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Research article

Unveiling the phytochemical profile, antioxidant and antibacterial activities, acute toxicity insight and analgesic effect of *Retama dasycarpa* stems: An unexplored endemic plant from Morocco

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ABSTRACT

Retama dasycarpa is an endemic shrubby leguminous plant of Morocco used in traditional folk medicine. The plant has never been studied for either its phytochemical or pharmacological properties. This study represents the first investigation of the phytochemical profile as well as the antioxidant, the antibacterial, the analgesic effects and the oral acute toxicity of *Retama dasycarpa*. Watery and hydromethanolic stems plant macerates have been investigated. Secondary metabolites quantitative analysis was achieved through spectrophotometric techniques. Antioxidant effect was explored through DPPH, ABTS and FRAP trials. Antibacterial activity was investigated using a micro-plates dilution assay. Analgesic activity was explored through acetic acid-induced writhing and tail-flick methods. Acute oral toxicity was investigated on mice. Phytochemical analysis was achieved through UHPLC connected to diode array and mass spectrometry detectors.

The obtained results showed significant contents in total phenolics, flavonoids and tannins in both extracts especially the hydromethanolic extract whose contents were slighlty higher than the aqueous one resulting in a remarkable antioxidant activity. Compared to the aqueous extract, the H_2O:MeOH (1:1) one showed notable antibacterial activity against the tested strains. The acute toxicity in mice revealed the non-toxicity of the extracts along with a promised starting material of central and peripheral analgesics. The UHPLC analysis revealed the presence of several bioactive phytochemicals pertaining to phenolic acids, flavonols, flavones and isoflavones.

The obtained results demonstrate the richness of this endemic and unexplored plant in terms of bioactive compounds and their associated activities, making it a promising source of pharmacological ingredients.

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1. Introduction

Herbal remedies have been used to treat illnesses for many years. Throughout history, humans have used plants to prevent and treat a variety of diseases. Nowadays, plants-derived products are still used as remedies against a wide variety of health problems and diseases despite the notable advancement of contemporary or modern therapy. Many botanical species are thus still used for health-care purposes around the world [1].

Research on medicinal plants for drug development involves an integrated multidisciplinary approach combining ethnobotanical, pharmacological and phytochemical research fields. In these broad disciplines, exploring botanical species used in ancestral popular care is of great importance and plays a crucial role in the production of bioactive compounds of potent biological activities. In connection with this aspect, more attention should be brought to endemic unexplored species that could be useful to discover and produce bioactive compounds which could be further exploited and used for drug development.

Due to its Mediterranean climate and eco-geographical position, Morocco constitutes a significant area of plants diversity. Moroccan diversified flora is characterized by a high rate of endemism exceeding five hundred species of the 7000 species and sub-species of the Moroccan medicinal plants [2]. Moroccan flora is also characterized by a notable endemism which puts the country among those recognized for their richness in endemic plants [3,4].

As part of a research program on medicinal plants in Morocco, we were focused to investigate the phytochemical profile and some biological effects of *Retama dasycarpa*. The species belongs to the genus *Retama*, the *Fabaceae* family, the sub-family Faboideae, and the tribe Genisteae. This genus includes four species sporadically distributed in the Mediterranean basin, namely *Retama monosperma*, *Retama sphaerocarpa, Retama raetam* and *Retama dasycarpa.* The latter is an endemic unexplored species from Morocco. *Retama* species can tolerate severe drought conditions and can therefore grow in various climates and habitats including deserts, scrub, and coastal dunes [5].

Retama species present an excellent source of phytochemicals and the genus *Retama* has recently received much attention for screening bioactive molecules from different parts. The literature reveals that *Retama* species contained several bioactive products known for their numerous biological properties mainly anti-ulcer, aseptic, anti-inflammatory, antioxidant, antitumor, antiviral, and hepatoprotective effects [6–11]. Among the four above indicated *Retama* species, *Retama dasycarpa* is an endemic Moroccan plant defined by the vernacular name '*R'tem*' or '*Algu*' [12]. With a large botanical repartition in the High Atlas Mountains, the plant is traditionally used against urological and nephrological diseases [13]. The species is a perennial plant growing in the interior valleys of the Moroccan High Atlas Mountains with cool and cold semi-arid bioclimates. This upright shrub can attain a height exceeding 3 m, accompanied by a trunk measuring 3.5–6 cm in diameter. Characterized by ovoid pods, the plant boasts a dense covering of hairs and houses yellow seeds [14].

The study of this Moroccan endemic plant is of great and crucial importance since bibliographic research showed that only one study dealing with the multiplicity of microsymbionts that nodulate this plant and their different phenotypes and symbiotic properties was reported [12]. Until now, no published investigations were reported on the phytochemical analysis, the biological or the pharmacological potential of the plant. The present research was then initiated to determine the proximate chemical composition of the aqueous and H_2O:MeOH (1:1) extracts from *Retama dasycarpa* using UHPLC-ESI-MS analysis together with the phytochemical analysis, the antioxidant prospects, and antibacterial and analgesic effects. This study constitutes then the first investigation of the plant and gave new and original findings on the phytochemical content and some biological activities of the plant. The outcomes derived from this study will provide valuable insights into the ethnomedicinal aspects of *Retama dasycarpa*, shedding light on its traditional medicinal uses and practices. The newfound results hold the potential to significantly contribute to the sustainable use of *Retama dasycarpa* in both pharmacological and phytomedicine domains.

2. Material and methods

2.1. Plant material

Retama dasycarpa stems were collected on November 11, 2021 during the harvest season from the Marrakesh region (N 31°28',7°24′ W), located at the west of the High Atlas Mountains hills in Morocco. The species was botanically characterized by the Moroccan Scientific Institute using species descriptions of the regional flora at the Botany and Plant Protection laboratory. A voucher specimen (RAB 114674) has been placed at the herbarium of the Moroccan Scientific Institute. The stems of *Retama dasycarpa* were air-dried at room temperature in shade during seven days and then grounded using an electric mill (Taurus, Barcelona, Spain). The grinded samples were stored in airtight glass vial till further use.

2.2. Extraction and sample preparation

The watery extract was obtained through mixing 50 g of plant powder with 500 mL of distilled water for 24 h at ambient temperature. The mixture was then properly sieved through Whatman filter paper and the solvent was reduced. The resulting extract was lyophilized, weighed, and stored at 4 °C until use.

In addition to water, organic solvents either singly or with water are frequently used for extracting antioxidants. Exploring the data reported on this subject evidenced that extraction efficiency depends on various criteria such as polarity, temperature, …. etc. Examination of literature results in addition to our previous works on extraction conditions optimisation suggested aqueous methanol as

an adequate extraction solvent giving highest yields of antioxidants such as polyphenols. This prompted us to prepare an aqueous methanolic extract from the investigated plant.

The hydromethanolic extract was obtained using methanol:water (1:1). The plant material was first submitted to soxhlet extraction with methylene chloride for 6 h to eliminate apolar products. The plant remainder was then suspended in methanol/water (50/50) at ambient temperature with agitation for 24 h. The obtained suspension was filtered through Whatman's paper and the resulting filtrate was vacuum-evaporated on a rotavapor (Buchi R-210, Switzerland), and the extracts were freeze-dried. The dried extracts were weighed and stored at 4 °C until use. The extraction outputs were given as a percentage of dry-weight material (% DW).

2.3. Phytochemical analysis

2.3.1. Total phenolics content (TPC)

The amounts of total phenolic compounds in *Retama dasycarpa* extracts were determined using the Folin-Ciocalteu reagent [15]. Briefly, 100 μL of each sample was blended with 0.1 mL of an aqueous Folin-Ciocalteu solution. After incubation for 5 min, 10 mL of Na_2CO_3 (7.5 %) was added and the mixture was incubated at ambient temperature for 1 h. The absorbance was assessed at 765 nm with a PerkinElmer spectrophotometer. The total amount of polyphenols was determined using a standard gallic acid calibration line obtained with concentrations ranging from 0 to 0.225 mg gallic acid/mL. The results were thus given as mg GAE per g dry extract.

