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Antioxidant and Antimicrobial Potentials of *Retama Sphaerocarpa.*

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ABSTARCT

The present work studies the antioxidant activities and antimicrobial properties of the aerial part of *Retama sphaerocarpa.* The secondary metabolites (polyphenols) were extracted using different solvents: chloroform, ethyl acetate and *n*-butanol. In each fraction, total polyphenols and flavonoids were respectively quantified using Folin-Ciocalteu test and aluminium chloride colorimetric method. The concentration of polyphenols was higher in the chloroform extract (121.4 ± 0.3 mg GAE/g), than in n-butanol extract (111.7 ± 0.1 mg GAE/g) and ethyl acetate extract (73.0 ± 0.4 mgGAE/g). The evaluation of flavonoids content also showed a high value of flavonoid in chloroform extract (20.9 ± 0.2 mg QE /g DM), whereas the ethyl acetate and the n-butanol extracts of *Retama sphaerocarpa* leaves contained similar quantity of flavonoids with 16.5± 0.8 mg QE /g DM and 15.3± 0.7 mg QE /g DM, respectively. The obtained extracts of *R. sphaerocarpa* were also evaluated for their antioxidant activities using DPPH radical-scavenging method. The three extracts (*i.e.* chloroform, ethyl acetate and n-butanol extracts) showed very high free radical scavenging activity: 73 ± 0.8, 75.6 ± 1.9 and 81.5 ± 0.3 (%), respectively. We also investigated the antimicrobial activities of the three extracts using the disc diffusion method. Each of the three extracts were active against *Bacillus sp.*

Keywords: *Retama sphaerocarpa, P*olyphenols, Flavonoids, Antioxidant properties, Antimicrobial properties

Abbreviations: GAE-Gallic Acid Equivalents: QE-Quercetin Equivalents: DPPH2-2-DiPhenyl-1-Picryl-Hydrazyl: BHT-Butylated Hydroxy Toluene: DM-Dry Matter

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INTRODUCTION

Plants contain mixtures of various organic compounds such as polyphenols. These molecules coming from the secondary metabolism of plants are becoming increasingly important due to their beneficial effects on health [1,2]. Polyphenolic substances such as flavonoids, polyphenolic acids and tannins are considered major contributors to the antioxidant capacity of plants. Related to their radical scavenging properties, they possess health properties to act against cancer, allergies [3,4], inflammation [5, 6] and microorganisms [7].

Algerian flora empirically known for its species diversity is promising for the discovery of new chemicals of interest. Indeed, these plants might contain different secondary metabolites with high bioactive properties [8] and could be used for the treatment of different diseases [9].

The genus *Retama* belongs to the family *Fabaceae*, which is located in the Mediterranean area, in North Africa and in the Canary Islands [10]. This genus has been the subject of several pharmacological studies especially, *Retama raetam* [11-13], *Retama monosperma* [14, 15] and *Retama sphaerocarpa* [16-21].

Retama genus is used in traditional medicine in many countries, such as purgative, vermifuge [22], antihelmintic, disinfectant, abortive [23] and antidiabetic [24, 25]. However, its toxicity was investigated by Algandaby [26]. This former author confirmed that at low concentration, the subacute toxicity potential of the methanolic extract of *Retama raetam* fruit was insignificant. However, it might have hepatotoxic, nephrotoxic and mutagenic effects at higher doses.

Retama sphaerocarpa (L.) Boissier (*Fabaceae*) is a perennial shrub widespread in the Iberian Peninsula and North East Africa [27]. The aerial parts are empirically used as an emmenagogue and abortifacient and are also used in the treatment of warts and constipation [17].

Hence, the aim of this study was to discover new bioactivities in *Retama sphaerocarpa* related to the presence of polyphenols and flavonoids especially, antioxidant and antimicrobial activities.

EXPERIMENTAL

Chemicals

All chemicals (Butylated hydroxytoluene (BHT), Folin-Ciocalteu phenol reagent, the free radical 2,2'-diphenyl-1-picryl-hydrazyl (DPPH), gallic acid, sodium carbonate (Na_2CO_3), aluminum chloride solution, methanol, petroleum ether, ethyl acetate, n-butanol, ascorbic acid and quercetin) were purchased at Sigma-Aldrich and were used of analytical grade.

