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PHYTOCHEMICAL STUDY, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF FLAVONOIDS AND DIETHYL ETHER EXTRACTS FROM LEAVES AND SEEDS OF MEDICINAL PLANT OF ALGERIA FLORA: *Retama monosperma* (L.) Boiss.

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Abstract
The objective of this study is to determine the total phenol content of *Retama monosperma* (L.) Boiss. obtained by maceration and to test their antioxidant and antimicrobial capacity. The phenolic content was determined by the Folin-Ciocalteu reagent method, and the test of the free radical DPPH• was used to evaluate the antioxidant activity, while the evaluation of the antimicrobial power of the extracts was determined by the agar diffusion method. The crude extracts of *Retama monosperma* showed a high total phenol content of 74.75 mg EAG / g extract for the seeds and 155.61 mg EAG / g extract for the leaves. The results of antimicrobial activity shows that the curd extract has a variable activity which differs according to the strain tested. It has been shown to be inactive against meticillin-resistant *Staphylococcus aureus*, while *Candida albicans* yeast is highly susceptible.

Keywords: *Retama monosperma*, polyphenols, antioxidant activity, antimicrobial activity, antifungal effect.

Introduction

The use of plants for their medicinal properties is a very old practice. Many natural compounds isolated from these have demonstrated a broad spectrum of biological activities (Jang *et al*., 2004). The extraction of bioactive molecules, especially the case of polyphenols, is becoming increasingly important because of their beneficial effects on health. Indeed, their role antioxidant is attracting more and more interest in the prevention and treatment of many

diseases (Bouayed *et al*., 2007; Aberoumand & Deokule, 2008). *Retama monosperma* (L.) Boiss. is a member of the family Fabaceae. This species is widely used in folk medicine (Fdil *et al*., 2012).

The aim of this work is the phytochemical study and determined the antioxidant and antimicrobial capacity of leaves and seeds of an endemic plant from the Mediterranean basin of the *Retama monosperma* from Northeastern Algeria.\

Methodology

Plant material

The leaves and seeds of *Retama monosperma* were collected in the coastal dune zone of the Echatt region located east of Annaba in Northeastern Algeria. After drying the plant material in a ventilated area in the shade and away from moisture.

Preparation of extracts

500gr of the matter plant are subjected to the extraction of crude extract and flavonoids compounds, adopting the hydro-alcoholic method by maceration. The total depletion of the drug of its active ingredients is carried out by renewal of the solvent (methanol / water, 75:25, v / v) every 48 hours at the rate of three repetitions.

To obtain dry extracts, the filtrates are evaporated under vacuum using a Rotavapor type Buchi at a temperature not exceeding 40°C. After filtration and concentration of the solution, 200 ml of warm distilled water is added; the solution is left to stand overnight, filtered, and carried out successive extractions. The filtrate undergoes liquid-liquid extraction in a separatory funnel using solvents of increasing polarity, starting with diethyl ether, ethyl acetate and finally n-butanol (Chouia *et al*., 2018). Each extraction is repeated three times. The organic phases thus obtained are dried with anhydrous sodium sulphate (Na_2SO_4) and filtered, concentrated under reduced pressure to dryness and weighed.

The same procedure was applied for the drug in the form of seeds (Bruneton, 1999).

Content of phenolic compounds

The determination of the phenolic compounds is carried out, according to the method of Wolfe (Wolfe *et al*., 2003), by the application of the Folin-Ciocalteu reagent. The oxidation of phenols reduces this reagent to a blue-colored compound (Ribéreau-Gayon, 1968), the intensity of the color is proportional to the level of oxidized phenolic compounds.

The 1.5 ml of diluted (3 times) Folin-Ciocalteu reagent is added to 200µl of the sample solution. The mixture is stirred vigorously and incubated in the dark for 5 min at 25ºC. Then

1.5 ml of Na_2CO_3 (6%) is added to the solution. The mixture is incubated in the dark for 90 min at 20°C, and then the optical density is read spectrophotometrically at a length of 725 nm.