2.3.2. Total flavonoids content (TFC)

TFCs were estimated through colorimetry by the aluminum chloride method [16]. Briefly, 500 μL of each sample were combined with 1.50 mL of ethyl alcohol 95 %, 0.10 mL aluminum chloride 10 %, 0.10 mL sodium ethanoate 1M and 2.80 mL distilled water and the absorbance was then measured at 415 nm. Final TFC determination was carried out with quercetin as standard calibration line and results were given as mg QE per g dry extract.

2.3.3. Total condensed tannins content (TCTC)

TCTCs were quantified through the previously reported vanillin-HCl technique [17]. Shortly, to an aliquot (50 μL) of *Retama dasycarpa* samples or standard solution, 1.5 mL of vanillin (4 % in MeOH) and 750 μL of HCl solution (12 M) were successively added and the obtained solution was thoroughly blended. After dark incubation for 20 min at room temperature, the absorbance was assessed at 500 nm. TCTC was determined using a (+)-catechin standard calibration curve. The results were thus expressed as mg CE/g dry extract.

2.4. Antioxidant activity assays

2.4.1. DPPH effect test

Retama dasycarpa extract's capacity to trap the DPPH radicals was estimated, with few adjustments, according to previously reported method [18]. DPPH was solubilized in methyl alcohol and added to 50 μL of samples at varied concentrations. Following dark incubation during 90 min at ambient-temperature, the absorbance was measured at 515 nm. The equation below was used to calculate the DPPH inhibition percentage:

$$\%\ \text{inhibition} = (A_0\text{-}A_1)\ /\ A_0 * 100$$

where A_0 and A_1 are the absorbances of the blank and the assayed samples respectively. The IC_{50} was determined by plotting the inhibition percentages against the concentrations of the sample. Quercetin was used as positive control.

2.4.2. ABTS free radical scavenging activity

The ABTS experiment was carried out using the previously described protocol where the ABTS radical was produced through oxidation of ABTS with potassium persulfate [19]. Briefly, when the potassium persulfate (70 mM) and the ABTS solution (7 mM) were mixed in equal volume and allowed to sit in the dark for 16 h at room temperature, ABTS radical cations were produced. Before being utilized in the assay, the ABTS radical cation was diluted with methanol to an initial absorbance of roughly 0.7 at 734 nm. Various quantities of each extract (100 μL) were mixed with 2 mL of the ABTS solution, the absorbance was measured at 734 nm and the percentage inhibition was calculated. Ascorbic acid was used as positive control.

2.4.3. Ferric-reducing antioxidant power test (FRAP)

The FRAP of the plant samples were spectrophotometrically evaluated through previously reported method [20]. Each investigated sample at various concentrations or a standard was blended with 2.5 mL of 0.2 M sodium phosphate buffer (pH = 6.6) solution and potassium ferricyanide ($K_3Fe(CN)_6$). After incubation for 20 min at 50 °C, 2.5 mL of 10 % (w/v) trichloroacetic acid was added to the mixture. To a volume of 2.5 mL of each concentration, 0.5 mL of 0.1 % (w/v) ferric chloride ($FeCl_3$) and 2.5 mL of distilled water were added. The blue-green color's intensity was quantified at 700 nm. (+)-Catechin (0.65–21.39 g/mL) was used as a positive control.

2.5. Antibacterial activity

The antibacterial activity of *Retama dasycarpa* extracts was evaluated counter Gram-negative (*Escherichia coli*, *Pseudomonas*

aeruginosa) and the Gram-positive (*Staphylococcus aureus*) strains using a micro-plates dilution assay. The three bacteria were choosen as common bacterial pathogens pertaining to both Gram-positive and Gram-negative species.

The MICs were assayed in a liquid medium (Mueller-Hinton) using sterile plastic 96 wells microplates. Each extract (100 mg/mL) was dissolved in DMSO at a 10 % concentration. 100 μL of Mueller-Hinton liquid culture medium was introduced to each well before adding 100 μL of the explored extracts and subsequent dilutions were carried out. 10 μL of each microorganism suspension is added to each well at an ultimate inoculum concentration of 10^8 CFU/mL. The plates were then covered and incubated for 24 h at 37 °C. 10 μL of bacterial growth indicator (resazurin) was added to each well for 2 h of further incubation at 37 °C. A color change from purple to pink revealed bacterial growth. The well concentration shortly before the first purple-colored well is known as the MIC [21]. The minimal bactericidal concentration (MBC) was evaluated by inoculating 3 μL from each negative well, which was spotted on LB plates and incubated at 37 °C for 24 h. All experiments were assayed three times and tetracycline was used as positive control.

2.6. Acute oral toxicity

The acute toxicity of the explored extracts was assessed following the OECD guidelines code 423 [22]. The body weight of each animal was determined after a 3–4 h fast in order to determine the dosage to be orally administered (in mg extract per kg body weight). Three groups of six mice each were randomly selected among the animals. Three female mice, each weighing between 20 and 30 g, were housed separately and individually in sterile polypropylene cages for each extract. During the experimental trial, each *Retama dasycarpa* extract was orally given using an esophageal probe at a rate of 2000 mg/kg, while the untreated control group received distilled water as a vehicle and was also assayed. The dose of extracts was determined by the guide based on the chemicals' potential for toxicity. Following the extracts ingestion, each animal was maintained under observation for 14 days during which body weight variations, mortality rate and any physical and behavioral changes have been noted in addition to clinical symptoms such as lethargy, diarrhea, salivation, convulsions, sleep and coma.

2.7. Analgesic activity

The analgesic effect of *Retama dasycarpa* extracts was explored using the acetic acid-induced writhing model in mice technique [23]. Animals were housed at the Faculty of Sciences, Rabat, Morocco. Animals had free access to food and water and were reared in standard usual conditions. Swiss mice were chosen one day before each trial and distributed into three groups of six mice each. One group (G1) served as the control and received no treatment. The second group was given aspirin (125 mg/kg) while the other groups were treated with test samples (250 and 500 mg/kg). All animals received intraperitoneally 10 mL/kg of 1 % acetic acid solution. After treatment, the number of abdominal writhings during 30 min was recorded. Aspirin was used as positive control and the analgesic activity was calculated in % as follow:

$$\text{Analgesic activity } (\%) = [\{\text{Wr (Control) - Wr (test compound)}\} / \text{ Wr (Control)}] \times 100$$

Wr = Mean number of writhings.

The tail immersion test was carried out according to formerly established method [24]. Wistar albino rats (160–250 g) were distributed similarly to those of the abdominal writhing test. The lower 6 cm part of the rats' tail was soaked in a water bath maintained at 55 ± 0.5 °C. The time between tail immersion and deflection was noted at 0, 30, 60, and 120 min after treatment with morphine or extracts using a digital stopwatch. Ten seconds is the time maintained to abstain from animal injury and morphine (5 mg/kg) was used as positive control.

2.8. UHPLC/ESI-MS analysis

Using liquid chromatography with PDA and MS detectors, the phytochemical profile of the investigated extract under optimized conditions has been explored. The analysis was performed on an Ultimate 3000 UHPLC apparatus including an ESI source interface, a data collection and processing system, an LCQ Advantage ion trap mass Thermo scientific spectrometer provided by Orbitrap analyzer and a surveyor quaternary pump connected to a PDA detector (200–600 nm). As stationary phase, a BDS Hypersil C18 column (length:150 mm, particle size: 5 μm, ID: 4.6 mm) was used. Flowing phase composed of H_2O (A) and CH_3CN (B) containing formic acid (0.1 %) formed with a flow of 0.48 mL/min. The used elution program was: 1 % B in 5 min, from 1 to 4.5 % B in 3 min, from 4.5 to 12 % B in 12 min, from 12 to 12.8 % B in 2 min, from 12.8 to 13.3 % B in 5 min, from 13.3 to 14.5 % B in 6 min, from 14.5 to 30 % B in 15 min, from 30 to 100 % B in 7 min and from 100 to 1 % B in 4.5 min followed by 5 min for column's re-equilibration for five minutes [11]. Compounds UV spectra were acquired and collected from 200 to 600 nm and the column temperature was 45 °C.