Extraction of secondary metabolites from *Retama sphaerocarpa* plant

Figure 1: *Retama sphaerocarpa* specie

The freshly cut leaves of *Retama sphaerocarpa* (Figure 1) were collected in June 2014 from natural population in Kabylia, region located in the north of Algeria. They were air-dried at room temperature and stored in the dark until analysis. Then, leaves of *Retama sphaerocarpa* were reduced into powder using a grinder.

Twenty grams (20 g) of dried powder were macerated in 200 mL of a mixture of methanol/ water (80/ 20, v/v) for 48 hours. At the end ofthisperiod, mixture wasfiltered through afilterpaperWhatmanN°1. The aqueous filtrate was pooled and evaporated to remove methanol, then a petroleum ether liquid-liquid extraction was used for chlorophyll elimination on the dry extract.

The former aqueous phase was submitted to extraction solvents of increasing polarity, namely: chloroform then ethyl acetate and finally n-butanol. Each extraction was repeated three times.

After each extraction, the organic phase was dried in arotary evaporator to eliminate the solvent extraction and each dry extract was dissolved in methanol for further analysis.

Quantification of secondary metabolites

Total polyphenolic compounds

The total polyphenolic compounds present in each extract (*i.e.* chloroform, ethyl acetate and n-butanol extracts) were quantified according to the method described by Singleton et al. [28] using the Folin-Ciocalteu reagent.

Each sample (0.2 mL) was mixed with 1 mL of Folin-Ciocalteu reagent (10 % v/v) and maintained at room temperature. After 5 min, 0.8 mL of sodium carbonate solution (Na_2CO_3, 7.5% w/v) was added and mixed vigorously. Then, the samples were incubated for 60 min at room temperature (25°C) in the dark. The absorbance was measured at 760 nm with a UV/visible spectrophotometer (Shimadzu, 160 A).

Results were expressed in mg gallic acid equivalents (GAE)/g dry matter (DM). The calibration curve was obtained with gallic acid prepared in various concentrations (0-0.12 mg/mL). The blank was performed with methanol. For each sample, the Folin-Ciocalteu test was triplicated and standard deviation calculated.

Flavonoids content

The total flavonoids content was measured spectrophotometrically using the aluminium chloride colorimetric method similarly to Chang et al. [29].

One mL of each extract was mixed with 1 mL of aluminium chloride solution ($AlCl_3$. 2% w/v). After 30 min incubation carried out at room temperature and in the obscurity, absorbance was measured at a wavelength of 430 nm.

The calibration curve was performed with quercetin, which was prepared at various concentrations (0-0.2 mg/mL). Results were expressed as mg quercetin equivalents (QE)/ g dry matter (DM). The blank was performed with methanol. Samples were analyzed in triplicate.

Determination of the radical scavenging activity with the DPPH method

The dry residue collected after each solvent evaporation (*i.e.* chloroform, ethyl acetate, n-butanol) was dissolved in methanol at various concentrations (0-0.1 mg/mL).

The free radical-scavenging activity of each *Retama sphaerocarpa* extract was carried out using a modified DPPH test adapted from the literature [30-32].

One mL of DPPH solution (24 µg/ mL of MeOH) was added to 500 µL extract prepared in methanol and remained in the dark for 30 minutes. After this incubation period, the absorbance was measured by

spectrophotometry at a wavelength of 517 nm. The DPPH radical-scavenging activity (%) was calculated using the following equation:

$$Scavenging\ activity(\%) = \frac{A_0 - A_s}{A_0} \times 100$$

With: A_0, the control absorbance and A_s, the sample absorbance.

Antimicrobial activity

The antimicrobial activity of the different extracts was evaluated with the agar disc diffusion method [33]. Various clinical bacterial strains isolated directly from patients from the "Hospital University Ibn Badiss" of Constantine (Algeria) were used. *Bacillus sp.* was studied as Gram positive bacteria, while *Escherichia coli, Klebsiella pneumonioa* and *Pseudomonas aeruginosa* were studied as Gram negative bacteria.

