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Total Phenolics, Flavonoids and Tannins Contents and *in Vitro* Antioxidant and Antibacterial Effects of *Arenaria Rubra* Extracts

Mourad Boudjouref *[1], Rachid Belhattab[2]

[1]Department of Nature and Life Sciences, Faculty of Exact Sciences and Nature and Life Sciences, Larbi Ben M'Hidi University, Oum El Bouaghi-04000, Algeria
[2]Department of Biochemistry, Faculty of Nature and Life Sciences, Ferhat Abbas –Setif 1 University, Setif-19000, Algeria.
***Corresponding Author:** MouradBoudjouref**, Email:** mourad.boudjouref@gmail.com, Boudjouref.Mourad@univ-oeb.dz

ABSTRACT

This research was conducted in order to establish content of total phenolics, flavonoids, flavonols and flavonss, and tanninsof extracts from theflowers of *Arenaria rubra*. In addition, antioxidant and antimicrobial activities were evaluated. The DPPH radical trapping technique andferric reducing antioxidant power (FRAP) were examined *in vitro* for antioxidant activity.Theinhibitory effects of the extracts against species of gram-positive and gram-negative bacteria, and speciesof fungi were determined using the disk diffusion method.The highest DPPH radical-scavenging activity was found in the methanol-water extract,the antibacterial activity was expressed only on *Escherichia coli*, for the antifungal activities the yeast *C. albicans*was sensitive to all extracts tested.
Keywords:-*Arenaria rubra*, Antioxidant activity, Antimicrobial activity.

Introduction

In order to prolong the storage stability of foods and to reduce damage to the human body, many synthetic preservatives, such as butylatedhydroxytoluen (BHT, butylatedhydroxyanisole (BHA)have been used (Demir et *al.,* 2009). However, these synthetic antioxidantsare suspected to be toxic and/or carcinogenic (Szabo et *al.,* 2010). In addition, Microbial contamination is another important issue in the field of food, beverage, cosmetic, and pharmaceutical industries (Ebrahimabadi et *al.,* 2010). For this reason, there is a growing interest in studies ofnatural products with both antioxidant and antimicrobial activities (Tianet *al.,* 2009). In Algeria, phytotherapy is an integral part of the local culture; population has an important indigenous knowledge acquired empirically through the generations. Its geographical location and climatic diversity have allowed the development of a very rich and highly diversified flora; which was used since time immemorial to treat several diseases (Bouasla,2017)

The Caryophyllaceaegenus is one of the largest genera of the world's flora (Bechkri et *al.,* 2022). Plant species belonging to this family are diverse in the eastern parts of the Mediterranean Basin and in southwestern Asia(Moiloa et *al.*, 2021). In Algeria, this genus is represented by ten species (Quezel and Santa 1963)

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Caryophyllaceae species are known for their rich content in bioactive metabolites, such astriterpene saponins, flavonoids,phytoecdysteroids and oligosaccharides. Moreover they possess interesting pharmacological activities such as antitumour, anti-inflammatory, antiviral,cytotoxic, analgesic and antipyretic (Allaoua et *al.*, 2016)

Arenaria rubra L. known by the vernacular name "fatete lahjar" is a medicinal plant of the Caryophyllaceae family, widely used in traditional Algerian medicine and as a food condiment (Ouldyerou et *al*., 2022)

The aerial parts of the *Arenaria rubra* are widely used as an infusion due to its diuretic properties (Teğin, 2018).*A. rubra* has biological and pharmacological properties such asantidiabetic, anticholinesterase, and antioxidant activities (Kim et *al*., 2014)

The present study focused on the quantification of the total phenol, flavonoids and tannins contents, and also to evaluate antioxidantand antimicrobial activities of the different extracts from the leaf of *Arenaria rubra*.

Materials and methods

Plant collection and storage

Theflowers samples of A.*rubra*were purchased from local markets.Theplant species was identified and a voucher specimen was depositedin the Research Laboratory, University of Oum El Bouaghi.The flowers were dried at room temperature for about a week, then ground. The dried plant flowers were ground to a fine powder using a blender and stored in the dark until extraction.

Extraction

The powdered material (40 g) was successively macerated at room temperature for 72h withdifferent solvents700 mL of increasing polarity namely dichloromethane (DCM),methanol andWater/Methanol (V/V, 1:5).The macerates werefiltered, and the organic solvents removed using a rotary evaporator at temperature of 40 °C. The obtained extracts were stored in a refrigerator at 4 °C until further analysis.

Quantification of phytochemicals

Phenolic content evaluation

The concentration of the extracts in polyphenols was evaluated using the Folin-Ciocalteu reagent according to Li et *al.* 2007).To a volume of 200µl of plant extract solutions was added 1.0 mL of tentimes diluted Folin-Ciocalteu reagent, after 4 min, an amount of 800 µL of aqueous Na_2CO_3 (7.5%) was added to the mixture.The sample was incubated for 2h at ambient temperature then the absorbance wasmeasuredat 765 nm. gallic acid was employed as a reference and results were reported as mg of gallic acid equivalent (GAE)/g of extract.