Sample extract was dissolved in MeOH:H_2O (50:50) and the obtained solution was filtered and injected to the UHPLC apparatus. Based on the acquired and collected UV and MS spectra in negative ionization mode and by comparing the observed spectroscopic features with those formerly reported data, the different detected phytochemicals were characterized.

2.9. Statistical analysis

The data were expressed as mean ± standard error. The independent *t*-test assessed the significance of means obtained for yield, TPC, TFC, and TCTC for the two used extraction solvents. One-way ANOVA followed by the Tukey post hoc test assessed the difference

between recorded antioxidant activities of both solvent extracts. Two-way analysis of variance was used to identify significant differences between means obtained under different treatments and times. All statistical analyses were performed using GraphPad Prism V9.5. A *p*-value less than 0.05 was regarded as indicating statistical significance.

3. Results and discussion

3.1. Quantitative phytochemical analysis

The results regarding the extraction yields of both extracts in addition to their quantitative phytochemical composition are gathered in Fig. 1A–D. Fig. 1A showed that the hydromethanolic extract yield higher (29.6 %) than the aqueous one (18.4 %). This was confirmed by the unpaired *t*-test showing a statistically significant difference between the yields obtained with the two solvents (*p*-value <0.001). The obtained results were similar to those observed for other Moroccan *Retama* species where yields of 18.91 and 28.97 % were respectively obtained for *Retama sphaerocarpa* and *Retama raetam* while no study was found on *Retama dasycarpa* [25,26].

The results concerning the TPC, TFC and TCTC in both extracts are depicted respectively in Fig. 1B, C and 1D. The total phenolics recovery from hydromethanolic extract was remarkably high, with a value of 198.59 mg GAE/g, while the aqueous extract revealed a slightly lower amount of 154.9 mg GAE/g. It is frequently stated that a water/methanol mixture gave higher phenolics recovery. Similar results were obtained from our previous work using methanol at concentrations close to 50 % to maximize yield from *Retama* species. This supports the fact that the extraction yield depends strongly on the extraction conditions used.

The above indicated results were comparable to those recently described for other Moroccan *Retama* species where amounts of 154.09 and 256.42 mg GAE/g mg were respectively observed for *Retama sphaerocarpa* and *Retama reatam* [25,26]. In general, polar solvents and hydromethanolic mixture have been often described to be efficient in phenolic compounds extraction [27–29]. Since methanol and water have been suggested due to their significant effectiveness in extracting soluble chemicals, variation in phenolic contents between species may be attributed to plant species, extraction method and geo-climatic conditions.

Regarding the total amount of flavonoids, the results in Fig. 1C illustrated that the hydromethanolic extract showed higher content (74.35 mg QE/g) compared to the aqueous one (38,68 ± 0.23 mg QE/g). The same trend was observed for total condensed tannin levels (Fig. 1D), with a relative richness of the hydromethanolic extract (12.5 ± 0.36 mg EC/g) compared to the aqueous one (5.52 ± 0.24 mg EC/g). The fact that the hydromethanolic mixture was more efficient for flavonoids and condensed tannins could probably be attributed to methanol being more effective in extracting lower molecular weight polyphenols such as flavonoids, which is in agreement with literature [30]. The difference between aqueous and hydromethanolic extract was statistically significant for the yields, TPC, TFC, and TCTC (*p*-value<0.001).

3.2. Antioxidant activity

The antioxidant capacity of the explored extracts was assessed using DPPH, ABTS, and FRAP methods. Table 1 summarizes the IC_{50} obtained results related to DPPH and ABTS assays and EC_{50} of the FRAP assay of the tested extracts and standards. The obtained results suggested that *Retama dasycarpa* hydromethanolic extract showed potent antioxidant activity through DPPH and ABTS assays with

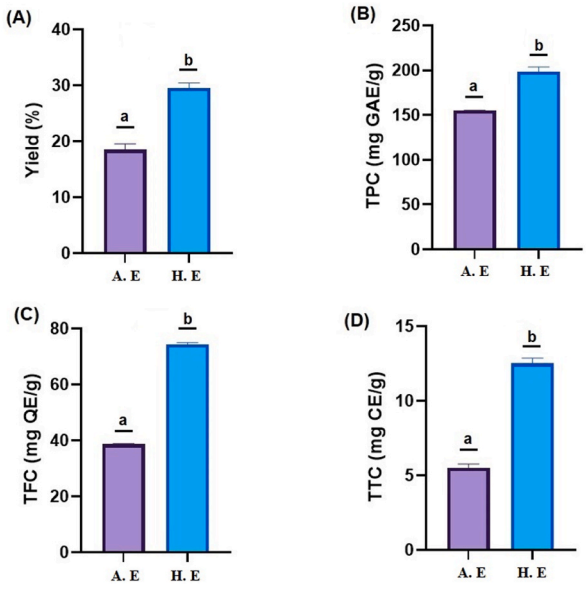

Fig. 1. Extraction yield (A), total phenolic (B), total flavonoid (C) and total condensed tanins (D) contents in aqueous (A.E) and hydromethanolic (H.E) extracts of *Retama dasycarpa*.

IC_{50} values of 152.46 and 109.43 μg/mL respectively. In the same conditions, values of 179.85 and 145.43 μg/mL were obtained for the aqueous extract respectively for DPPH and ABTS assays. This overall efficient activity of the MeOH/H_2O extract was predictable considering its relative abundance in antioxidants such as polyphenols, flavonoids and condensed tannins as shown in the above obtained quantitative analysis results.

On the other side, the aqueous extract showed higher antioxidant activity through the FRAP trial with an EC_{50} value of 263.55 μg/mL compared to 313.35 μg/mL obtained for the hydromethanolic extract.

The observed differences in the antioxidant effectiveness of the tested samples could arise to the peculiar chemical profile of the two extracts. This could also suggest that additional non-phenolic substances with more potent antioxidant properties might be involved and contribute to the observed activity through mutual synergistic and/or antagonistic interactions. Besides chemical structure, the concentrations used may also affect phytoconstituents overall activity [31]. Finally, the observed difference could also be due to the involved antioxidant mechanism involved in each used assay.

The difference in means obtained for the three tests, DPPH, ABTS, and FRAP, by both solvents and standards were statistically significant (p-value <0.05), and the Tukey post-hoc test observed no homogenous means.

3.3. Antibacterial activity

The antibacterial activity of *Retama dasycarpa* extracts was assessed versus 3 common bacterial pathogens pertaining to both Gram-positive and Gram-negative species (*Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus* aureus) using the microdilution method. Various extract concentrations were tested to yield MIC and MBC values for each strain. The obtained results indicated in Table 2 showed that *Retama dasycarpa* samples counteract the growth of the three tested strains with MIC values ranging from 12.5 to 50 mg/mL according to each assayed strain. Table 2 also showed that both tested extracts demonstrated a bactericidal effect against the studied strains.

Comparison between the two investigated samples effect showed that the hydromethanolic extract exhibited higher effect versus *Escherichia coli* and *Staphylococcus aureus* with MIC values of 12.5 mg/mL while that observed for *Pseudomonas aeruginosa* was 25 mg/mL. In the same conditions, MIC values of 12.15, 25, and 50 mg/mL were respectively observed for *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* in the case of the aqueous extract.

The observed sensitivity of the used strains to both extracts suggested the obvious existence of bioactive compounds like polyphenols, flavonoids or other products in their composition. The difference in secondary metabolites contents observed above could be the cause of the observed distinct antibacterial activity observed for the two explored extracts. The relatively observed potent activity of the hydromethanolic extract could be due, at least partly, to its content in phenolic compounds (polyphenols, flavonoids, condensed tannins) relatively higher than the aqueous extract.

