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RESEARCH ARTICLE

Evaluation of the content of polyphenols, flavonoids and tannins, the antioxidant capacity and the antimicrobial activity of different organic and aqueous fractions of stems of *Retama monosperma*

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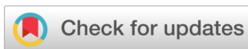

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Abstract

Retama monosperma is an endemic plant of the Mediterranean region that has been traditionally used in folk medicine to treat various ailments. It contains a variety of bioactive phytochemicals and exhibited several biological activities. This study aimed to assess the phytochemical screening, total phenolic, total flavonoid, and total tannin compounds, as well as the antioxidant capacity and antimicrobial activity. The phytochemical screening involved color reactions, characteristic reagents, and precipitation methods. Total phenolic, flavonoid, and tannin compounds were quantified using colorimetric methods across four fractions. Antioxidant capacity was assessed using 2,2-Diphenyl-1-picrylhydrazyl radical scavenging, ferric reducing antioxidant power, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging, and phosphomolebdenum assay. The antimicrobial activity was evaluated through disc diffusion method and the microdilution assay. Qualitative phytochemical tests revealed the presence of flavonoids, tannins, terpenoids, alkaloids, and sterols. Quantification of total phenolic, flavonoid, and tannin compounds confirmed the richness of polyphenolic compounds in all fractions. The antioxidant capacity measurements revealed that the ethanol fractions exhibited the highest antioxidant capacity in 2,2-Diphenyl-1-picrylhydrazyl, ferric reducing antioxidant power assays, and phosphomolebdenum assay. Conversely, the aqueous fraction showed highest activity in the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay. Regarding antimicrobial activity, the ethyl acetate fraction demonstrated superior efficacy against *Staphylococcus aureus*, and *Bacillus cereus*. These findings suggest that *R. monosperma* could serve as a valuable source of antioxidant and anti-infective phytocompounds.

Keywords

Retama monosperma; polyphenols; flavonoids; tannins; antioxidant capacity; antimicrobial activity

Introduction

Retama monosperma or *Genista monosperma* (common local name: R'tem), is a leguminous shrub belonging to Fabaceae family. It features small white flowers, deciduous leaves, and photosynthetic cladodes. This species grows

spontaneously and abundantly in many Moroccan natural forests and coastal areas with sandy soils, where it has long been recognized for its medicinal plant (1). The genus Retama comprises four species (*Retama monosperma* (L.) Boiss., *Retama raetam* (Forssk.) Webb., *Retama sphaerocarpa* (L.) Boiss., and *Retama dasycarpa* Coss.). These species are geographically distributed across the Canary Islands, North Africa, and the Mediterranean Basin (2, 3). The populations of various countries in North Africa and the Mediterranean Basin have traditionally used these plants in folk medicine to treat a wide range of diseases. They are commonly employed for skin care, joint pain, and inflammation, as well as for aiding in the healing of circumcisions, treating rheumatism, and alleviating eczema (4). The Retama genus is administered in small doses to treat hyperlipidemia, diabetes, and hypertension (4). Additionally, it is known for its calming properties in the treatment of local wounds and for its functions as an abortifacient, purgative, antihelminthic, and vermifuge (1, 5).

Several studies on the chemical composition of different parts of *R. monosperma* have reported its richness in various bioactive substances. The main constituents of this species includes dipiperydine and quinolizidine alkaloids such as Sparteine, Ammodendrine, Cytisine, and Anagyrine (6), as well as aglycons and glycosides of flavonoids including Kaempferol, Genistin, Genistein, Luteolin, Rutin, and Daidzin (3), along with pinitol as a cyclitol (7). Previous pharmacological studies have indicated that extracts obtained from different parts of *R. monosperma* exhibit a variety of pharmacological activities. Alkaloids have demonstrated antifungal activity (8), antibacterial properties (9), *in vitro* antioxidant capacity (10), anticancer activity against cervical cancer cell lines (11), *in vivo* anti-inflammatory effect (12), and the anti-aging capacity (3).

Hence, the current study aims to investigate the antioxidant activity using four complementary methods, each employing different mechanisms. The DPPH and ABTS assays are based on electron and proton H transfer, while FRAP and PMA are based on electron transfer reaction. Additionally, the study aims to evaluate the antimicrobial activities *in vitro* by two methods: the disc diffusion method and the microdilution assay. This investigation focuses on an endemic herbal plant of the Mediterranean basin, *R. monosperma,* as well as conducting phytochemical screening and determining its total phenolic, flavonoids, and tannin contents.

Materials and Methods

Study area and identification of the plant

The stems of *Retama monosperma* were collected from the Mehdia forest (Rabat-Sale-Kenitra region) in Morocco in July 2021. The plant material was authenticated by a botanist from the Herbarium of the Botany Department at the Scientific Institute of Rabat, Morocco (Voucher Specimen: RAB113533), and it was deposited in the Institute's Herbarium. The stems were cleaned and dried at room temperature, powdered, and stored in glass bottles kept in the dark to preserve them from light and moisture until future use.