Trapping test of the free radical DPPH•

This method is based on the reduction of a very stable free radical: dark violet-colored 2,2'-diphenyl-1-picrylhydrazyl (DPPH•) which turns into a yellowish color in the presence of a donor antioxidant (Sanchez-Moreno *et al*., 1998). The latter is reduced to the hydrazine form by accepting a hydrogen atom. Reduction of the free radical (DPPH) by an antioxidant can be followed spectrophotometrically, by measuring the decrease in absorbance at 517nm caused by the presence of a hydrogen donor (Gulcin *et al*., 2003; Molyneux *et al*., 2004; Roginsky *et al*., 2005). The DPPH test was performed according to the Brand- Williams *et al* (1995). Preparation of the DPPH• solution at the concentration of 2.4mg in 100ml of methanol; a series of concentration of extract is prepared in methanol; preparation of the ascorbic acid range in methanol (standard) at different concentrations (0.2-7.5µg/ml); a volume of 50µl of the different concentrations of each extract is added to 1950µl of the DPPH solution; for each concentration, a blank (negative control) is prepared by mixing 50µl of methanol with 1950µl of the DPPH solution. After an incubation period of 30 min at room temperature in the dark, the absorbance are read at 517nm using a spectrophotometer (Maisuthisakul *et al*., 2007). The inhibition of the free radical of DPPH in percentage (I%) was calculated in the following way:

$$\mathbf{I\% = [(Ac\text{-}As) / Ac] \times 100}.$$

Ac: absorbance of the control **As**: absorbance of sample

A curve of the concentrations of the extract as a function of percentage inhibition (I%) was drawn in order to obtain the IC_{50} value, corresponding to the concentration of the substrate which ensures the reduction of 50% of the activity of DPPH• (Samarth *et al*., 2008) .

Calculation of the anti-radical power

The IC_{50} is inversely proportional to the anti-radical capacity of a compound, the lower the IC_{50} value, the greater the anti-radical activity of a compound (Prakash *et al*., 2007; Chew *et al*., 2009).

$$\mathbf{ARP = 1/IC_{50}}$$

ARP: Anti-Radical Power.

IC_{50}: Concentration of the extract necessary to reduce to 50% the initial concentration of the DPPH radical.

Antimicrobial activity

Preparation of the diluted solutions

The extract was dissolved in dimethylsulfoxide (DMSO) in order to prepare the different concentrations with successive dilutions. The final concentration range thus obtained corresponds to 0.5, 0.25, 0.125, 0.0625 mg/ml.

Preparation of the suspensions

The strains microbial (bacterial and yeast) obtained from university hospital of Annaba; An isolated colony was taken from the microbial strains by hospital previously revived. pathogenic yeast *Candida albicans* and a multidrug-resistant bacterial strain *Staphylococcus aureus* by a swab and discharged into a screw tube containing sterile physiological water (suspension at a concentration of 1.5x108 cells/ml) adjusted to a standard concentration of 0.5 Mc Farland (Nalubega *et al*., 2011).

Inoculation

Under sterile conditions, the Mueller Hinton medium is poured into Petri dishes, whose agar thickness is 4 mm, after cooling and solidification of the medium on the sterile bench, the suspensions of the strains previously prepared from the Young cultures in physiological saline are taken using a sterile swab and then seeded by the standardized method NCCLS.

The same procedure is performed for yeast *Candida albicans* with the use of a suitable medium that is the chloramphenicol-Sabouraud.

Filing disks

With the aid of a micropipette, the discs are loaded by the dilutions of the extract prepared previously by replacing the mouthpiece during each use.

Another disk was impregnated with the crude extract to test the activity of the pure extract.

Using a sterile forceps, the discs are placed in the middle of the boxes by pressing lightly so that they adhere well to the agar.

Incubation

All the Petri dishes are first left for 30 minutes before being incubated at 37°C (for the bacterial strain) and 28°C (for yeast) in an oven for 24 to 48 hours.

Determination of inhibition diameters and the Minimum Inhibitory Concentration (MIC)

The halo around the disk represents the zone of inhibition whose diameters were measured using a Vernier caliper. The measurement was taken directly on the bottom of the closed box and the results are expressed in millimeters. The diameters of the zones of inhibition indicate

the degree of sensitivity of the bacteria. The minimum inhibitory concentrations are correlated with the minimum diameters of the inhibition zones (Shahidi *et al*., 2003).

Determination of Minimal Bactericidal Concentration (BMC)

The minimum bactericidal concentration is determined after seeding by streak of suspension recovered with a swab from the zones of inhibition. Seeding is carried out on the surface of the nutrient agar poured into a petri dish and then incubated at 37°C for 24hours. On reading, the CMB of the extract is deduced from the first box devoid of bacteria (Nalubega *et al*., 2011).

