each. Group I (negative control) were administered intraperitoneally (i.p) with normal saline (10 mL/kg), group II (positive control) received 200 mg/kg of acetylsalicylic acid in 0.5 mL of water orally, while groups III, IV, V and VI received orally 200 mg/kg of hydroalcoholic extracts (in 0.5 mL of water) of the leaves, bark, green and ripe fruits, respectively. One hour after drug (ASA) and extracts administration, 3% acetic acid solution (10 mL/kg) was administered intraperitoneally (i.p) to all the mice to induce abdominal contortions or writhing.
Each mouse was then placed in a transparent observation box and the number of abdominal constrictions (writhes) for each mouse was counted for 20 minutes, immediately after intraperitoneal injection of acetic acid. The percentage writhing inhibition was calculated using the following formula:

$$\text{Percentage of inhibition of writhing} = \frac{\text{Mean of control group - Means of treated group}}{\text{Means of control group}} \text{ X } 100$$

Ethical consideration
All biological experiments were carried out following the provisions of the standard operating procedures in force at the Center for Studies for Biological Research and Evaluations of the Institute of Pharmacy and Food of the University of Havana, Cuba. No alterations were detected that could affect the integrity of the results. All animals received an exact dosage according to weight and the route of administration used.[25] At the end of the test, the animals were sacrificed using an atmosphere saturated with ether, always considering the refinement techniques currently proposed to carry out the tests with experimental animals.[26]
The researchers who participated in the study respected the ethical principles that govern animal experimentation, guaranteeing their welfare and protection, both for human sensitivity to animal suffering and for guaranteeing the validity of the results obtained, complying with the Bioethical Norms and Biosecurity established.[26]

Statistical analysis
For the analgesic activity, the data were expressed as arithmetic mean ± standard deviation. One way analysis of variance (ANOVA) was used to determine the differences between groups, followed by Tukey post hoc test with level of significance taken at $p \leq 0.05$ at 95% confidence interval. In the toxicological study, the weights of the rats at different times were statistically processed to obtain the mean and standard deviation, data were subjected to one-way analysis of variance and the Student Newman Keuls test ($p < 0.05$). The statistical program SPSS for Windows version 8.0 was used for the statistical analysis.

Results and Discussion

Phytochemical Analysis
Tables 1 and 2 list the compounds identified for each extract. The predominance of fatty acids was observed in the leaves and bark extracts, with the main components being stearic acid (13.96% and 16.75%) and palmitic acid (6.02% and 7.89%), for the leaves and bark extracts, respectively.
Dodecanoic acid and threonic acids were detected in the leaves, but not found in the bark extract, while in the latter, pentanoic acid, benzoic acid, 9,12-octadecadienoic acid ZZ and trans-9-octadecenoic acid were detected. It is noteworthy that unsaturated fatty acids present in the bark extract were detected in the leaves extract. The α-amyrin acetate, a pentacyclic triterpenoid was also detected in both extracts.
Figure 2 presents the gas chromatograms of the hydroalcoholic extracts of the fruits in the two stages of maturation, in which both qualitative and quantitative differences were observed. Tables 3 and 4 list the compounds identified for these extracts.
It was observed that in the extracts of the fruits (green and ripe) the fatty acid compounds predominated. In both extracts, linoleic, succinic, lauric, margaric, palmitic, oleic and xylonic acids -γ-lactone and the ethyl ester of palmitic acid were identified. The triterpenoid squalene, and L-proline 5-oxo-1 were also detected in the two extracts. Glucitol and mannonico acid were the exclusive components of the extract of the ripe fruits.
There are reports of the chemical composition for other species of the genus such as *M. elengi*, *M. cafra* and *M. zeyheri*, in which fatty acids such as palmitic,[27] oleic, linoleic and stearic acids have been identified.[28-30] Similarly, squalene and α-amyrin acetate have been detected in the unsaponifiable fractions as well as saturated and unsaturated fatty acids in the saponifiable fractions of *M. coriacea* seed oils, in investigations carried out by Bustamante *et al*.[16] However, the presence of L-proline 5-oxo-1 is reported for the first time for the species under study, the genus *Mimusops* and Sapotaceae family.