Preparation of the extracts

The collected stems of *R. monosperma* were shade-dried and powdered. 50 g of the powdered material were introduced into a cotton cellulose cartridge and then extracted using the Soxhlet apparatus with 400 mL of different solvents. Primarily, we used hexane, followed by ethyl acetate from the pomace of the stems of *R. monosperma*, and finally ethanol to obtain three fractions. The termination of Soxhlet extraction was determined by the appearance of colorless solvents in the siphon tube, which occurred after 6 hrs for hexane and 8 hrs for ethyl acetate and ethanol, following the method described by a previous study with some modifications (13). Additionally, the pomace was macerated with 1000 mL of distilled water for 8 hrs in the dark at room temperature. The crude extracts obtained were filtered through Whatman filter paper and concentrated using a rotary evaporator at a bath temperature of 35-40°C and reduced pressure with a rotational speed of 120 rpm. Subsequently, the concentrated aqueous extract was frozen at -80°C for 24 hrs and then freeze-dried to obtain the extract in powder form. The fractions obtained were stored at 4°C until use.

Phytochemical screening

The preliminary phytochemical screening of the vegetable powder and hexane, ethyl acetate, ethanol, and aqueous fractions (1 mg/mL) of *R. monosperma* stems was conducted using standard procedures for flavonoids (14), tannins (15), anthocyanins (16), coumarins (17), quinones (18), terpenoids (17), alkaloids (19), anthraquinones (20), saponins (14), and sterols (21). The qualitative phytochemical screening aimed to detect the presence or absence of various bioactive compounds, and the results were analyzed accordingly.

Phytochemical assays

Determination of total phenolic content

The total phenolic content (TPC) of each fraction of stems of *R. monosperma* was determined spectrophotometrically using the Folin-Ciocalteu colorimetric method, as described in a previous study with some modifications (22). Briefly, a 200 µL aliquot of the extract solution was mixed with 1000 µL of 10% of Folin-Ciocalteu reagent and 800 µL of 7.5% sodium carbonate Na_2CO_3 solution. The reaction was incubated in the dark at room temperature for 30 min. The absorbance of each sample was measured against the blank at 765 nm using UV/VIS Spectrophotometer (Model: UV-1800APC). A calibration curve was prepared using gallic acid as a phenol standard to determine the concentration of total polyphenols. The results of TPC were expressed in milligrams of gallic acid equivalent per gram of extract (mg GAE/g extract).

Determination of total flavonoid content

The aluminum chloride colorimetric assay, as described in a previous study with some modifications, was used to determine the total flavonoid content (TFC) (23). Initially, 500 µL of aluminum chloride $AlCl_3$ solution (2%) was added

to 500 µL of each fraction or standard solution of quercetin. After incubating at room temperature for an hour, the absorbance was recorded at 420 nm against a blank using a UV/VIS Spectrophotometer (Model: UV-1800APC). The TFC was expressed as milligram equivalent of quercetin per gram of extract (mg EQ/g extract) using quercetin as the standard curve.

Determination of total tannin content

The procedure described by (24) was utilized to determine the total tannin content (TTC) of each fraction. In this method, 1.5 mL of a 4% methanolic solution of vanillin and 750 µL of concentrated hydrochloric acid (HCl, 37%) were mixed with aliquots of 50 µL from each extract. After incubating for 20 min at ambient temperature in the dark, the absorbance was measured at 500 nm using a UV/VIS Spectrophotometer (Model: UV-1800APC) against a blank. A standard curve was prepared using catechin, and the TTC was expressed as milligrams of catechin equivalent per gram of extract (mg EC/g extract).

Antioxidant capacity

DPPH free radical-scavenging activity

The free radical scavenging capability of the four fractions against the DPPH radical was tested according to the procedure described by (25) with some modifications. In this method, a methanolic solution of DPPH (0.0023%), was prepared, along with separate preparations of all fractions, and ascorbic acid as an antioxidant standard. Briefly, 50 µL of each extract, ascorbic acid (positive control) at different concentrations, or methanol (negative control) was mixed with 2 mL of DPPH solution. After homogenization, the samples were kept in the dark at ambient temperature for 20 min. The color changed from dark violet to bright yellow, and the absorbance of the samples was measured against a blank at 517 nm using a UV/VIS Spectrophotometer (Model: UV-1800APC). The following equation was used to calculate the percentage of inhibition (I%) of radical scavenging capacity:

$$I\% = [(A_{control} - A_{sample}) / A_{control}] \times 100 \quad \text{(Eqn. 1)}$$

$A_{control}$: Absorbance of negative control;

A_{sample}: Absorbance of the sample.

Ferric reducing antioxidant power assay

The antioxidant capacity of *R. monosperma* fractions was also investigated using the ferric reducing antioxidant power (FRAP) assay, employing the ferricyanide-ferric chloride method as previously described by (26). Briefly, 2.5 mL of phosphate buffer (pH = 6.6; 0.2M) was added to 0.2 mL of each fraction and catechin as a positive control, which were previously prepared with different concentrations. This mixture was then combined with 2.5 mL of 1% potassium ferricyanide ($K_3Fe(CN)_6$). After incubating at 50°C for 20 min in a water bath, the reaction was stopped by adding 2.5 mL of 10% trichloroacetic acid. Finally, 2.5 mL of distilled water and 0.5 mL of 0.1% iron chloride ($FeCl_3$) were added to 2.5 mL of the reaction mixture. The absorbance of the reaction mixtures was measured at 700 nm using a UV/VIS Spectrophotometer (Model: UV-1800APC).