These phytochemicals could act against bacteria by changing their morphology and structure or creating metabolic imbalances. Phenolic compounds were thus showed to interact with different places in the bacterial cell due to their hydroxylic groups [32]. This could explain the results observed which differ according to the targeted bacteria. This agreed with previously reported data for *Retama sphaerocarpa* hydroalcoholic extract where a potent antibacterial effect versus *Staphylococcus aureus* and *Pseudomonas aeruginosa* specis was observed [11]. The observed variation between species are correlated with the abundance of specific bioactive components.

3.4. Acute oral toxicity

The findings about the acute toxicity of *Retama dasycarpa* extracts through oral administration to mice are reported in Fig. 2 and showed no toxicity at an amount of 2000 mg/kg. The obtained results also showed that the LD_{50} is higher than 2000 mg/kg. During the total period of the experiments, no death or morbidity clinical signs were noticed and the tested mice lived with similar normal behavior between treated and control animals. During follow-up period, the mean body weight of each group did not significantly change (Fig. 2). From the results indicated above, and based on the EOCD 423 guidelines, the tested *Retama dasycarpa* extracts are considered as non-toxic for single oral ingestion at 2000 mg/kg.

3.5. Analgesic tests

There is no reported specific ethnobotanical use of *Retama* species for anti-inflammatory activity. However, the pharmacological

Table 1
Antioxidant activity of *Retama dasycarpa* extracts and standards using DPPH, ABTS and FRAP assays.

Extracts/Standards	DPPH (IC_{50} in μg/mL)	ABTS (IC_{50} in μg/mL)	FRAP (EC_{50} in μg/mL)
Aqueous extract	179.85^{c}	145.43 ± 0.63^{c}	263.55 ± 1.05^{c}
Hydromethanolic extract	152.46^{b}	109.43 ± 0.12^{b}	313.35 ± 4.19^{b}
Quercetin	5.49 ± 0.02^{a}	–	–
Ascorbic acid	–	2.52 ± 0.02^{a}	–
(+)-Catechin	–	–	13.90 ± 0.03^{a}

Means with different letters were significantly different at the level of $p < 0.05$.

Table 2
Antibacterial activity (MIC/MBC values) of *Retama dasycarpa* extracts and standard.

Strains	Hydromethanolic extract (mg/mL)	Aqueous extract (mg/mL)	Tetracycline (μg/mL)
Escherichia coli	12.5/12.5	25/25	256/256
Staphylococcus aureus	12.5/12.5	12.5/12.5	128/128
Pseudomonas aeruginosa	25/25	50/50	256/256

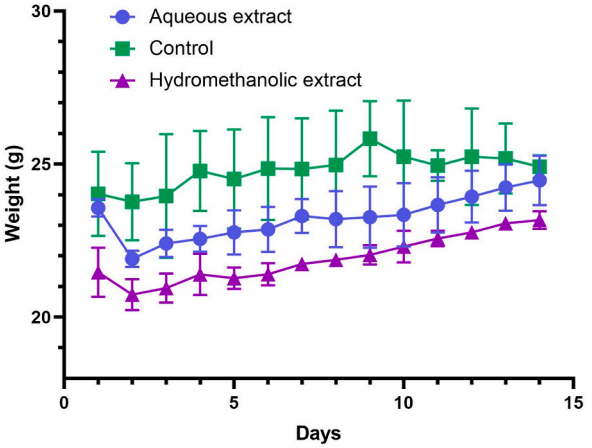

Fig. 2. Weight monitoring of mice after oral administration of 2000 mg/kg of *Retama dasycarpa* extracts.

activity involving inflammation was validated *in vitro* [5]. The plants biological benefits such as anti-inflammatory antioxidant and analgesic potentials have been shown to be mediated by secondary metabolites such as phenolic compounds [33]. This has prompted researchers to explore *Retama* species and its analgesic benefits.

In this work, animal models were used to explore for the first time the analgesic effects of *Retama dasycarpa*. Among these models, the writhing test was used to assess the antinociceptive effects of the plant extracts by inducing pain in the peripheral pathway. In this model assay, the injection of ethanoic acid into the mice peritoneal cavity causes abdominal contractions which could be inhibited using analgesic agents.

The results obtained with ethanoic acid writhing test method are reported in Table 3 showing the observed animal cramps number after intraperitoneal injection of ethanoic acid in the presence or absence of the plant extracts. The results expressed in percentages showed that the number of ethanoic acid-induced cramps was notably diminished by the hydromethanolic extracts and a dose-dependent inhibition on the writhing response.

Thorough examination of the indicated results showed that at a dose of 500 mg/kg, both explored aqueous and hydromethanolic extracts caused a significant writhings inhibition attaining 64.32 % and 75.23 % respectively. The obtained data pointed thus that the studied *Retama dasycarpa* extracts act peripherally. Considering the role played by ethanoic acid in inflammatory response through elevating prostaglandins, the extract phytochemicals could counteract the enzymes implicated in the production of such derivatives and their effects [34]. Previous reports showed that isolated isoflavones compounds, comprising biochanin, pratensein, 3-*O*-methy-lorobol, genistein and its 6-hydroxy derivative remarkably diminished the ventral writhing number caused by intra-peritoneal ethanoic acid injection [35].

The analgesic potential of the investigated extracts was further explored through tail immersion model assay and the attained data using the plant extracts and morphine are shown in Fig. 3. The results are given as the mean time required for tail withdraw at 0.5, 1.0, and 1.5 h. The two-way ANOVA findings indicated a statistically significant difference between the two studied factors, type of treatment (aqueous extract, hydromethanolic extract, morphine and control), and time (0, 30, 60, and 120 min) on the mean time of

Table 3
Analgesic activity of *Retama dasycarpa* extracts and aspirin on acid-induced writhing in mice.

Treatment	Dose (mg/kg)	Number of writhing	Inhibition (%)
Aqueous extract	250	17.00 ± 0.79	48.17
Aqueous extract	500	11.70 ± 1.15	64.32
Hydromethanolic extract	250	14.50 ± 0.80	55.79
Hydromethanolic extract	500	8.35 ± 0.60	75.23
Aspirin (125 mg/kg)	125	5.16 ± 1.16	84.26
Control	–	32.80 ± 1.30	–

Data represent the mean ± standard deviation of five independent experiments.

the tail withdraw movement (p-value <0,0001). Treating animals with aqueous and hydromethanolic extracts, at a dose of 500 mg/kg, alters rats latency to painful thermal stimulus in the tail flick test.

The findings presented in Fig. 3 demonstrated a notable increase in the anti-sensitive outcomes of the used extracts and morphine compared to negative control with a relatively prolonged tail flicking time in rats, with the highest values observed at 60 min. Subsequent to this time point, the recorded values stand at 13.31 ± 0.14 s and 8.37 ± 0.22 s for hydromethanolic and aqueous extracts, respectively, in comparison to 6.58 ± 0.35 s for the negative control and 12.45 ± 0.67 s for morphine. By the 120-min mark, the effect of the positive control (morphine) begins to diminish, reaching only 5.61 ± 0.55 s, while the antinociceptive effect of the hydromethanolic extract maintains its efficacy over this extended period of time, registering a value of 11.3 ± 0.6 s. The increase in response latency demonstrated the important antinociceptive activity of both extracts compared to positive and negative control. These findings suggest the presence of biologically active compounds noteworthy for further investigation to elucidate their mechanism of action by which they exert their antinociceptive properties.

The different observed analgesic activity of the two explored extracts observed for both used assay could be explained by the different observed phytochemical composition with higher secondary metabolites contents for the hydromethanolic sample in comparison to the aqueous one.