Total flavonoids content evaluation

The aluminum chloride colorimetric technique was used to calculate the total flavonoids in the plant extracts.A volume of 1 mL of aluminium chloride 2% alcoholic solution was added to 1 mL of each extract. The absorbance was measured at 430 nm after 10 minutes of incubation at room

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temperature.Quercetin was employed as a reference and results were reported as mg of quercetin equivalent /g of extract (Kosalec et *al*., 2004).

Flavones and flavonols content evaluation

The aluminum chloride colorimetric test was adopted for the estimation of flavones and flavanols. About 0.5 mL of various extracts of *A.rubra*was mixed with a volume of 1.5 mL of methanol, and then to this mixture were added 0.1 mL of the 10% methanolic aluminum chloride solution and 0.1 mL of sodium acetate and 2.8 mL of distilled water.

The reaction mixture was left to react forhalf an hour and then the absorbance was calculated at 415 nm (Kosalec et *al*., 2004). The flavones and flavonols concentration was measured by using quercetin as a reference compound.

Tannins content evaluation

The content of total condensed tannins was determined using a modified vanillin assay.A volume of 3 mL of methanolic solution of vanillin (4%)were added with 1.5 mL of concentrated HCl and mixed with 50 µl of samples or reference, the resulting solution was allowed to stand for 15 minutes, and the absorbance was read at 500 nm. Catechin was employed as a reference and results were reported as mg of catechin equivalent /g of extract (Singh and Patra, 2018).

Measurement of the antioxidative potential of A. *rubra*

Assay for reducing DPPH radical

The DPPH technique was used to assess the free radical scavenging activity of *A. rubra*extracts, following the protocol published by Bounatirou et *al.*,(2007) with small changes.

0.1 mL of each extract solutionof different concentrations was added to2 mL of 0.1 mM DPPH methanol solution. The solution was mixed and then maintained at room temperature for 30 minutes. The absorbance was measured at 517 nm against a blank.Ascorbic acid was used as standard control forcomparison. The DPPH radical trapping activity was calculated using the following equation and reported as the percentage of free radicals inhibited by the sample.

% inhibition of DPPH = (A control − A sample /Abs control) × 100

With Abs control represents the absorbance of the DPPH solution that doesn't contain the extract and A sample represents the absorbance of the extract.

Ferric reducing antioxidant power (FRAP) assay

The reduction potential of several extracts from the plants under investigation was determined according toBougandoura and Bendimerad, (2013).

0.1mL of various concentrations of extracts weremixed withphosphate buffer (2.5mL, 0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide $K_3Fe(CN)_6$ solution (1%). After 20 min of incubation under 50°C, 2.5 mL of trichloroacetic acid (10%) were added to these mixtures, which were then centrifuged at 3000 rpm during 10 min. Finely 2.5 mL of the supernatant from each concentration were stirred with a mixture consisting of 2.5 mL of distilled water plus 0.5 mL of $FeCl_3$0.1%. The absorbance was determined at 700 nm.

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Antimicrobial assay

Evaluation of the antibacterial activity by the disc diffusion test

The antimicrobial activity of *A. rubra* extract was evaluated against a panel of pathogenic bacteria and fungi :*Escherichia coli*,*Pseudomonas aeruginosa*, *Staphylococcus aureus and Candida albicans*.

Disc diffusion method was used toinvestigatetheantimicrobial activity of the plant extracts.

The test micro-organisms, were grown onsolid Muller−Hinton (MH)agar (for bacteria) or Sabouraud dextrose agar (for fungi).

Disks of Whatman filter paper were prepared usinga regular paper puncher (6 mM) and were sterilized. Then 20µL of various concentrations of extract weredropped in the center of each filter paper disk. before placing them on agar.Thepetri dishes were then incubated at 37°C for 24 hours. Equal volumes ofethanol and methanol were used as a negative control,and ciprofloxacin (2 mg/mL) was used as a positive control.After incubation,thediameter of thegrowthinhibitionzone(mm)surroundingthe disks showed the inhibitory effect.The experiment was performed in duplicate (Bolou et *al.*, 2011).

Results and Discussion

Phytochemical composition

The determinations were performed fordichloromethane (DCM),methanol anda mixture ofWater/Methanol extracts obtained from A*renaria rubra* flowersand results are presented in Table 1. The values are the mean of three determinations ± standard error mean.The concentration of total phenolics varied from29.54to37.83mg GAE/g, flavonoids 2.76–30.8 mg QE/g, flavones and flavonols 0.066– 0.142 0 mg QE/g and tannins 18.50–69.5 mg CE/g.In fact,methanol extract showed the highest contentquantity of polyphenols, flavonoids and tannins followed by dichmoromethane and methanol/water extracts.