All bacteria species were cultured overnight at 37°C in Mueller Hinton medium (Bio-Rad). Suspensions of the tested micro-organisms (0.1 mL of 10^7-10^8 cells/mL) were spread over the surface of the medium in Petri dish. The agar plates were stored at 4°C for further use. Filter paper discs (Whatman N°1; 6 mm in diameter) were impregnated with 20 µL of the *Retama* extract (*i.e.* chloroform, ethyl acetate and n-butanol extracts) and placed onto the inoculated agar plates.

The plates containing the bacteria were incubated for 24 h at 37°C. The resulting inhibition zones diameters (IZD) were measured in millimetres [34]. Control experiment was carried out on solvent alone (*i.e.* methanol). All experiments were performed in triplicates.

RESULTS AND DISCUSSION

Total polyphenolics content

The total polyphenolic content of each extract of *Retama sphaerocarpa* leaves was evaluated using the Folin-Ciocalteu method (Table 1). The calibration with gallic acid showed a linear relation with a high correlation coefficient (r^2> 0.99).

Table 1: Total polyphenolic and Total Flavonoids content in various extracts of *Retama sphaerocarpa* leaves

Extracts	Total Polyphenolic Content (mg GAE/g extract)	Total Flavonoids Content (mg QE /g DM))
Chloroform	121.4 ±0.3	20.9 ± 0.2
Ethyl Acetate	73.9 ±0.3	16.5 ±0.8
n-Butanol	111.7 ± 0.1	15.3 ± 0.6

The chloroform extract had the highest polyphenolic content with 121.4 mg GAE/g followed by the n-butanol extract (111.7 mg GAE/g). The Ethyl Acetate extract showed also a significant content of polyphenolics compounds but lower than the previous one (73.0 mg GAE/g).

Aqueous extracts of various parts of *Retama raetam* plant (stems, flowers, roots and fruits) showed lower content of polyphenolics compounds (25.19, 51.68, 11.11 and 11.11 mg GAE/g extract respectively [12]) than the aerial part of *Retama sphaerocarpa*. Hence, the extracts of *Retama sphaerocarpa* leaves are much more interesting in terms of total polyphenolic compounds than the former *Retama raetam* specie.

Flavonoids content

The total flavonoids content present in the extracts of *Retama sphaerocarpa* leaves is presented in quercetin equivalents /g dry matter (Table 1). Under the assay conditions described, the calibration curve carried out on quercetin showed a linear relation with a high correlation coefficient (r^2>0.97).

The chloroform extract had the highest flavonoids content with 20.9 mg GAE/g DM. The ethyl acetate extract and the n-butanol extract of *Retama sphaerocarpa* leaves contained similar quantity of flavonoids with 16.5 mg QE /g DM and 15.3 mg QE /g DM, respectively.

In vitro radical scavenging activity of Retama sphaerocarpa extracts

The free radical-scavenging activity of different extracts of *Retama sphaerocarpa* leaves was determined by measuring the absorbance decrease of DPPH free radical at 517 nm in the presence of various concentrations of extracts and compared to BHT (Table 2).

Table 2: DPPH radical scavenging activity (%) of various *Retama sphaerocarpa* extracts and BHT (positive control)

Concentrations (mg/mL)	BHT	Chloroform extract	Ethyl acetate extract	n-Butanol extract
0.00	0	0	0	0
0.02	23 ± 2	10 ± 1.2	22± 4	18 ± 0.5
0.04	56 ± 2	22 ± 1.6	35± 1.5	29 ± 0.8
0.06	77 ± 2	39± 1.2	53 ± 2	41 ± 2
0.08	83 ± 1	68 ± 1.9	51 ± 5	71 ± 1
0.1	85 ± 0.7	73 ± 0.8	76 ± 2	82 ± 0.3

The absorbance decrease was concentration-dependent. At low concentration, the ethyl acetate extract shows the most efficient radical scavenger as compared to n-butanol and chloroform extracts. However, for a higher concentration, the radical scavenging activity of n-butanol extract is best compared to that of ethyl acetate and chloroform extracts.