3.6. UHPLC-MS analysis

3.6.1. Qualitative analysis

After exploring the acute toxicity and some biological activities of *Retama dasycarpa*, the phytochemical profile of the hydromethanolic sample was explored by UHPLC with PDA and MS detectors. The results presented above showed that MeOH/H_2O (1:1) extract presents relatively higher activity than the aqueous one. The analysis allowed the detection and the tentative structural elucidation of 21 phytochemicals pertaining to different families including flavonoids (flavones, flavonols, isoflavonoids) and non-flavonoids (organic acids, phenolic acids). An example of the obtained chromatographic profile is presented in Fig. 4. Characterization of the detected products was achieved based on the interpretation of the acquired spectroscopic UV and MS results and their comparison with formerly published data. The different detected compounds with their spectral characteristics and their tentative identification with the corresponding references are shown in Table 4 and discussed below. The structures of some major evidenced products are gathered in Fig. 5.

3.6.1.1. Organic acids. Compound **1** (Rt = 3.83 min) yielded the pseudomolecular ion $[M-H]^-$ at *m/z* 209. An additional signal was noticed at *m/z* 191 and attributed to the $[M\text{-}H\text{-}H_2O]^-$ moiety yielded through dehydration of the pseudomolecular ion. Considering the obtained UV spectral characteristics of compound **1** and the previously reported cleavage scheme, compound **1** was characterized as glucaric aid [36,37].

Compound **2** (Rt = 5.35 min) revealed the pseudomolecular ion signal $[M-H]^-$ at *m/z* 191. A further moiety signal was observed at *m/z* 173 attributed to the release of a water molecule from the pseudomolecular ion $[M\text{-}H\text{-}H_2O]^-$. An additional peak appeared at *m/z* 111 attributed to $[M\text{-}H\text{-}2H_2O\text{-}CO_2]^-$ fragment in agreement with quinic acid fragmentation pattern [38,39]. This was confirmed by the obtained UV maxima consistent with quinic acid previously reported in *Retama* species [25].

3.6.1.2. Phenolic acids. Compound **3** (Rt = 10.40 min) was characterized as quinic acid caffeoyl ester. The mass spectrum revealed a pseudomolecular ion signal at *m/z* 353 matching to the deprotonated ion. The latter suffered a loss of caffeoyl moiety yielding a peak at *m/z* 191 confirming the proposed structure and consistent with previously reported MS and UV properties of caffeoylquinic [40,41,56]. Identification of compound **3** was also established through comparison of its retention, UV and MS properties with caffeoylquinic acid previously detected and identified in *Retama sphaerocarpa* extract analyzed in the same conditions [25]. Quinic acid derivatives

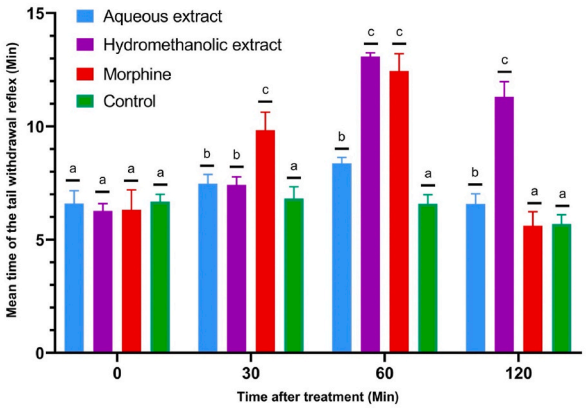

Fig. 3. Mean time of the tail withdrawal reflex after treatment with aqueous and hydromethanolic extracts (500 mg/kg) as well as negative and positive (morphine) controls at 30, 60 and 120 min.

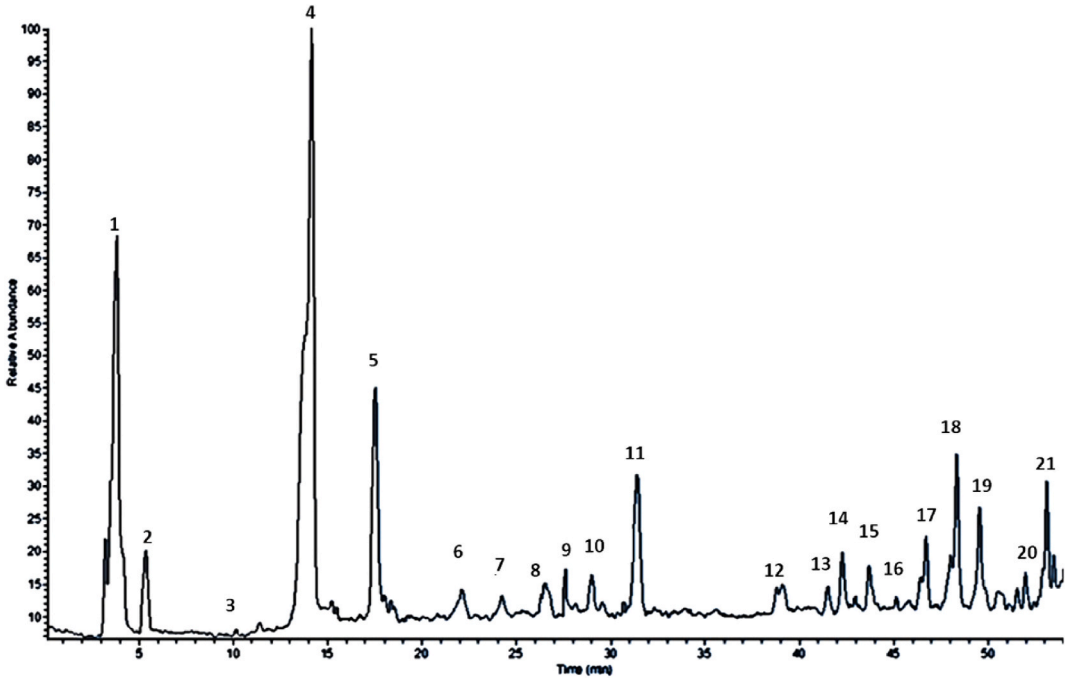

Fig. 4. UHPLC/ESI-MS chromatographic profile recorded in the negative ion mode of *Retama dasycarpa* hydromethanolic extract.

Table 4
Characterization of compounds detected through UHPLC–ESI-MS in *Retama dasycarpa* hydromethanolic extract (NI: not identified).

Peak	Rt (min)	UV (nm)	$[M-H]^-$ (*m/z*)	MS (*m/z*)	Identification	References
1	3.83	228	209	191, 173	Glucaric acid	[36,37]
2	5.35	246	191	173, 129, 111	Quinic acid	[25,38,39]
3	10.40	219, 305	353	191	Caffeoylquinic acid	[40,41]
4	14.15	224, 275	255	193, 179, 165	Piscidic acid	[11,25]
5	17.53	239, 306	593	447, 301	Quercetin di-*O*-rhamnoside	[11,42]
6	24.23	235, 268, 330	563	443, 431, 327, 311, 269	Apigenin 8-*C*-hexoside-7-*O*-pentoside	[43,44]
7	26.52	238, 271, 332	593	431, 311	Vitexin *O*-hexoside	[11,25,45]
8	27.59	232, 321	593	449, 285, 257, 197	Luteolin derivative	[11,46,47]
9	29.01	230, 348	491	445, 283	Calycosin *O*-hexoside	[25,48]
10	30.69	230, 348	593	473	Apigenin 6,8-di-*C*-hexoside	[25,49,50]
11	31.42	264, 310	431	311	Genistein C-hexoside	[51]
12	38.81	260, 340	575	477, 431	Genistein 3-hydroxy-3-methylglutaroyl	[51]
13	39.11	260, 326	477	431, 269	Genistin	[48,52,53]
14	41.51	264	765	765, 663, 457, 353	NI	
15	42.28	232, 321	577	503, 353, 473, 253	Apigenin 8-*C*- rhamnoside-6-*C*-glucoside	[39,54]
16	43.70	241, 325	607	447, 255	NI	
17	42.95	234, 266, 318	575	431, 311, 269	Vitexin 3-hydroxy methyl glutaroyl	[11,25]
18	46.43	261, 325	739	431, 269	Apigenin 7-O-glucosylrhamnoside 6-C-glucoside	[46,49]
19	46.70	240, 323	445	283	Calycosin *O*-hexoside	[25,48]
20	48.35	240, 325	735	285	Luteolin derivative	[11,39,55]
21	53.13	234, 267, 340	973	269	Apigenin derivative	[11,39]

with caffeoyl groups were reported to have higher antioxidant activities than the free acid form [57].