Table 1: Compounds identified in the hydroalcoholic extract of *M. coriacea* leaves

RT (min)	Compounds	MF	MM g/mol	% Abundance
13.87	Butanodioic acid (Succinic acid)	$C_4H_6O_4$	118.09	0.24
19.67	Threonic acid	$C_4H_8O_5$	136.10	0.18
22.17	Dodecanoic acid (Lauric)	$C_{12}H_{24}O_2$	200.32	0.35
26.40	Tetradecanoic acid (myristic)	$C_{14}H_{28}O_2$	228.37	0.73
30.28	Hexadecanoic acid (palmític acid)	$C_{16}H_{32}O_2$	256.40	6.02
32.07	Pentadecanoic acid (pentadecyl)	$C_{15}H_{30}O_2$	242.39	0.49
33.85	Octadecanoic acid (stearic)	$C_{18}H_{36}O_2$	284.48	13.96
37.08	Eicosanoic acid (arachidic)	$C_{20}H_{40}O_2$	312.53	0.14
43.87	n-Nonacosane	$C_{29}H_{60}$	408.00	1.16
42.49	α Amyrin acetate	$C_{31}H_{48}O_3$	468.72	2.78

Legend: RT = Retention Time, MF = Molecular Formula, MM = Molecular Mass

Table 2: Compounds identified in the hydroalcoholic extract of *M. coriacea* bark

RT (min)	Compounds	MF	MM g/mol	% Abundance
13.81	Butanodioic acid (Succinic acid)	$C_4H_6O_4$	118.09	0.15
26.40	Tetradecanoic acid (myristic)	$C_{14}H_{28}O_2$	228.37	0.77
28.37	Pentadecanoic acid (pentadecyl)	$C_5H_{10}O_2$	102.13	0.16
28.56	Benzoic acid	$C_7H_6O_2$	122.12	0.08
30.28	Hexadecanoic acid (palmític acid)	$C_{16}H_{32}O_2$	256.40	7.89
32.07	Heptadecanoic acid (margaric)	$C_{17}H_{34}O_2$	270.45	0.46
33.20	9,12 Octadecadienoic Acid (Linoleic)	$C_{18}H_{30}O_2$,	278.43	0.20
33.32	Octadecenoic acid (oleic)	$C_{18}H_{34}O_2$	282.47	0.53
33.85	Octadecanoic acid (stearic)	$C_{18}H_{36}O_2$	284.48	16.75
37.08	Eicosanoic acid (arachidic)	$C_{20}H_{40}O_2$	312.53	0.20
43.87	n-Nonacosane	$C_{29}H_{60}$	408.62	0.39
51.61	α Amyrin acetate	$C_{32}H_{52}O_2$	468.80	1.89

Legend: RT = Retention Time, MF = Molecular Formula, MM = Molecular Mass

The changes in the composition of the extract of the ripe fruits with respect to, that of the green fruits can be explained because during maturation, a set of changes in the physicochemical characteristics are revealed such as texture, colour, flavour and aroma, as well as the reduction of firmness,[31,32] which allows defining the different stages of maturity.

Acute oral toxicity

The validation of the non-toxicity of medicinal plants is of great value, since it a way of guaranteeing the safety of the population that consumes them.

One parameter evaluated is the body weight of the animals, which is of great value in the toxicological evaluation of a substance since its decrease could be considered as an indicator of possible organic damage.[33] This variable is directly influenced by the consumption of food and water intake by the animals.[34] In this study, weight gain was observed in the experimental animals, which could be indicative of the non-toxic nature of the extracts evaluated. Table 5 shows the body weights of the rats during the experiment.

No clinical alterations were observed with respect to the respiratory, circulatory, autonomic, and central nervous system of the animals. No hair shedding, tremor, seizure, salivation, sedation, and drowsiness was observed. From the macroscopic point of view, no damage was seen in the samples of the selected organs (lung, heart, spleen, kidneys, and stomach). Under the experimental conditions evaluated, it is evident that the acute oral administration of the hydroalcoholic extracts of the leaves, bark, green and mature fruits of *M. coriacea*, at a dose of 2000 mg/kg did not produce toxic effects on the animals tested.