ABTS radical scavenging assay

The experiments were conducted using an improved ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonicacid)) decolorization test following the method described by (27). The $ABTS^{\cdot+}$ radical cation was generated by reacting 7 mM ABTS and 70 mM potassium persulfate in methanol. The mixture was allowed to sit for 16 hrs at room temperature in the dark before usage. Methanol was added to the ABTS reaction solution to adjust the absorbance at 734 nm to a range between 0.700 and 0.734 before use.

Appropriate volumes of the ABTS solution (2 mL) were mixed with 100 µL of each test sample and the control blank. After 1 min of incubation at room temperature, the absorbance was measured at 734 nm using a UV/VIS Spectrophotometer (Model: UV-1800APC). Ascorbic acid was used as the standard inhibitor. The 50% inhibitory concentration (IC_{50}) values of the ABTS radical scavenging activity were calculated following the method described for the DPPH test.

Phosphomolebdenum assay

The total antioxidant capacity (TAC) of each *R. monosperma* fraction was evaluated using the phosphomolebdenum assay (PMA) according to the method described by (28). An aliquot of 0.2 mL of each extract was mixed with 2 mL of the reagent solution (0.6 M sulfuric acid, 4 mM ammonium molybdate, and 28 mM sodium phosphate). The mixture was then heated to 95°C in a water bath for 90 min. The absorbance of each sample was measured using a UV/VIS Spectrophotometer (Model: UV-1800APC) at 695 nm against a blank. Ascorbic acid was used to create the standard curve, and the results were expressed in milligrams of ascorbic acid equivalent per gram of extract (mg EAA/g extract).

Antimicrobial activity

The antimicrobial effects of *R. monosperma* stem fractions were evaluated *in vitro* by two methods: disk diffusion for qualitative assessment and broth microdilution for the determination of minimal inhibitory concentration (MIC) and minimal lethal concentration (MLC).

Test organisms

The fractions of *R. monosperma* were examined for their antimicrobial activity against five bacteria strains and one yeast strain (Table 1). The pathogens were obtained from the Moroccan Coordinated Collection of Microorganisms (CCMM). The bacterial strains were cultivated at 37°C on Muller-Hilton agar plates, while the yeast strain was cultivated at 28-30°C on Sabouraud dextrose agar plates.

Antimicrobial screening

The bacterial suspension was prepared, and the susceptibilities of the fractions were determined using the disc diffusion method on Mueller Hilton agar and Sabouraud dextrose agar, as modified from the method described by (29). The recommendations of the National Committee to Clinical Laboratory Standards were used to interpret the results (30). Consequently, 100 µL of the microbial suspen-

sions, with approximately equal concentration or density as 1 McFarland standards, were evenly spread on nutrient agar plates. The fraction's solution (10 µL) was absorbed into sterile standard blank discs (6 mm) in sterile petri dishes. The plates were then incubated at 37°C for 24 hrs. and 28°C for 48 hrs. for bacteria and fungi, respectively. For negative control, solvents (30% of DMSO) alone were included, while Penicillin (10 µg/mL), Tetracycline (30 µg/mL), Ampicillin (10 µg/mL), and Amphotericin B (250 µL) were used as a positive control. After incubation, the antimicrobial activity was determined by comparing the zones of inhibition, including the diameter of the disc measured in millimeters produced by *R. monosperma* fractions with those of the controls.

Determination of minimum inhibitory concentration

Table 1. Strains of microorganisms used for testing the antimicrobial activity.

		Names of strains	CCMM code	ATCC
Bacterial species	Gram-positive	*Staphylococcus aureus*	B804	ATCC 43300
		Bacillus cereus	B1167	ATCC 14579
		Enterococcus faecalis	B392	ATCC 19433
	Gram negative	*Escherichia coli*	B803	ATCC 11775
		Pseudomonas aeruginosa	B612	ATCC 90027
Yeast species		*Candida albicans*	L60	-

The determination of the minimum inhibitory concentration (MIC) was carried out through microdilution using the (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) (MTT) colorimetric method, as previously described (31). For the analysis, 96-well microplates were used. The wells were filled with 160 µL of Muller Hilton broth, and 20 µL of each fraction solution was added to the wells in the first row. From the first well, 90 µL was transferred from well to well in double dilution. Each well received 10 µL of a bacterial suspension adjusted to 1 McFarland. A negative control was included. Similarly, serial dilutions of antibiotics (Ampicillin and Tetracycline) were prepared in distilled water and used as positive controls. After 24 hrs of incubation at 37°C, the MICs of the samples were detected by the addition of 10 µL of MTT at 0.4 mg/mL and incubation for 10 to 30 min at 37°C. Viable bacteria transformed the yellow compound into a blue-violet color. The minimum inhibitory concentration (MIC) was defined as the lowest sample concentration that prevented the color change and completely inhibited microbial growth.

Determination of the minimum bactericidal concentration

To determine the minimum bactericidal concentration (MBC) (32), a portion of the liquid from each well that did not change the color of MTT was streaked on solidified nutrient agar plates, then incubated at 37°C for 24 hrs. The lowest concentration of the extract that yielded no growth after this sub-culturing was considered bactericidal.