Compound **4** (Rt = 14.15 min) presented mass spectral data similar to those of piscidic acid which was previously detected and identified in other *Retama* species [11,25]. The obtained MS results yielded the pseudomolecular ion $[M-H]^-$ at *m/z* 255 which further gave a fragment signal at 193 attributed to the $[M\text{-}H\text{-}CHO_2\text{-}OH]^-$ moiety. The latter further release a 28 uma unit (-CO) yielding a peak at *m/z* 165 in consistent with data formerly published on piscidic acid [58]. It may be noted that piscidic acid, which was identified among the major components in *Retama sphaerocarpa* [25], is well known for its powerful antioxidant effect and is rarely naturally encountered, being limited to plants exhibiting crassulacean acid metabolism (CAM) [58].

3.6.1.3. Flavonoids. Along phenolic and organic and acids, examination of the analysis results indicated the occurrence of several flavonoid derivatives. The occurrence of such derivatives was confirmed through peaks UV–visible spectra with typical absorptions

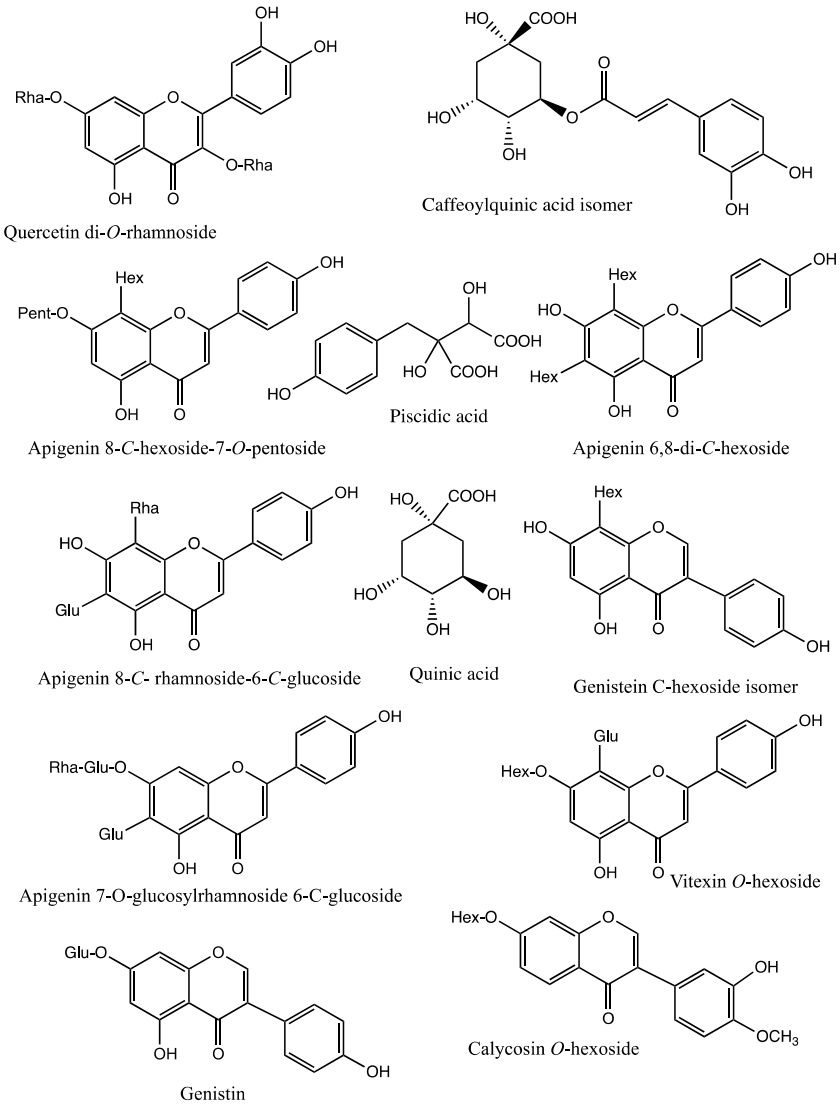

Fig. 5. Structures of the major phenolics evidenced in *Retama dasycarpa* hydromethanolic extract (Rha: rhamnose, Glu: glucose, Hex: hexose, Pent: pentose).

maxima with two major (BI and BII) bands due to the cinnamoyl (300–350 nm) and the benzoyl (230–280 nm) moieties. The glycosylation type was determined based on the mass spectra fragmentation and ion products relative intensities [59–61]. The identified compounds have been divided in flavonols, flavones and are discussed below.

3.6.1.3.1. Flavonols. Compound **5** (Rt = 17.53 min) which gave deprotonated ion $[M-H]^-$ signal at *m/z* 593 was concluded to be a quercetin derivative. Indeed, the mass spectrum also showed an ion signal at *m/z* 301 as base signal matching the aglycone quercetin ion [42]. This fragment was obtained through a first loss of 146 amu giving an ion signal at 447 followed by a second 146 amu loss. These losses could probably correspond to two rhamnose moieties suggesting that compound **5** is a quercetin derivative with two rhamnose moieties. From the obtained MS data along with the UV visible characteristics which were analogous to those formerly published on *Retama sphaerocarpa* [11], compound **5** was concluded to be quercetin di-*O*- rhamnoside.

3.6.1.3.2. Isoflavones. Compound **9** delivered at (Rt = 29.01 min) was assigned as an isoflavone derivative from its UV spectroscopic properties. Its MS characteristics showed signal at *m/z* 491 assigned to the $[M\text{-}H + HCOOH]^-$ ion. Its pseudomolecular ion $[M-H]^-$ was also noticed at *m/z* 445. The last-mentioned ion yielded the aglycone moiety at *m/z* 283 by losing the hexose moiety (−162 amu). Considering the UV data of product **9** characteristic of isoflavone derivatives and previously reported data of *Retama* specis [8,25,48], this product was concluded to be calycosin *O*-hexoside.

Compound **11** (Rt = 31.42 min) gave a deprotonated pseudomolecular ion signal $[M-H]^-$ at *m/z* 431. An additional ion signal at *m/z* 311 matching the $[M\text{-}H\text{-}120]^-$ ion was noticed suggesting a *C*-hexoside flavonoids [11,45,51]. Based on these results along with the obtained UV spectrum characteristic of isoflavone skeleton and previously reported data on *Retama* species compound **11** was

concluded to be genistein *C*-hexoside.

In a similar manner, compound **12** (Rt = 38.81 min) was deduced to be a derivative of genistein from its UV spectral characteristics and its previously reported occurrence in *Retama* species. Its deprotonated molecular ion signal $[M-H]^-$ appeared at *m/z* 575. The MS also revealed the occurrence of a base signal at *m/z* 431 matching the $[M\text{-}H\text{-}144]^-$ ion released through the loss of the neutral 3-hydroxy-3-methylglutaroyl moiety [51]. Compound **12** was thus assigned to genistein-3-hydroxy-3-methylglutaroyl.

Compound **13** eluted at 39.11 min presented UV spectral characteristic of isoflavones. The mass spectra revealed the molecular ion $[M-H]^-$ signal at 431. This was also confirmed through the presence of the $[M\text{-}H + HCOOH]^-$ peak which was observed at *m/z* 477. Further cleavage of the $[M-H]^-$ released the fragment at *m/z* 269 consistent with a genistein aglycone ion $[M\text{-}H\text{-}162]^-$. The latter was obtained from the deprotonated ion by losing the hexose group. Product **13** was then assigned as genistin which is mainly encountered in Leguminosae as a glucoside form of genistein [53]. The 7-*O*-glycosides derivatives of these compounds were described to be used by plants [52].