All extracts of *Retama sphaerocarpa* leaves (*i.e.* chloroform, ethyl acetate and n-butanol extracts) showed high radical scavenging ability. Yet, these ones were slightly lower compared to that of BHT (85% at 0.1 mg/mL) in the same concentration level.

The high free radical scavenging activity of ethyl acetate and n–butanol extracts (76% and 82%, respectively, at 0.1 mg/mL) correlated relatively well to their high content in polyphenolic compounds determined by the Folin-Ciocalteu assay (73.0 and 111.7 mg GAEs / g extract, respectively; Table 1).

However, the free radical scavenging activity of chloroform extract was the lowest one (73% for the same concentration), while its polyphenolic and flavonoids contents were higher than the two other extracts (ethyl acetate and n-butanol extracts; Table 1, respectively). The chloroform extract may contain some polyphenols and flavonoids with insignificant antioxidant capacity.

Djeddi et al. [12] showed that the antioxidant activity of *Retama raetam* aqueous extract of different parts of the plant (roots, stem, fruits and flowers) was lower than the one of BHT. Similarly to our work, former authors correlated the assessed antioxidant activity to the total polyphenolic content. Besides, the flowers oils of *Retama raetam* cultivated in Tunisia presented higher DPPH radical scavenging activity than BHT, which was attributed to the relative high percentage of monoterpenes contained in the essential oils [35].

Belmokhtar et al. [14] evaluated the relation between the antioxidant activity determined by DPPH and the phenolic contents, *i.e.* the flavonoids and the condensed tannin present in *Retama monosperma*. A significant high correlation (r^2=0.91) was evidenced between flavonoids content and antioxidant activities for the different fractions (chloroform, ethyl acetate and butanol) of the hydromethanolic extract of the different parts of this plant.

In vitro antimicrobial effects of Retama sphaerocarpa

The antimicrobial activity was evaluated on the three extracts of *Retama sphaerocarpa* (*i.e.* chloroform, ethyl acetate and n-butanol extracts) using disk diffusion method by measuring inhibition zone diameters [33].

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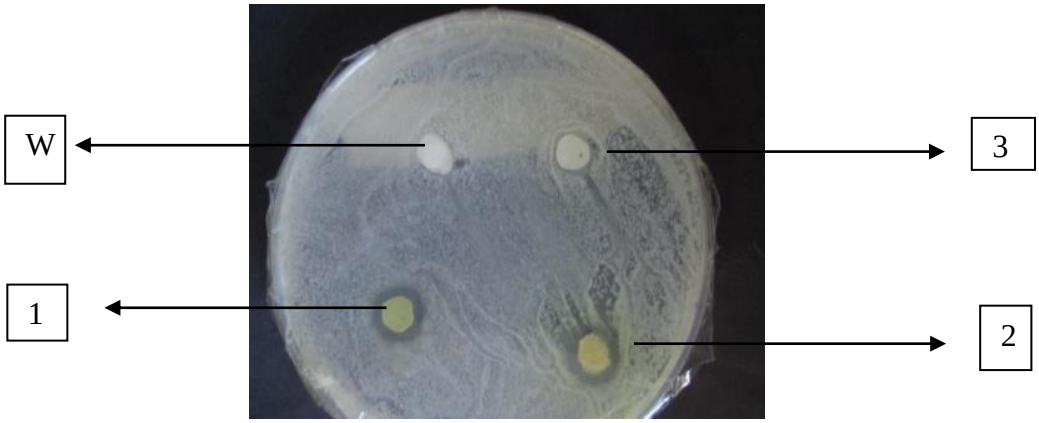

Figure 2: Antibacterial activity against *Bacillus sp*.

(W: witness (methanol), 1: chloroform extract, 2: ethyl acetate extract, 3: n-butanol extract)

At the highest dilution, all extracts of *Retama sphaerocarpa* leaves (*i.e.* chloroform, ethyl acetate and n-butanol extracts) showed a strong antibacterial activity against *Bacillus sp.* (Figure 2). On the other hand, no antibacterial activity was observed against *Escherichia coli, Pseudomonas aeruginosa,* and *Klebseilla pneumoniae* (Table 3).