Statistical analysis

Experiments were conducted in triplicate, and the results were expressed as mean±SD. Statistical analysis and comparison of means were performed using one-way analysis of variance (ANOVA) followed by the Tukey test. Differences between the mean values were considered statistically significant when $p < 0.05$. The analysis was carried out using GraphPad Prism 8.

Results and discussion

Phytochemical screening

The present study demonstrated that the powder of *R. monosperma* stems and their fractions were rich in various classes of the secondary metabolites. The results of the phytochemical screening analysis are presented in Table 2. Various chemical tests were performed, revealing the presence of different bioactive secondary metabolites, namely, flavonoids, tannins, coumarins, quinones, terpenoids, alkaloids, and sterols in the four fractions and stem powder. Saponins were found only in the ethanol extract, aqueous fraction, and vegetable powder. Anthocyanins and anthraquinones were absent in the stem powder and their fractions.

These findings are consistent with previous studies, which have reported that the Retama genus is very rich in secondary metabolites such as flavonoids, alkaloids, terpenoids, and saponins in *R. raetam* (33, 34). The methanol crude extract of *R. monosperma* stems was found to be devoid of anthraquinones, quinones, and coumarins but rich in alkaloids, flavonoids, tannins, saponins, and terpenoids (35). These various secondary metabolites exhibit a wide range of biological properties, including antioxidant, antimicrobial, anti-inflammatory, and antidiabetic activity.

Table 2. Secondary metabolites contents in stems of *R. monosperma* and their extract.

	Vegetable powder	Hexane fraction	Ethyl acetate fraction	Ethanol fraction	Aqueous fraction
Flavonoids	+	+	+	+	+
Tannins	+	+	+	+	+
Alkaloids	+	+	+	+	+
Quinones	+	+	+	+	+
Coumarins	+	+	+	+	+
Terpenoids	+	+	+	+	+
Sterols	+	+	+	+	+
Saponins	+	-	-	+	+
Anthocyanins	-	-	-	-	-
Anthraquinones	-	-	-	-	-

+: present; -: absent.

Total phenolic, flavonoid, and tannin contents

The extraction yield (%w/w) for the stems of *R. monosperma* was 2.46% for the hexane fraction, 4.61% for the ethyl acetate fraction, 10.50% for the ethanol fraction, and 18.12% for the aqueous fraction. The contents of total phenols, flavonoids, and tannins were determined from the calibration curves (y=0.009×+0.0958; R^2=0.9923), (y=0.0293×+0.028; R^2=0.996) and (y=0.00005×+0.0212; R^2=0.9901) using Gallic acid, Quercetin, and catechin as standard, respectively. Depending on the number of phenolic groups, flavonoids, and tannins present, different reactions can be observed in terms of the color change due to the reaction with the reagents used. This color change can be detected by a spectrophotometer and quantified in terms of mg Gallic Acid Equivalent per gram of extract (mg GAE/g extract), mg Quercetin Equivalent per gram of extract (mg QE/g extract), and mg Catechin Equivalent per gram of extract (mg CE/g extract), for the total phenols, flavonoids, and tannin content, respectively. The total TPC, TFC and TTC of different fractions of the stems of *R. monosperma* are summarized in Table 3.

Table 3. Total phenolic, flavonoid and tannin content of *R. monosperma* fractions.

	Hexane fraction	Ethyl acetate fraction	Ethanol fraction	Aqueous fraction
Yield of extraction %	2.46	4.61	10.50	18.12
TPC (mg GAE/g extract)	47.11±0.55^a	218.95±0.22^b	212.75±0.34^c	236.31±1.57^d
TFC (mg QE/g extract)	21.98±0.12^a	75.73±0.05^b	26.38±0.14^c	8.89±0.44^d
TTC (mg CE/g extract)	79.36±0.59^a	35.68±0.96^b	7.14±0.18^c	4.70±0.15^d

Different letters in the same line (**a** to **d**) indicate the significant difference ($p < 0.05$). values were compared by using one way ANOVA followed by multple comparison test. **TPC**: total phenolic content; **TFC**: total flavonoid content; **TTC**: total tannin content; **mg GAE/g extract**: mg Gallic Acid Equivalent per gram of

All the fractions were found to be important sources of phenolic compounds, known for exhibiting significant biological activities. The quantitative estimation of the total phenolic contents revealed that the highest amount was found in the aqueous fraction (236.31±1.57 mg GAE/g extract), followed by the ethanol fraction, ethyl acetate, and hexane fraction 212.75±0.34 mg GAE/g extract, 218.95±0.22 mg GAE/g extract, and 47.11±0.55 mg GAE/g extract, respectively. The concentration of flavonoids in all fractions ranged from 8.89 to 75.73 mg QE/g extract. A high concentration was determined in the ethyl acetate fraction, accounting for 75.73±0.05 mg QE/g extract, while concentrations of 26.38±0.14, and 21.98±0.12 mg QE/g extract were found in the ethanol and ethyl acetate fractions, respectively. The lowest flavonoid concentration was determined for the aqueous fractions (8.89±0.44 mg QE/g extract). Additionally, the fractions of *R. monosperma* also contained significant tannin contents. The highest tannin content was recorded in the hexane fraction (79.36±0.59 mg CE/g extract) compared to the other fractions, with 35.68±0.96 mg CE/g extract, 7.14±0.18 mg CE/g extract and 4.70±0.15 mg CE/g extract in the ethyl acetate, ethanol, and aqueous fraction, respectively.