Another glycosidic methylated isoflavones, namely calycosin *O*-hexoside, was eluted at Rt 46.70 min (compound **19**). Its mass spectrum showed the pseudomolecular ion $[M-H]^-$ at *m/z* 445. Another ion was noticed at *m/z* 283 obtained through losing 162 mass unit group and matching the release of an hexose unit. Considering the UV spectrum of compound **19** characteristic of isoflavone derivatives and previously reported data of *Retama* species [8,25,48], this product was concluded to be calycosin *O*-hexoside.

The presence of genistein and calycosin adducts in *Retama dasycarpa* confirmed the previously reported data where such isoflavones are considered as representatives among Fabacae and particularly the *Retama* genus [8].

3.6.1.3.3. Flavones. Compound **6** eluted at 24.23 min presented UV spectrum characteristic of flavone with two band maxima located at 330 and 268 nm matching respectively the cinnamoyl and the benzoyl absorption groups. The deprotonated ion $[M-H]^-$ signal was observed at *m/z* 563 along with another ion signal at *m/z* 431 corresponding to $[M\text{-}H\text{-}132]^-$ and resulting from the release of a pentoside unit [43]. An additional minor signal was located at *m/z* 443 released through the loss of 120 amu suggesting a *C*-hexosyl flavones confirming formerly reported data [45]. This was also supported by the occurrence of a signal located at *m/z* 327 resulting from a fragmentation occurring involving the *C*-hexosyl moiety. The fragment signal resulting from the successive losses of the two above indicated moieties $[M\text{-}H\text{-}132\text{-}120]^-$ was also revealed at *m/z* 311. Finally, the signal at *m/z* 269 ion $[M\text{-}H\text{-}132\text{-}120]^-$ produced through the successive losses of the pentose and hexose moieties was observed releasing the apigenin deprotonated ion signal. The presence of apigenin skeleton was reinforced by the UV characteristics of compound **6** with the two above indicated characteristic absorption maxima. The *C*-hexosyl unit was deduced to be bounded to the carbon 8 based on the fact that the $[M-H\text{-}120]^-$ ion signal was of a weak intensity along with the non-appearance of the $[M\text{-}H\text{-}90]^-$ signal in agreement with previously reported data [43,44]. Concerning the *O*-pentoside group, the likely linkage position would be 7-hydroxyl unit as previously reported [62]. Compound **6** was then assigned as apigenin 7-*O*-pentoside-8-*C*-hexoside.

Compound **10** (Rt = 30.69 min) was also deduced to be a flavone *C*-glycoside derivative. Its MS results showed a pseudomolecular $[M-H]^-$ signal at *m/z* 593, that through further fragmentation released a product signal at *m/z* 473 $[M\text{-}H\text{-}120]^-$ with 100 % relative abundance suggesting the occurrence of a *C*-hexoside group. Based on the obtained mass and UV spectral results and previously reported data for di *C*-glycosylflavone derivatives compound **10** was deduced to be apigenin 6,8-di-*C*-hexoside [25,49,50].

Compound **15** (Rt = 42.28 min) displayed a molecular ion signal $[M-H]^-$ at *m/z* 577. Further signals appeared at *m/z* 503 and 473 assigned respectively to $[M\text{-}H\text{-}74]^-$ and $[M\text{-}H\text{-}104]^-$ suggesting the occurrence of a rhamnose unit. Additional peaks were located at *m/z* 353 and 253 indicating a di *C*-glycosyl flavone cleavage scheme [44]. Considering the obtained results along with the UV spectral characteristics, compound **15** was deduced to be apigenin 8-*C*-rhamnoside-6-*C*-glucoside [39,46,56].

Product **18** (Rt = 46.43 min) showed a pseudomolecular ion $[M-H]^-$ signal at *m/z* 739. Two other signals were observed at *m/z* 577 and 431 resulting respectively from the successive losses of an hexosyl and rhamnosyl groups and suggesting the existence of a glucosyl rhamnoside moiety. An additional abundant signal appeared at *m/z* 269 suggesting a *C*-glycosyl apigenin moiety in agreement with the obtained UV spectral characteristics. The linkage positions of these glycosyl moieties were tentatively concluded to be the 6 position for the *C*-glucoside and the 7 position for the *O*-glucosyl rhamnoside moieties taking into account the obtained signals intensities and the reported results concerning such compounds [27,44–46,49,63]. Compound **18** was then concluded to be apigenin 7-*O*-glucosylrhamnoside 6-*C*-glucoside [49].

Compound **21** (Rt = 53.13 min) gave a pseudomolecular ion at *m/z* 973 giving a portion signal at *m/z* 269 typical of apigenin aglycone. This was confirmed through the obtained UV characteristics analogous to that of apigenin. Compound **21** was thus assigned as apigenin derivative [11,39].

In the same manner, compounds **8** (Rt = 27.59 min) and **20** (Rt = 48.35 min) yielding $[M-H]^-$ signals at *m/z* 593 and 735 respectively were concluded to be a luteolin derivatives through their UV and MS acquired data. The luteolin aglycone moiety ion signal appeared at *m/z* 285 for both compounds [46,48]. Compound **8** released the luteolin skeleton by losing 308 amu corresponding probably to an hexosyl and a deoxyhexosyl moieties suggesting a disaccharide nature of this product. Luteolin derivatives were previously reported in the *Retama* [5,39]. Compound **7** (Rt = 26.52 min) was deduced to be a vitexin *O*-hexoside on the basis of its mass and UV spectral results. Its $[M-H]^-$ signal appeared at *m/z* 593. This ion yielded a portion $[M\text{-}H\text{-}162]^-$ signal at *m/z* 431 through the release of an *O*-hexose moiety. The *C*-hexoside group was supported through the existence of peaks at *m/z* 341 and 311 (−90 and −120 Da) in agreement with a vitexin skeleton [11,25,45].

Compound **17** (Rt = 42.95 min) was tentatively identified as vitexin 3-hydroxy-3-methylglutaroyl. Its UV spectrum was comparable to those of vitexin isomer. Its acquired MS results displayed a pseudomolecular $[M-H]^-$ signal at *m/z* 575 along with a product ion at *m/z* 431 in agreement with the proposed structure [11]. An additional release of 144 amu was also observed suggesting the existence of 3-hydroxy-3-methylglutaroyl moiety [63].

Finally, compounds **14** and **16** which were observed at 41.51 and 43.70 min were not fully characterized due to the insufficient

obtained results for their unambiguous identification.

3.6.2. Relative quantitative analysis

After the structural elucidation of the products detected in the hydromethanolic sample, the relative percentage of each detected product was performed. The attained results, given as percentages calculated from the characterized product peaks surfaces, are assembled in Table 5. The relative composition of *Retama dasycarpa* phytochemical composition is characterized by the presence of piscidic acid as the major compound representing 22.91 % of the extract. The explored extract contained also relatively high amounts of flavonoids (58.8 %) such as flavones (34.29 %), flavonols (6.64 %) and (17.65 %). Flavones were dominated by apigenin (24.5 %) and luteolin (9.79 %) glycosides and isoflavonoids adducts consisting of genestein (10.14 %) and calycosin (7.51 %) glycosides while quercetin glycosides (6.64 %) were the only detected flavonols. Among the other detected compounds, organic acids such as glucaric acid (8.5 %) and quinic acid (3.42 %) and caffeoylquinic acid (0.57 %) were reported.

As indicated above *Retama* genre encompasses four botanical species distributed in the Mediterranean area. Examination of literature data showed results of the phytochemical composition of *Retama monosperma* [5,64–66], *Retama sphaerocarpa* [5,11,25,67] and *Retama raetam* [5,51,53].

The previously reported data showed that Retama species have a broad spectrum of bioactive compounds including volatile and non-volatile compounds such as phenolics notably flavonoids. No previous results are reported on the Moroccan endemic specie *Retama dasycarpa* for which the investigation detailed above represents the first phytochemical study. Juxtaposition of the obtained outcomes on this specie with the previously mentioned data on other *Retama* species showed an overall similar composition from a qualitative point of view.