Results

The Table 1 groups the values of the yields obtained at the different parts of the plant. The best yield is recorded with the solvent n-butanol in the two herbal drugs "seeds and leaves" at the rate of 4.7% and 3.5% respectively.

Table 1. yield and color of the extracts of different parts of *Retama monosperma*.

Drugs	Extraction Solvent	Yield%	Color
leaves	Diethyl ether	0.1	Greenish
	Ethyl acetate	1.46	Greenish
	n-butanol	3.5	Yellowish
Seeds	Diethyl ether	0.5	Greenish
	Ethyl acetate	2.44	Brown
	n-butanol	4.7	Brown

Content of phenolic compounds

The total polyphenol content is calculated from the equation of the calibration curve of Gallic acid ($y = 5.553x + 0.0099$ with $R^2 = 0.9997$). The total polyphenol contents are expressed as mg of Gallic acid per gram of extract (mg EAG/g extract).

The polyphenol content at the plant level differs from one plant part to another as well as the organic solvent used. According to Fig. 1 reveals that the leaves of *Retama monosperma* contain the highest amounts in relation to the seed fraction.

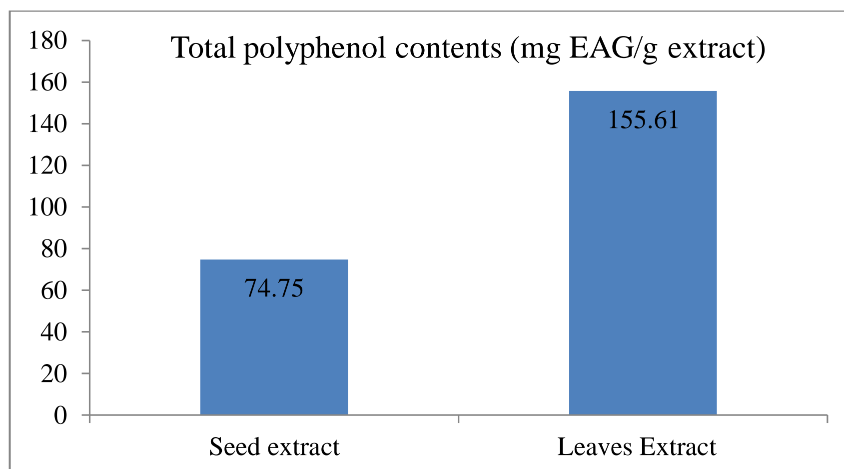

Fig. 1 Total polyphenol content of Diethyl ether extracts in different plant organs of *Retama monosperma*.

Anti-radical activity

The ethyl acetate extract showed an antiradical power (Table 2) the most important (71.2 µg/mL) for the leaves and (84.7µg/mL) for the seeds compared to the butanolic extract of the two drugs at 88.5µg/mL and 105.25µg/mL respectively. But this value (IC_{50}=71.2 µg/mL) of activity remains significantly lower than that of the positive control (Ascorbic acid) which represents (IC_{50}= 5.91µg/mL) of inhibitory activity.

Table 2. IC_{50} values (µg/mL) and Anti-Radical Power (ARP) of extracts of *Retama monosperma* and Ascorbic acid.

Drug	leaves			Seeds			Ascorbic acid
Extracts	Diethyl ether	AcOEt	n-BuOH	Diethyl ether	AcOEt	n-BuOH	
IC_{50}	118.32	71.2	88.5	123.7	84.7	105.25	5.91
ARP	0.0085	0.014	0.0113	0.0081	0.0118	0.0095	0.1692

Anti-Microbiological activity

To raise the problem of antimicrobial resistance to antibiotics, most work is currently directed towards other natural antimicrobial agents with a specific mode of action. For this reason, the antibacterial activity of *Retama monosperma* has been tested on pathogenic microorganisms responsible in most cases for urinary infections and nosocomial diseases encountered in hospital departments. The sensitivity of the bacteria to the extracts is determined according to the inhibition halo diameter around the discs containing the extracts at different concentrations by the agar diffusion method. In our study the Diethyl ether extracts and the flavonoids extracts of seeds not showing any responses of strains used(Inhibition zones = 00 mm), the data obtained are shown in the Table 3 for flavonoids extracts of *Retama monosperma* leaves.

Table 3. Inhibition zones (in mm) of flavonoids extracts of *Retama monosperma* leaves.