The polyphenol content generally varies depending on the solvent used and its polarity. It's evident that all fractions of *R. monosperma* stems contain an important source of phenolic compounds, with variability among the four fractions. Differences in polarity and extractability of antioxidants can explain variations in extraction yield and antioxidant capacity. Furthermore, the solvent polarity is a crucial factor in increasing the solubility of phenolic compounds (36). Comparing with other studies, our results are similar to those obtained by (10), although the tannin content in our study is lower than the analysis carried out by (35). Our results are also approximately similar to those found in *R. raetam*. According to these authors, the fraction extracted with ethyl acetate has the highest concentration of flavonoids and tannin compounds (37). In another study, the hydromethanolic extract from stems of *R. sphaerocarpa* was found to be richer in total phenolic content compared to hydromethanolic extracts from seeds, with values of 336.5±0.8 and 125.8±2.0 mg GAE/g extract, respectively (38). In addition to the solvent type, genotypic traits, and current environmental conditions such as temperature, precipitation, and altitude, may impact the synthesis of metabolites (39, 40). Phenolic substances function as reducing agents, enabling them to act as antioxidants. Their free radical scavenging activity is enhanced by hydroxyl groups, and the phenolic concentration could be used as a basis for rapid screening of antioxidant capacity. Flavonoids, as secondary metabolites of plants, exhibit antioxidant capacity dependent on the presence of OH groups, particularly 3-OH (41).

Antioxidant capacity

In the present study, the four fractions extracted from the stems of *R. monosperma* with different solvents, increasing in polarity, underwent screening for possible antioxidant capacity using four complementary methods: DPPH and ABTS assays, which are based on electron and proton H transfer mechanism, while FRAP and PMA are based on electron and transfer reaction. The results of the antioxidant capacity tests are illustrated in Table 4.

The four methods clearly demonstrated that the studied fractions of *R. monosperma* possess considerable antioxidant and antiradical properties. Overall, all fractions were able to reduce the stable violet DPPH and blue-green $ABTS^{.+}$ radical to the yellow DPPH-H and colorless ABTS with decreasing absorbance at wavelengths of 517 nm and 734 nm, respectively, except for the hexane fraction. In the FRAP assay and PMA, the yellow color of the test solution turns green, depending on the reducing power of each sample. The effective concentration for reducing 50% of DPPH was 125.40±0.55, 121.55±0.90, and

234.98±0.79 µg/mL for the ethyl acetate fraction, ethanol fraction, and aqueous fraction, respectively. Compared to the antioxidant standard, all extracts were less effective than ascorbic acid with IC_{50} = 1.67±0.01 µg/mL for the DPPH assay. The IC_{50} values of FRAP test were 417.94±3.80 µg/mL for the hexane fraction, 255.39±2.88 µg/mL for the ethyl acetate fraction, 247.10±1.32 µg/mL for the ethanol fraction, and 1059.53±4.12 µg/mL for the aqueous fraction. The results were compared with the commercially available antioxidants catechin (IC_{50} = 19.54±0.24 µg/mL). For ABTS, the IC_{50} values were 319.34±0.29, 353.10±0.23, and 208.58±0.77 µg/mL for the ethyl acetate fraction, ethanol fraction and aqueous fraction, respectively. The IC_{50} of ascorbic acid was 2.59±0.02 µg/mL. For PMA, the values were expressed in micrograms of ascorbic acid equivalent per gram of extract (mg EAA/g extract), with values of 114.90±1.08 mg EAA/g extract, 155.49±0.96 mg EAA/g extract, 216.66±0.87 mg EAA/g extract, and 42.00±0.39 mg EAA/g extract for the hexane, ethyl acetate, ethanol and aqueous fraction, respectively.

isolated flavonoids (genistein, quercetin, 6-methoxy-kaempferol, and kaempferol). The antioxidant capacity was tested using several methods such as DPPH scavenging activity, conjugated diene scavenging activity, and H_2O_2 scavenging assay (3). Additionally, there have been numerous publications on the antioxidant capacity of various *Retama* species. The fruits of *R. sphaerocarpa* extracts and the aerial parts of *R. raetam* extracts show antioxidant capacity in DPPH, FRAP, ORAC, and TEAC assays (33, 43). The presence of such compounds in extracts may explain the potent antioxidant activity observed. Phenolic chemicals are responsible for the majority of the antioxidant properties of plant origin (44). However, there are suggestions that phenolic compounds are not solely responsible for antioxidant capacity. Other secondary metabolites, in combination with a synergistic effect, could explain this behavior (45).

Table 4. Antioxidant capacity of *R. monosperma* fraction by DPPH, ABTS, FRAP test expressed as median inhibitory concentration (µg/mL) and PMA methods expressed as mg AAE/g extract.