Analgesic activity

Models that investigate anti-nociception can be peripheral or central analgesia. The acetic acid-induced abdominal writhing reflex pain model in mice was used in this study, which is used to investigate peripheral or local analgesic activity.[35]

In this test, a twisting reflex is produced in laboratory animals by the activation of chemo sensitive nociceptors. The percentage reduction in the number of abdominal contortions indicates the level of analgesia in the model.[36]

As a positive control, acetylsalicylic acid was used, a carboxylic acid derivative from the group of non-steroidal anti-inflammatory drugs that is highly effective as an analgesic, antipyretic and anti-inflammatory agent. Table 6 shows the results of the analgesic activity.

The acetylsalicylic acid-treated animals, there was a significant decrease in the number of abdominal contortions produced by acetic acid. The total number of contortions (writhing) was significantly lower than the rest of the groups tested with a 93.54% inhibition.

The hydroalcoholic extracts of the leaves and bark decreased the number of writhing at almost the same magnitude (as acetylsalicylic acid) with percentage inhibition of 92.63 and 92.47%, respectively.

The extracts of the fruits, likewise, reduced the number of contortions, but significantly less than that of the leaves and bark extracts. The ripe fruit extract showed less analgesic activity than the green fruit extract.

Intraperitoneal injection of acetic acid causes the release of endogenous substances such as prostaglandins E2 and F2α, serotonin, and histamine in the peritoneal area, exciting nerve endings for pain.[37] This suggests that the analgesic effect of the extracts obtained from the *M. coriacea* may be the result of a synergism between its components, in whose actions peripheral mechanisms of analgesia that prevent the synthesis of prostaglandins may be involved, thus causing a symptomatic relief of the pain.

Many plant-derived extracts are rich in secondary metabolites with important pharmacological properties. Fatty acids have been shown to have a significant analgesic effect in the acetic acid-induced contortion model.[38] Polyunsaturated fatty acids in recent years have shown beneficial effects on health, they have achieved positive results in situations related to inflammatory pain in the joints, knee osteoarthritis,[39] acute pain,[40] rheumatoid arthritis, neck, back, or shoulder pain,[41] neuropathic pain,[42] musculoskeletal injury, dysmenorrhea, and chronic headaches.[43,44] Terpenoids are a large family of natural products with highly important biological activities. Many monoterpenes have exhibited analgesic effects,[45] and triterpenoids derived from the lupane skeleton have also shown analgesic effect in the acetic acid-induced contortion model with inhibition percentages of 73 and 94%, respectively.[46,47]

Table 3: Compounds identified in the hydroalcoholic extract of *M. coriacea* green fruits

RT (min)	Compounds	MF	MM g/mol	% Abundance
13.81	Butanodioic acid (Succinic acid)	$C_4H_6O_4$	118.09	2.37
14.31	Propanoic acid	$C_3H_6O_2$	74.08	1.18
18.62	L-proline 5-oxo-1	$C_5H_9NO_2$	115.13	10.87
21.71	Xilonic acid-γ-lactone	$C_5H_{10}O_6$	166.13	2.96
22.18	Dodecanoic acid (Lauric acid)	$C_{12}H_{24}O_2$	200.318	1.18
26.40	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37	1.12
28.56	Benzoic acid	$C_7H_6O_2$	122.12	11.53
29.06	β-D-glucopiranose	$C_6H_{12}O6$	180.15	4.13
29.29	Hexadecanoic acid ethyl ester	$C_{18}H_{36}0_2$	284.48	1.18
30.31	Hexadecanoic acid (palmític acid)	$C_{16}H_{32}O_2$	256.40	15.25
32.06	Heptadecanoic acid (margaric acid)	$C_{17}H_{34}0_2$	270.50	1.78
33.20	9,12-octadecadienoic acid	$C_{18}H_{32}O_2$	282.45	5.92
33.33	Trans-9-octadecenoic acid	$C_{18}H_{34}O_2$	282.46	2.85
33.85	Octadecanoic acid	$C_{18}H_{36}O_2$	284.48	11.83
42.59	Squalene	$C_{30}H_{50}$	410.73	5.92/0.01
51.60	α Amyrin acetate	$C_{32}H_{52}O_2$	468.80	5.61