Thus, organic acids such as quinic acid which has been detected along with its caffeoyl derivative in *Retama dasycarpa*, has been already reported as one of the major compounds in grains and cladodes of *Retama sphaerocarpa* from Tunisia [11] and Morocco [25]. This organic acid was also identified in the aerial organs of *Retama raetam* from Tunisia [51] and in the stems of *Retama monosperma* from Algeria [64].

Phenolic acids such piscidic acid identified herein in the stems of *Retama dasycarpa* was also identified as main components in other *Retama* species such as *Retama sphaerocarpa* [11,25], *Retama raetam* [51] and *Retama monosperma* [64].

In addition to organic and phenolic acids, this study showed that flavonoids which are often abundantly reported in plants were also predominant in *Retama dasycarpa*. This is in line with *Retama* species phytochemical composition where such phenolics were indicated as common in the genus [5,65,66]. Within this phenolic family, quercetin di-*O*-rhamnoside (morin) which is a flavonol derivative has been identified in the studied *Retama dasycarpa*. This compound has been also detected among the major compounds in the stems extracts of *Retama spaerocarpa* from Tunisia [11]. Besides morin other flavonols such kaempferol and isorhamnetin adducts were formerly reported in *Retama sphaerocarpa* [11,25] and *Retama monosperma* [64].

Over and above flavonols, the investigated *Retama dasycarpa* was shown to be qualitatively rich in flavones with several luteolin and apigenin *C*- and *O*- mono-ad di-saccharides. This prevalence was also observed for other *Retama* species where apigenin adducts were identified in *Retama monosperma* [64], *Retama raetam* [53], *Retama sphaerocarpa* [11,25]. Luteolin based flavones were detected in *Retama sphaerocarpa* [11,25] and finally chrysin glycoside was evidenced in *Retama sphaerocarpa* [25].

In addition to flavones and flavonols, isoflavonoid derivatives were also identified in the explored *Retama dasycarpa* stems extract with genestein and calycosin as aglycones. Such aglycones derivatives have been already reported in other *Retama* species. This is the case of *Retama monosperma* [64], *Retama raetam* [51,53], *Retama sphaerocarpa* [11,25] which were indicated to contain genestein

Table 5
Relative quantitative composition of *Retama dasycarpa* hydromethanolic extract.

Peak	Rt (min)	Compound	Percentage (%)
1	3.83	Glucaric acid	8.50
2	5.35	Quinic acid	3.42
3	10.40	Caffeoylquinic acid	0.57
4	14.15	Piscidic acid	22.91
5	17.53	Quercetin di-*O*-rhamnoside	6.64
6	24.23	Apigenin 8-*C*-hexoside-7-*O*-pentoside	4.11
7	26.52	Vitexin O hexoside	3.35
8	27.59	Luteolin derivative	3.77
9	29.01	Calycosin *O*-hexoside	2.94
10	30.69	Apigenin 6,8-di-*C*-hexoside	1.88
11	31.42	Genistein C-hexoside	5.77
12	38.81	Genistein 3-hydroxy-3-methylglutaroyl	1.92
13	39.11	Genistin	2.45
14	41.51	NI	1.86
15	42.28	Apigenin 8-*C*- rhamnoside-6-*C*-glucoside	4.06
16	43.70	NI	4.61
17	42.95	Vitexin 3-hydroxy methyl glutaroyl	3.13
18	46.43	Apigenin 7-O-glucosylrhamnoside 6-C-glucoside	2.81
19	46.70	Calycosin *O*-hexoside	4.57
20	48.35	Luteolin derivative	6.02
21	53.13	Apigenin derivative	5.16

adducts while calycosin glycosides were identified in *Reatama raetam* [51,53], *Retama sphaerocarpa* [11,25]. Besides calycosin and genestein glycosides, other isoflavonoid aglycone derivatives have also been reported in *Retama* species. This is the case of licoisoflavone B identified in *Retama monosperma* [64] and daidzin derivatives reported in *Retama monosperma* [5,65,66] and *Retama sphaerocarpa* [5].

It may be noted that, even if the phytochemical compounds identified herein in *Retama dasycarpa* were almost already detected in other *Retama* species, obvious quantitative variations could be observed between species. These are generally due to the different climatic and geographical conditions. This could also be attributed to the used extraction techniques and the used plant organs as well as genetic, environmental conditions in addition to harvesting time.

4. Conclusion

In the current research investigation, we investigated the phytochemical composition and some biological activities of the unexplored endemic Moroccan plant *Retama dasycarpa*. Extraction with aqueous and hydromethanolic solvents showed that the latter was more effective with higher yields in various phytochemicals such as phenolic compounds, flavonoids and condensed tanins. While no acute toxicity was observed with both extracts, the hydromethanolic sample showed significant antioxidant, antibacterial and analgesic activities than the aqueous one.

The obtained results showed thus that *Retama dasycarpa* exhibited potential biological properties due to its richness in phenolic compounds. These have been evidenced through LC-ESI/MS analysis which showed the occurrence of various phenolics such as piscidic acid, genistein isoflavonoids in addition to luteolin and apigenin flavone glycosides as major compounds.

Our study represents the first investigation on *Retama dasycarpa* and presents thus new and original information related to its phytochemical composition and biological activities. The obtained results thus constitute a scientific root for the use of this perennial plant in traditional and indigenous herbal remedies. Due to the investigated beneficial therapeutic properties, the plant could then be recommended for potential applications in pharmacology and phytomedicine. The phenolics concentration and potent biological activity of the plant make it suitable and appropriate for further investigation and analysis such as separation, purification and pharmaceutical effect of the major ingredients with new perspectives and use in drug and food industries. Overall, this research endeavors to bind the common traditional information and contemporary scientific advancements, fostering a holistic approach to the sustainable use of *Retama dasycarpa* in the broader field of medicine.

Ethics statement

All animal experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Animal Ethics Committee (Local Institutional Research Committee, UM5/ND/70/8647).

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Data availability statement

Data included in article/supp. material/referenced in article.

CRediT authorship contribution statement

Aafaf El Baakili: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Mouhcine Fadil:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Fatima-Ezzahrae Guaouguaou:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Conceptualization. **Mustapha Missbah El Idrissi:** Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Khalid Taghzouti:** Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Mohamed Jeddi:** Methodology, Formal analysis, Data curation, Conceptualization. **Kawtar Fikri-Benbrahim:** Resources, Methodology, Conceptualization. **Nour Eddine Es-Safi:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:The author Nour Eddine ES-SAFI is an Associate Editor for Heliyon and was not involved in the editorial review or the decision to publish this article. This research was supported by ANPMA/UM5R/CNRST (Project number PMA2019-4). If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

List of abbreviations

A.E	Aqueous extract
A_0	Absorbance value of the blank
A_1	Absorbance values of the sample
ABTS	2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
ANOVA	Analysis of variance
BDS	Base deactivated silica
CAM	Crassulacean acid metabolism
CE	(+)-Catechin equivalent
CFU	Colony forming unit
DMSO	Dimethylsulfoxide
DPPH	2,2-Diphenyl-1-picrylhydrazyl
EC50	Effective concentration necessary to decrease the radical concentration by 50 %
ESI	Electrospray
FRAP	Ferric-reducing antioxidant power
GAE	Gallic acid equivalent
H.E	Hydromehanolic extract
IC50	Concentration providing 50 % DPPH inhibition
LCQ	Liquid chromatography quadrupole
LD50	Dose at which a substance is lethal for 50 % of animals tested
MBC	Minimal bactericidal concentration
MIC	Minimum inhibitory concentration
Min	Minute
MS	Mass spectrometry
NI	Not identified
OECD	Organization of economic cooperation and development
PDA	Photodiode array detector
QE	Quercetin equivalent
Rt	Retention time
TCTC	Total condensed tannins content
TFC	Total flavonoids content
TPC	Total phenolics content
UHPLC	Ultra high performance liquid chromatography
UV	Ultraviolet
Wr	Mean number of writhings

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