	Hexane fraction	Ethyl acetate fraction	Ethanol fraction	Aqueous fraction	Ascorbic acid	Catechin
DPPH IC_{50} (µg/mL)	ND	125.40±0.5^a	121.55±0.9^b	234.98±0.79^c	1.67±0.0^d	-
FRAP IC_{50} (µg/mL)	417.94±3.80^a	255.39±2.88^b	247.10±1.32^b	1059.53±4.12^c	-	19.54±0.24^d
ABTS IC_{50} (µg/mL)	ND	319.34±0.29^a	353.10±0.23^b	208.58±0.77^c	2.59±0.02^d	
PMA (mg AAE/g extract)	114.90±1.08^a	155.49±0.96^b	216.66±0.87^c	42.00±0.39^d	-	-

ND: not determined; Different letters in the same line (**a** to **d**) indicate the significant difference ($p < 0.05$). values were compared with the standard by using one way ANOVA followed by multiple comparison test. **DPPH**: DPPH Free Radical-Scavenging Activity; **FRAP**: Ferric Reducing Antioxidant Power Assay; **ABTS**: ABTS Radical Scavenging Assay; **PMA**: Phosphomolebdenum Assay.

However, in the three antioxidant assays DPPH, FRAP and PMA, the ethanol fractions demonstrated higher antioxidant potency than the other fractions, while the aqueous fraction exhibited the highest scavenging activity for the $ABTS^{\cdot+}$ test. Moreover, the lowest activities were noted for the aqueous fraction in the DPPH, FRAP, and PMA assays, while the ethanol fraction showed lower activity for ABTS assay.

In general, antioxidant capacity is a complex process that occurs through various mechanisms and is influenced by a variety of factors. It cannot be fully described by a single method. Thus, it is essential to determine antioxidant capacity using two or more methods to account for the different mechanisms of antioxidant action (42).

The difference observed between the fractions in antioxidant capacity may be attributed to variations in chemical composition. To the best of our knowledge, only one study has been conducted regarding the antioxidant capacity of *R. monosperma* stems. According to a study performed in Algeria, the ethyl acetate fraction showed significant antioxidant capacity, with an IC_{50} value of 0.15±0.11 mg/mL for DPPH assay and 197.95±0.98 mg EAA/g extract for PMA (10). Furthermore, Zefzoufi *et al* demonstrated the antioxidant capacity of the ethyl acetate extract of seeds, ether diethyl extract of flowers, and

Correlation between antioxidant capacity and total phenolic, flavonoid, and tannin contents

Pearson's correlation coefficients among TPC, TFC, TTC, DPPH, ABTS, FRAP, and PMA are showed in the Table 5. Correlation coefficients may be characterized as low correlation (r < 0.5), moderate (0.7 > r > 0.5), high (0.9 > r > 0.7), and particularly high (r > 0.9).

From the presented coefficients, it might be noticed that the majority of coefficients were particularly high and/or high, with correlation coefficients higher than 0.7. There were also coefficients among TPC and TTC, DPPH, and ABTS, as well as between DPPH and FRAP and PMA. Negative correlation among TPC, TFC, and TTC and antioxidant capacity suggests that activity increased with the content of those compounds (46).

Antimicrobial activity

Disc diffusion method

The antimicrobial activity of *R. monosperma* fractions (Hexane, ethyl acetate, ethanol, and aqueous fraction) was assessed against three Gram-positive bacterial strains: *Staphylococcus aureus*, *Bacillus cereus*, and *Enterococcus faecalis*, and two Gram-negative bacteria: *Escherichia coli* and *Pseudomonas aeruginosa*, as well as one yeast, *Candida albicans*. The evaluation was conducted using the disc diffusion method to determine their MIC and MBC values. Table 6 presents the antimicrobial activity

Table 5. Pearson's correlation coefficients among TPC, TFC, TTC, DPPH, ABTS, FRAP, and PMA.

Test	TPC	TFC	TTC	DPPH	ABTS	FRAP	PMA
TPC	1						
TFC	0.2004	1					
TTC	-0.9237	0.1502	1				
DPPH	0.9747	-0.6799	-0.5341	1			
ABTS	-0.9995	0.5256	0.3639	-0.9812	1		
FRAP	0.2412	-0.6324	-0.3439	0.9998	-0.9767	1	
PMA	0.0460	0.4352	-0.0485	-0.9488	0.9919	-0.9003	1

TPC: total phenolic content; **TFC**: total flavonoid content; **TTC**: total tannin content; **DPPH**: DPPH Free Radical-Scavenging Activity; **ABTS**: ABTS Radical Scavenging Assay; **FRAP**: Ferric Reducing Antioxidant Power Assay; **PMA**: Phosphomolebdenum Assay.

Table 6. Antimicrobial activity of *R. monosperma* fractions expressed as inhibition zones diameters (mm).