Legend: RT = Retention Time, MF = Molecular Formula, MM = Molecular Mass

Table 4: Compounds identified in the hydroalcoholic extract of *M. coriacea* ripe fruits

RT (min)	Compounds	MF	MM g/mol	% Abundance
13.87	Butanodioic acid (succinic acid)	$C_4H_6O_4$	118.09	3.32
14.30	Propanoic acid	$C_3H_6O_2$	74.08	0.13
19.01	L-proline 5-oxo-1	$C_5H_9NO_2$	115.13	8.2
21.71	Xilonic acid-γ-lactone	$C_5H_{10}O_6$	166.13	1.73
22.19	Dodecanoic acid (lauric acid)	$C_{12}H_{24}O_2$	200.318	0.40
26.42	Ácido tetradecanoico (miristic)	$C_{14}H_{28}O_2$	228.37	1.99
28.02	Glucitol	$C_6H_{14}O_6$	182.17	5.58
28.56	Benzoic acid	$C_7H_6O_2$	122.12	10.23
28.93	Mannonic acid	$C_6H_{10}O_6$	178.14	0.40
29.07	β-D-glucopiranose	$C_6H_{12}O_6$	180.16	7.57
29.27	Hexadecanoic acid ethyl ester	$C_{18}H_{36}0_2$	284.48	0.27
30.30	Hexadecanoic acid (palmitic acid)	$C_{16}H_{32}O_2$	256.40	11.55
32.07	Heptadecanoic acid	$C_{17}H_{34}0_2$	270.50	0.40
33.21	9,12-octadecadienoic acid ZZ	$C_{18}H_{32}O_2$	282.45	0.66
33.33	Trans-9-octadecenoic acid	$C_{18}H_{34}O_2$	282.46	5.18
33,84	octadecanoic acid (estearic acid)	$C_{18}H_{36}O_2$	284.48	9.08
42.59	Squalene	$C_{30}H_{50}$	410.73	4.25
51.60	α Amyrin acetate	$C_{32}H_{52}O_2$	468.80	3.13

Legend: RT = Retention Time, MF = Molecular Formula, MM = Molecular Mass

All the extracts exhibited analgesic effect, but this was superior for the extracts of the leaves and bark, between which there was no significant difference. The fruit extracts showed differences in the analgesic effect (higher for ripe fruits) and this effect was significantly lower than those of leaves and bark extracts.

The analgesic effect demonstrated for this species is in agreement with studies carried out on other species of the genus such as *Mimusops elengi*, whose ethanol and methanol bark extracts showed a good analgesic effect at a dose of 200 mg/kg.[48,49]

The results of the study allowed the identification in the tested extracts of compounds with the potential to act as analgesic agents. Differences were found in the presence of some metabolites and in the relative abundance of other compounds in the extracts of the leaves and bark and of the green fruits in relation to the ripe ones.