Table 3: Effect of various extracts of *Retama sphaerocarpa* leaves on the growth inhibition of four various bacterial strains.

Bacteria / Extracts	*Bacillus sp.*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Klebseilla pneumoniae*
Chloroform	11 ± 1,1	--	--	--
Ethyl acetate	11 ± 0,3	--	--	--
n-Butanol	9 ± 0,6	--	--	--

--: negative result.

Edziri et al. [36] evidenced an antibacterial activity of two flavonoids from *Retama raetam* flowers against *E. coli*., *S. aureus* and a moderate antibacterial activity against *P. aeruginosa*. Our results complements the positive antimicrobial result against *staphylococcus aureus* of n-butanol extract of *Retama sphaerocarpa* leaves obtained by Louaar et al. [20].

It appears that levels and metabolic patterns vary depending on the part of the plant and metabolic profiles differ widely from one species to the other [37].

CONCLUSION

To conclude, the various extracts of *Retama sphaerocarpa* leaves (i.e. chloroform, ethyl acetate and n-butanol extracts) show high radical scavenging capacities, in correlation with their richness in polyphenols and especially flavonoids.

Hence, these results evidence for the first time that *Retama sphaerocarpa* leaves, empirically used in the traditional Algerian medicine, can be considered as a source of very efficient antioxidant compounds, with therapeutic and preventive applications. Besides, all extracts showed good antibacterial activity against *Bacillus* sp. as well and could possibly be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by this former pathogen.

A perspective of this research work will be the identification of secondary metabolites involved in antioxidant activity and responsible of antimicrobial action using liquid chromatography coupled with mass spectrometry (HPLC/MS).