Fractions		Diameter of the zone of inhibition (mm)					
		Bacterial species					Yeast species
		Gram-positive			Gram-negative		
	*CC	*Staphylococcus aureus*	*Bacillus cereus*	*Enterococcus faecalis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*
Hexane fraction	333	NE	NE	NE	NE	NE	NE
	165.5	NE	NE	NE	NE	NE	NE
Ethyl acetate fraction	333	11.5±0.5^{a}	10.5±0.5^{a}	NE	NE	9.5±0.5^{a}	NE
	165.5	9.5±0.5^{b}	8.0±0.0^{b}	NE	NE	8.0±0.0^{b}	NE
Ethanol fraction	500	NE	7.0±0.0^{c}	NE	NE	7.5±0.5^{c}	NE
	250	NE	7.0±0.0^{d}	NE	NE	7.0±0.0^{d}	NE
Aqueous fraction	500	NE	NE	NE	NE	NE	NE
	250	NE	NE	NE	NE	NE	NE
Penicillin	10	8.0±0.0^{c}	7.5±0.5^{e}	17.5±0.5^{a}	NE	NE	-
Ampicillin	10	8.5±0.5^{d}	9.0±0.0^{f}	20.5±0.5^{b}	11.5±0.5^{a}	NE	-
Tetracycline	30	27.5±0.5^{e}	20.5±0.5^{g}	24.5±0.5^{c}	7.0±0.0^{b}	NE	-
Amphotericin B	250	-	-	-	-	-	NE

NE: no effect; *Concentration of the fractions in mg/mL and Concentration of the antibiotic and antifungal in µg/mL; Different superscript letters in the same column (**a** to **g**) indicate the significant difference ($p < 0.05$). values were compared with the standard by using one way ANOVA followed by multiple comparison test.

expressed as inhibition zone diameters (IZD) of *R. monosperma* fractions. The hexane and ethyl acetate fractions exhibited IZDs at 165.5 and 333 mg/mL, respectively, while for the ethanol and aqueous fractions, the IZDs were at 250 and 500 mg/mL. These were tested against the five bacterial strains and oneyeast, and compared to penicillin, ampicillin, tetracycline, and amphotericin B. The ethyl acetate and ethanol fractions were found to effectively inhibit the growth of *S. aureus*, *B. cereus*, and *P. aeruginosa*. The ethyl acetate fraction demonstrated inhibitory effects on *S. aureus*, *B. cereus*, and *P. aeruginosa*, with IZDs of 11.5, 10.5, and 9.5 mm, respectively, at a concentration of 333 mg/mL. Additio-nally, the ethanol fraction exhibited antibacterial activity against *B. cereus* and *P. aeruginosa*, with IZDs of 7.0 and 7.5 mm, respectively, at 500 mg/mL. However, no activity was observed for all fractions against *E. faecalis*, *E. coli*, and *C. albicans*, indicating resistance of these pathogens to the four fractions. It is noteworthy that all three positive controls (penicillin, ampicillin, and tetracycline) demonstrated broad-spectrum antibacterial effects against all bacteria tested, except for *P. aeruginosa*. *E. coli* exhibited varying degrees of resistance against Penicillin. Moreover, as expected, the solvent used as the negative control showed no significant effect.

Determination of minimum inhibitory concentration

The MIC values are presented in Table 7, indicating that the ethyl acetate fraction exhibited the lowest MIC value (the best MIC value) at 0.52 mg/mL against *S. aureus* and *B. cereus*, compared to the other extracts. Additionally, the MIC values of the ethanol fraction against *B. cereus* and *P. aeruginosa* were 25 mg/mL. However, the MIC values of tetracycline against *S. aureus*, *P. aeruginosa*, and *B. cereus* were 0.12 µg/mL (Table 7).

Determination of the minimum bactericidal concentration

Further analysis using the MBC test (Table 8) revealed that the lowest MBC value was observed for the ethyl acetate

fraction against *B. cereus* (0.52 mg/mL), while 16.65 mg/mL was determined against *S. aureus* and *P. aeruginosa*. The MBCs of the ethanol fraction were 25 mg/mL and more than 50 mg/mL for *B. cereus* and *P. aeruginosa*, respectively. Additionally, the MBC of tetracycline against *S. aureus*, *P. aeruginosa*, and *B. cereus* were 0.47, 0.94, and 0.12 µg/mL, respectively (Table 8).

Based on a literature review of *R. monosperma*, there are few studies dealing with the antifungal effect (8), and the antibacterial effect by using the disc diffusion of the different parts of *R. monosperma* (stems, seeds, leaves, and flowers) (9, 35), but there is no report on its antibacterial activity with the determination of MIC and MBC. Consequently, the current study reveals for the first time the MIC

Table 7. Minimum inhibitory concentration of *R. monosperma* fractions and antibiotics against microorganisms.

	MIC				
	Staphylococcus aureus	*Bacillus cereus*	*Enterococcus faecalis*	*Escherichia coli*	*Pseudomonas aeruginosa*
Hexane fraction (mg/mL)	-	-	-	-	-
Ethyl acetate fraction (mg/mL)	0.52[a]	0.52[a]	-	-	16.65[a]
Ethanol fraction (mg/mL)	-	25[b]	-	-	25[b]
Aqueous fraction (mg/mL)	-	-	-	-	-
Ampicillin (µg/mL)	0.63[b]	>20[c]	0.04[a]	0.63[a]	5[c]
Tetracycline (µg/mL)	0.12[c]	0.12[d]	0.12[b]	0.12[b]	0.12[d]

Different letters in the same column indicate the significant difference ($p < 0.05$). values were compared with the standard by using one way ANOVA followed by multiple comparison test.