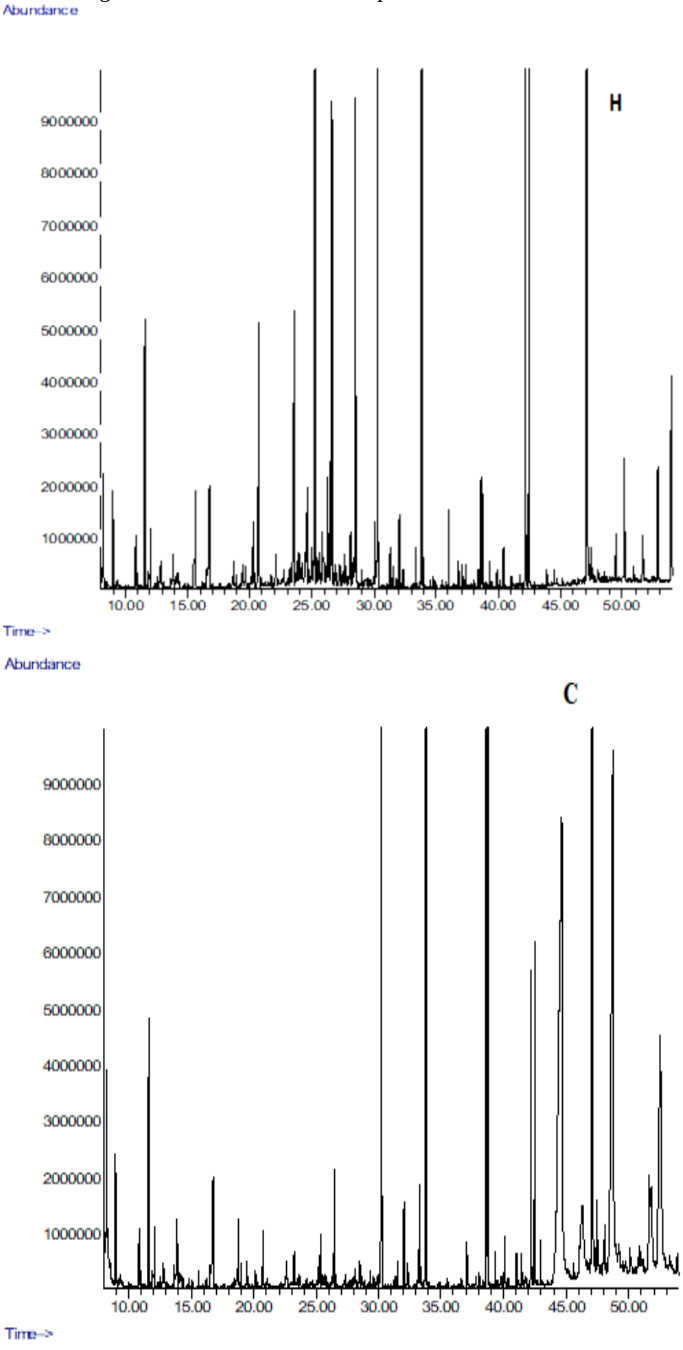

Figure 1: Gas chromatogram of the hydroalcoholic extracts of the leaves and bark of *M. coriacea*.
Legend: H - leaves; C – bark

Conclusion

The results of the present investigation provide the first findings on the demonstration of the analgesic effect of *M. coriacea*. The bioactive potential detected lays the foundations for the possible use of the species for analgesic purposes, and the absence of oral toxicity favours the traditional use of the plant.

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.