Concentration (mg/ml)		*Staphylococcus aureus* resistant to methicillin	*Candida albicans*
		Ethyl acetate extract	
C1	0.5	00	45
C2	0.25	00	42
C3	0.125	00	26
C4	0.0625	00	00
		n-butanol extract	
C1	0.5	00	51
C2	0.25	00	22
C3	0.125	00	20
C4	0.0625	00	00

Leaves extracts from the plant were found to be inactive against methicillin resistant *Staphylococcus aureus*, while they showed effects on *Candida albicans* (Fig. 2-5). On the other hand, the seed extracts are inactive for the two strains tested; this is probably due to the quality of the polyphenols stored in the storage organ which are different from those synthesized at the leaves level.

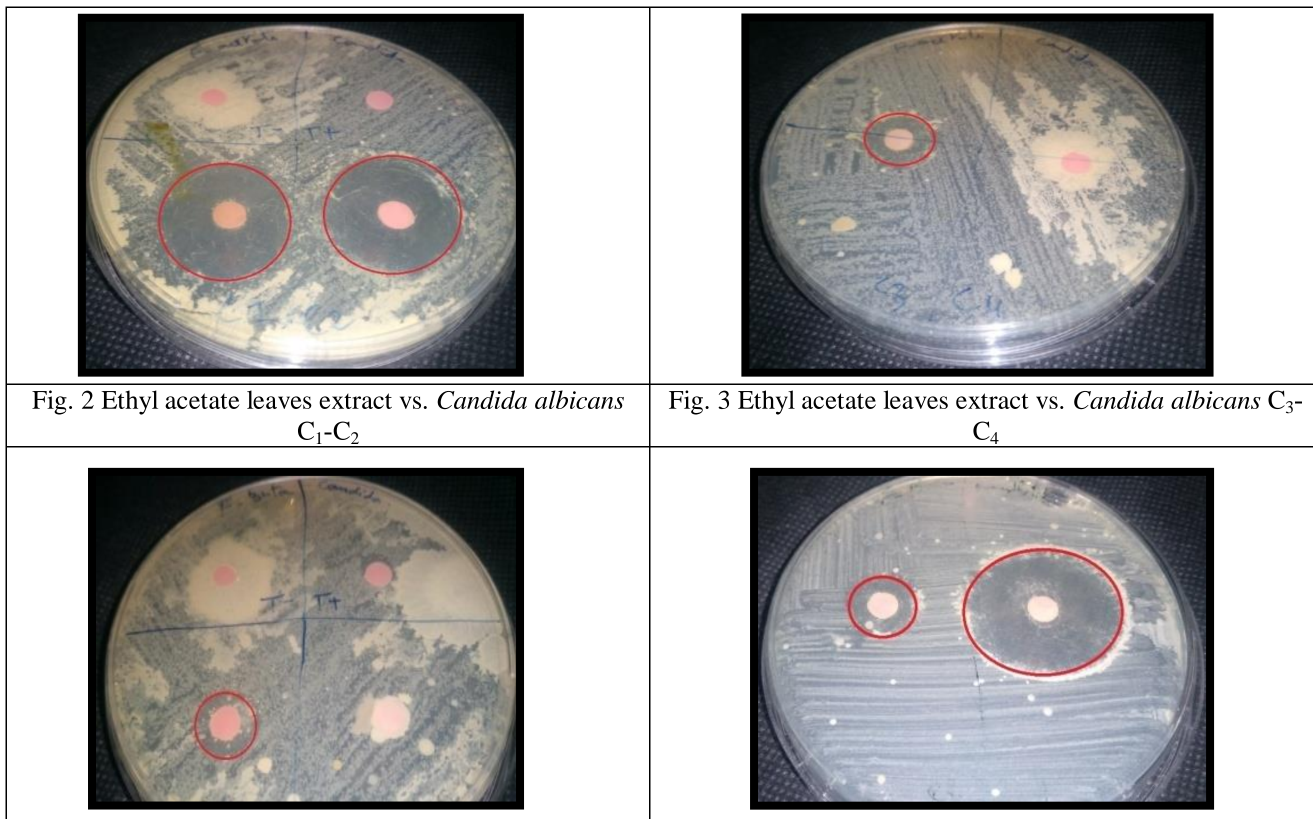

Fig. 2 Ethyl acetate leaves extract vs. *Candida albicans* C_1-C_2

Fig. 3 Ethyl acetate leaves extract vs. *Candida albicans* C_3-C_4

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Fig. 4 leaves n-butanol extract vs. *Candida albicans* C_3-C_4	Fig. 5 leaves n-butanol extract vs. *Candida albicans* C_1-C_2

The zones of inhibition appear at a concentration of 0.5 mg/ml with a diameter of 45 mm for the extract of ethyl acetate of the leaves and at the same concentration with a diameter of 51 mm for the extract of n-butanol of the leaves. The latter is considered the MIC (minimal inhibitory concentration).