Table 8. Minimum bactericidal concentration of *R. monosperma* fractions and antibiotics against microorganisms.

	MBC				
	Staphylococcus aureus	*Bacillus cereus*	*Enterococcus faecalis*	*Escherichia coli*	*Pseudomonas aeruginosa*
Hexane fraction (mg/mL)	-	-	-	-	-
Ethyl acetate fraction (mg/mL)	16.65[a]	0.52[a]	-	-	16.65[a]
Ethanol fraction (mg/mL)	-	25[b]	-	-	≥ 50[b]
Aqueous fraction (mg/mL)	-	-	-	-	-
Ampicillin (µg/mL)	5[b]	>20[c]	20[a]	0.63[a]	>20[c]
Tetracycline (µg/mL)	0.47[c]	0.12[d]	7.5[b]	0.12[b]	0.94[d]

Different letters in the same column indicate the significant difference ($p < 0.05$). values were compared with the standard by using one way ANOVA followed by multiple comparison test.

Our results demonstrate that the antibacterial capability varies depending on the nature of the solvent. Overall, the ethyl acetate fraction has the best activity against *B. cereus*, *S. aureus*, and *P. aeruginosa* compared to other extracts, followed by the ethanol fraction. According to study by Kuete in 2010, antimicrobial activity can be classified as weak activity (MIC > 625 µg/mL), moderate activity (100 < MIC < 625 µg/mL), and significant (MIC < 100 µg/mL) (47). According to this classification, it can be concluded that the ethyl acetate fraction exhibited moderate activity against *S. aureus* and *B. cereus*, with the same MIC value (520 µg/mL). The antibacterial activity of the *R. monosperma* fractions, as depicted in Tables 2 and 3, can be attributed to the presence of various bioactive compounds. Additionally, it may be explained by the synergistic effects of these secondary metabolites. In fact, previous investigations led to the identification of a variety of secondary metabolites which found effective antimicrobial effects against a wide array of micro-organisms. For instance, flavonoids for example taxifolin, genistin, apigenin, and kaempferol (3), and alkaloids such as sparteine, ammodendrine, and anagyrine (8). *R. monosperma*, rich in quinolizidine alkaloids, demonstrated significant activity against various bacterial strains, including *B. subtilis*, *S. aureus*, and *P. aeruginosa* (48).

and MBC against tested bacterial strains of the *R. monosperma* extracts. Previous studies have reported the antimicrobial activity of various extracts from plants in the genus Retama against human bacterial pathogens. Mariem and colleagues reported that the ethyl acetate extract of aerial part of *R. raetam* showed an appreciable antibacterial activity against *E. coli* and *B. cereus* with an inhibition zone diameter (IZD) of 12 mm (37). Furthermore, (49) have shown that *R. sphaerocarpa* displayed more antibacterial effect against *S. aureus*, and *R. raetam* has an interesting activity against *S. aureus*. Moreover, the essential oil obtained from the aerial part of *R. raetam*, during the fresh fruiting stage, exhibited excellent activity against *E. coli*, *B. cereus*, and *P. aeruginosa,* with an inhibition zone diameter of 10 mm (50). The hydromethanolic extracts of *R. raetam* exhibited strong activity against the Gram-negative *Aeromonas hydrophila*, *Vibro alginolyticus*, *Vibro vulnificus*, and *Vibro cholerae*, as well as the Gram-positive *Enterococcus faecalis*, *Listeria monocytogenes*, and *Micrococcus luteus*. However, they showed no activity against *E. coli*, *S. aureus*, *P. aeruginosa*, and *Staphylococcus epidermidis* (50).

Conclusion

In this study, we investigated the phytochemical screening, polyphenolic contents, antioxidant capacity, and

antimicrobial activity of the hexane, ethyl acetate, ethanol, and aqueous fractions of *R. monosperma* stems, a plant widely used in traditional medicine worldwide. The phytochemical screening of the four fractions of *R. monosperma* stems, based on specific tests, allowed the characterization of several bioactive secondary metabolites, including flavonoids, tannins, coumarins, quinones, terpenoids, alkaloids, and sterols, known for their remarkable therapeutic effects. Analysis of the concentrations of total polyphenolic contents revealed richness in all fractions with TPC, TFC, and TTC. Assessment of its antioxidant capacity *in vitro* using four methods (DPPH, FRAP, ABTS, and PMA) showed that all the fractions possess significant antioxidant and antiradical activities. Furthermore, the fractions tested *in vitro* against five bacterial strains and one yeast, exhibited variable antimicrobial activities. Specifically, the ethyl acetate fraction demonstrated moderate activity against *Staphylococcus aureus* and *Bacillus cereus*. To further improve our understanding, more studies, both *in vivo* and *in vitro*, should be encouraged to explore the pharmaceutical values of *R. monosperma* through extensive research methods to determine its safety and toxicity. Moreover, isolating bioactive compounds would aid in ascertaining its potency and safety as a lead candidate of antioxidants and antibacterial for pharmaceutical use.

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Authors' contributions

FZB designed the study, conducted the statistical analysis, and drafted the manuscript. AM, LO, MF, HB, and AD contributed to the statistical analysis. AZ ensured the formal analysis and supervised the process. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

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