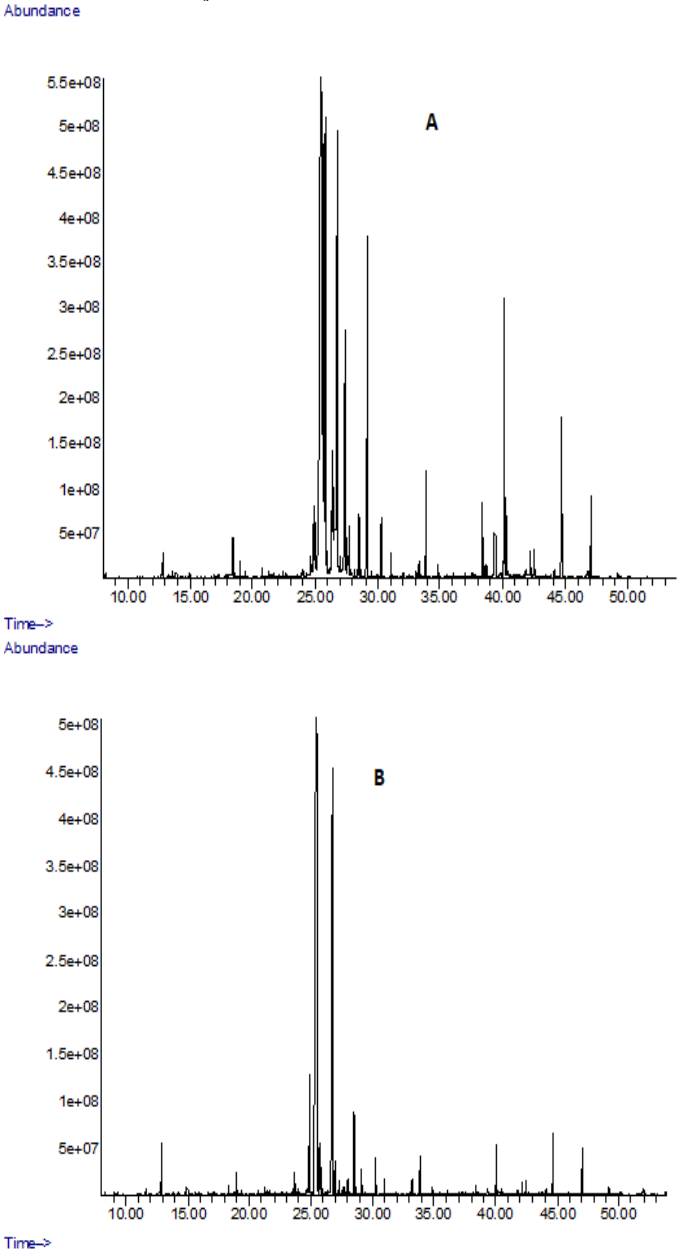

Figure 2: Gas chromatogram of the hydroalcoholic extracts of the green and ripe fruits of *M. coriacea*.
Legend: A - green fruits; B - ripe fruits

Table 5: Variation in body weight (g) of animals in the acute oral toxicity test of the hydroalcoholic extracts of the leaves, bark and fruits of *M. coriacea*

Samples	Groups	Average weight/Standard deviation		
		Time (days)		
		1	7	14
Leaves extract	I	194.6 / 8.0 [a]	214.6 / 17.6 [b]	217.3 / 16.7 [b]
2000 mg/kg	II	196.0 / 4.0 [c]	206.6 / 2.3 [c]	211.33 / 2.3 [d]
Bark extract	III	168.0 / 13.8 [e]	198.6 / 15.9 [f]	207 / 16.6 [f]
2000 mg/kg	IV	170.3 / 15.6 [g]	195.6 / 15.0 [h]	204 / 12.1 [h]
Green fruits extract	V	182.8 / 2.7 [i]	195.3 / 2.8 [j]	200.9 / 4.0 [j]
2000 mg/kg	VI	183.6 / 3.2 [k]	192.5 / 2.7 [l]	204.8 / 4.2 [m]
Ripe fruits extract	VII	194.9 / 4.78 [n]	199.1 / 4.8 [op]	205.2 / 4.7 [p]
2000 mg/kg	VIII	189.5 / 2.5 [q]	195.3 / 2.4 [r]	202.8 / 2.4 [s]

Different letters in a row represent significant differences ($p < 0.05$) according to Student Newman Keuls ($n = 3$)

Table 6: Analgesic activity of the hydroalcoholic extracts of the leaves, bark and fruits of *M. coriacea.*

Groups	Total contortions $\bar{X}/SD$	% Contortions inhibition
I. Negative control	19.08 / 3.53	-
II. Positive control (ASA) 200 mg/kg	1.13 / 0.83 [a]	94.07
III. Leaves extract 200 mg/kg	1.40 / 0.55 [b]	92.66
IV. Bark extract 200 mg/kg	1.40 / 1.67 [b]	92.66
V. Green fruits extract 200 mg/kg	5.60 / 1.63 [c]	70.64
VI. Ripe fruits extract 200 mg/kg	6.00 / 1.78 [d]	68.55

Legend: ASA: Acetylsalicylic acid; $\bar{X}/SD$: Average value of determinations ($n = 6$)/standard deviation
Different letters in a column represent significant differences ($p < 0.05$) according to Tukey post hoc test

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