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REFERENCES

[1] Aboul-Enein AM, Abu-Elalla F, Shalaby EA, El-Shemy HA. J. Med. Plants Res. 2012; 6(5): 689-703.
[2] Maiza K, Hammiche V, Maiza-Benabdesselam F. Life sciences Leaflets 2011; 16: 551 – 560.
[3] Chung KT, Wong TY, Huang YW, Lin Y. Critical Reviews in Food Science and Nutrition 1998; 38: 421–464.
[4] Gurib-Fakim A. Molecular Aspects of Medicine 2006; 27(1): 1-93.
[5] Bremnera P, Riverab D, Calzadoc MA, Obónd C, Inocenciob C, Beckwitha C, Fiebiche BL, Munozc E, Heinricha M. Journal of Ethnopharmacology 2009; 124: 295-305.
[6] Miguel M, Bouchmaaa N, Aazza S, Gaamoussi F, Badiaâ L, youssi B. Fresenius Environmental Bulletin 2014; 23, N°6.
[7] Edziri H, Mastouri M, Ammar S, Mata M, Gros P, Raida H, Mahjoub MA, Si Mohamed A, Gutmann L, Mighri Z, Mahjoub A. World J. Microbiol. Biotechnol 2008; 24: 2933–2940.
[8] Ziani BEC, Calhelha RC, Barreira JCM, Barros L, Hazzit M, Ferreiral CFR. Industrial Crops and Products 2015; 77: 582-589.
[9] Djeddi S, Boutaleb H. Journal of Advanced Sciences & Applied Engineering 2014; 01, N°01: 60-63.
[10] Quezel P, Santa S. Nouvelle flore de l'Algérie et des régions désertiques et Méridionales. CNRS Edition, Paris.2, 1963.
[11] Benmenine A, Ouahrani MR, Gherraf N, Sekirifa ML, Baameur L. Energy Procedia 2014; 50: 401 – 405.
[12] Djeddi S, Karioti A, Yannakopoulou E, Papadopoulos K, Chatter R, Skaltsa H. Rec. Nat. Prod. 2013 ; 7, 3: 169- 176.
[13] Kacem I, Majdoub H, Roudesli S. Journal of Applied Sciences 2008; 8(9): 1713-1719.
[14] Belmokhtar Z, Kaid Harche M. Natural Product Research 2014; 28 (24): 1-6.
[15] Belayachi L, Aceves-Luquero C, Merghoub N, Bakri Y, Fernandez de Mattos S, Amzazi S, VillalongaP. BMC complementary and alternative medicine 2014; 14, 38.
[16] Martin-Cordero C, López-Lázaro M, Gil-Serrano A, Rodriguez-Carvajal MA, Ayuso Gonzalez MJ. Phytochemistry 1999; 40: 1129-1131.
[17] López-Lázaro M, Carmen MC, Ma JA. Planta. Med. 1999; 65: 777-778.
[18] López-Lázaro M, Martin-Cordero C, Cortés F, Pinero J, Ayuso MJ. Z. Naturforsch 2000; 55c: 40-43.
[19] Louaar S, Akkal S, Bousetla A, Medjroubi K, Djarri L, Seguin E. Chemistry of Natural Compounds 2005; 41; 107.
[20] Louaar S, Akkal S, Laouer H, Guilet D. Chemistry of Natural Compounds 2007; 43(5): 616-617.
[21] Louaar S, Akkal S, Laouer H, Duddeck H. A new isoflavone glycoside from the aerial parts of *Retama sphaerocarpa*. Chemistry of Natural Compounds 2010; 5: 719-721.
[22] Bellakhdar J. La Pharmacopée Marocaine Traditionnelle : Médecine arabe ancienne et savoirs populaires. Saint–Etienne, Edit. IBIS, 1997, pp. 177
[23] [IUCN] International Union for the Conservation of Nature. Center for Mediterranean cooperation. A Guide to Medicinal Plants in North Africa 2005; 197-8.
[24] Eddouks M, Maghrani M, Lemhadri A, Ouahidi ML, Jouad H. J. Ethnopharmacol. 2002; 82: 97−10.
[25] Algandaby MM, Alghamdi HA, Ashour OM, Abdel-Naim AB, Ghareib SA, Abdel-sattar EA, Hajar AS. Food and Chemical Toxicology 2010; 48 : 2448–2453.
[26] Algandaby MM. Journal of the Chinese Medical Association 2015; 78 (12): 691-701.
[27] Guerrouj K, Ruiz-Diez B, Chahboune R, Ramirez-Bahena MH, Abdelmoumen H, Quinones MA, Missbah El Idrissi M, Velazquez E, Fernandez-Pascual M, Bedmar EJ, Peix A. Syst. Appl. Microbiol. 2013; 36: 218–223.
[28] Singleton VL, Orthofer R, Lamuela-Raventos RM. Methods Enzymol. 1999; 299: 152-178.
[29] Chang CC, Yang MH, Wen HM, Chern JC. J. Food Drug Anal. 2002; 10: 178-182.
[30] Molyneu P. Songklanakrin J. Sci. Technol. 2004; 26(2): 211–216.

[31] Lebeau J, Furman C, Bernier JL, Duriez P, Teissier E, Cotelle N. Free Radic. Biol. Med. 2000; 29(9): 900-912.
[32] Schwarz K, Bertelsen G, Nissen LR, Gardner PT, Heinonen MI, Hopia A. European Food Research and Technology 2001; 212 (3): 319-328.
[33] [NCCL] National Committee for Clinical Laboratory Standards. National Committee for Clinical Laboratory Standards. Wayne, Pa. USA, 1997.
[34] Jirovetz L, Buchbauer G, Stoyanova AS, Georgiev EV, Damianova ST. Journal of Agricultural and Food Chemistry 2003; 18 (51): 3854-3857.
[35] Edziri H, Mastouri M, Chéraif I, Aouini M. Webb from Tunisia. Nat. Prod. Res. 2010; 24: 789-796.
[36] Edziri H, Mastouri M, Mahjoub MA, Mighri Z, Mahjoub A, Verschaeve L. Molecules Journal 2012; 17:7284-7293.
[37] Fdil R, El Hamdani N, El Kihel A, Sraidi K. Ann. Toxicol. Anal. 2012; 24(3):139-143.