Discussion

The extraction yields differ significantly depending on the solvent and the part of the plant, according to Garcia-Salas *et al* (2010). The chemical nature of the phenolic compounds in the plant affects the solubility, which varies from a simple to highly polymerized compound. On the other hand, the solubility of phenolic compounds is affected by the polarity of the solvent used (Jokić *et al.*, 2010).

The uneven distribution of polyphenols is justified by the fact that the leaves are most exposed to solar radiation, which pushes them to synthesize protective substances against exogenous conditions and oxidation stress factors summarized in polyphenols (Gehin *et al.*, 2006).

The result of anti-radical activity can be explained, on the one hand, by the richness of this extract in flavonoids, and on the other hand by the fact that the activity of flavonoids to trap free radicals depends essentially on their individual structures (Cao *et al.*, 1997). Bi- and tri-glycosylated flavonoids (1-butanol extract) have lower antioxidant activity than mono-glycosylated flavonoids (ethyl acetate extract) (Sokol-Letowska *et al.*, 2007).

According to the works of Edziri et *al.* (2010) the anti-free radical activity of DPPH• obtained with the other species of same genus plant (*Retama raetam* (Forssk.) Webb.) growing in the arid zones of Tunisia, estimated of IC_{50}= 0.8 mg/ml and IC_{50}= 20µg/mL with BHT standard.

It can be explained that the methods, the extraction solvents and the zones of localization can be the cause of divergences in the richness and the quality of the flavonoids and their efficacies against the formation of the free radicals (Chouia *et al.*, 2018).

The study of antimicrobial activity shows that the extract used has a variable activity that differs according to the strain tested (Hammer *et al.*, 1999).

The antifungal power of the extracts of ethyl acetate and n-buthanol of the leaves against *Candida albicans* is directly related to their chemical compositions which agree with published studies which indicate that the compounds of polar terpenes and alcohols act as agent’s powerful antifungals (Knezevic *et al.*, 2016).

Our results on the antibacterial activity of *Retama monosperma* extracts are consistent with those obtained in Morocco by El Hamdani *et al*. (2016). The *Candida albicans* strain is susceptible to extracts from the leaves, the diameters of inhibition zones (18.66 ± 1.77mm, 13.33 ± 0.88mm, 9.66 ± 0.44mm and 00 mm). On the other hand the seeds extracts are inactive against this germ. The variation in the diameters of the zones of inhibition from one extract to another is mainly due to the diffusion capacity of the different compounds contained in the agar medium. Hence, this variation is proportional to the variation of the chemical composition and the polarity of the diffusible substances (Carneiro *et al*., 2008).

The results obtained in our study also agree with the data of Edziri *et al*. (2010) working on the scale of the Kerker region in Tunisia, whose MIC varied between 0.625 and 5 mg / ml. This slight difference with the local *Retam*, can be explained by the influence of the soil conditions and the extraction method (Hosni *et al*., 2013), the geographical origin (Chalchat *et al*., 1993; Pintore *et al*., 2002; Angioni *et al*., 2004) and environmental factors (Moghtader & Farahmand, 2013; Jordán *et al*., 2013).

This remarkable activity can be explained by the result of a synergistic effect between several active ingredients in the extract (Felice *et al*., 2005).

Conclusions

Few work done on the *Retama* especially in Algeria, for this reason, we carried out analyzes on *Retama monosperma* of a coastal dune region of Eastern Algeria, in order to unveil its therapeutic virtues especially as a plant to be able antioxidant.

The extraction and the determination of the total polyphenols allowed us to confirm the wealth of the latter in active ingredients, the main ones being polyphenols. The use of the DPPH method revealed that the extracts of the *Retama monosperma* represent the highest activity, the most important antioxidant activity of which is attributed to the ethyl acetate extract of the leaves.

From the study of the antimicrobial activity it appears that the extracts has a variable activity which differs according to the strain tested. It has been shown to be inactive against meticillin-resistant *Staphylococcus aureus*, while *Candida albicans* is highly susceptible.

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