These different results reflected the fact thatthe presence of phenolics, tannins and flavonoids is affected by thetype of plant part, maturity at harvest, growing conditions, plantvarieties and extraction methods (Deng et *al.*, 2015)

Table 1. Content of total polyphenols, flavonoids, flavones and flavonols and tannins in extracts of *A. rubra*

Extract	Total phenolics (mg GAE/g)	Flavonoids (mg QE/g)	Flavones and flavonols (mgQE/g)	Tannins (mg CE/g)
Dichloromethane	31.83±5.88	12.5 ± 0.36	0.142 ± 0.137	47.5 ± 3.53
Methanol	37.83±12.49	30.8 ± 2.05	0.066 ± 0.052	69.5 ± 4.94
Methanol:Water	29.54±0.93	2.76 ± 0.15	0.115 ± 0.01	18.5 ± 2.12

DPPH scavenging assay

The ability of antioxidants to scavenge DPPH radicals is related to their ability to donate hydrogen. When DPPH solution is mixed with a substance capable of donating a hydrogen atom, it causes a reduction in diphenylpicrylhydrazine which leads to the loss of its purple color. Free radical scavenging isone of the known mechanisms by which antioxidants inhibitlipid oxidation(Baharfar 2015).

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Table 2 illustrates the percent inhibition of DPPH radical bythe various extracts and the standard antioxidants at differentconcentrations.

The antiradical power of these extracts is dose dependent. At the concentration of 1mg/mL, the antiradical power of the methanol-water extractreached 82,18%, almost equal to that of ascorbic acid (83.87%), the dichloromethane and methanolic extracts present a moderate inhibitory activity: 64.27% and 42.18%, respectively, but relatively weak than that of ascorbic acid. The richness of the methanol-water extract in phenolic compounds is probably responsible for their potent antiradical activity.

Table 2. Anti-radical activity of *Arenaria rubra* extracts and ascorbic acid

Extracts	0,1mg/mL	0,25mg/mL	0,5mg/mL	0,75mg/mL	1mg/mL
DCM	1,08%	1,90%	2,90%	9,90%	25,08%
MeOH	12,90%	17,72%	22,36%	31,99%	42,18%
MeOH/H_2O	6,45%	25,36%	34,27%	65,63%	82,18%
Ascorbic acid	83,06%	83,26%	83,50%	83,70%	83,87%

Ferric reducing antioxidant power

The FRAP test was used to assess the antioxidant capability of extracts. For this test, ferric ions are transformed into ferrous ions by an antioxidant agent, which gives rise to a tripyridyltriazine ferrous complex (Fe^{2+}-TPTZ) with a blue color at pH 3.6. The transformation is followed spectrophotometrically at 593 nm (Ahmed et *al.*, 2015).

Compared to the standard used, this value is still higher than ascorbic acid value. The methanolic extract has a greater reducing power than the methanol/water and dichloromethane extracts. (Table 3)

The reducing power of our extracts is probably due to the presence of hydroxyl groups that can serve as electron donors. Therefore, antioxidants are considered as reducers and inactivators of oxidants(Bougandoura and Bendimerad, 2013).

Table 3. Reducing power of *Arenaria rubra* extracts and ascorbic acid

Extracts	0,1mg/mL	0,25mg/mL	0,5mg/mL	0,75mg/mL	1mg/mL
DCM	0,195	0,219	0,286	0,287	0,306
MeOH/H_2O	0,264	0,4	0,494	0,503	0,621
MeOH	0,421	0,421	0,503	0,677	0,716
Ascorbic acid	0,657	0,788	0,842	1,33	1,62

Antimicrobial test

The disk diffusion method for antimicrobial effect testing was investigated to determine the antibacterialactivities of the extracts againstgram-positive bacteria(*Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli*,*Pseudomonas aeruginosa*).

The results of antimicrobial activity of the crude extracts of*A.rubra*were shown in Tables4,5 6. In this study, it was found that all crude extractsshowed antimicrobial activity only against*Escherichia coli*. Both bacterial strains *S. aureus* and *P. aeruginosa* showed resistance against all extracts tested.Concerning the antifungal activity, the results showed that the yeast *C. albicans* is sensitive to all extracts.

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Table 4. Antimicrobial activity of *Arenaria rubra* dichloromethane extract expressed in diameters of inhibition zones

Test organism	500 µg/mL	1000 µg/mL	2000 µg/mL	4000 µg/mL	8000 µg/mL
E.coli	6±0	8±0	9.5±0.7	10.5±0.7	12.5±0.7
S.aureus	-	-	-	-	-
P.aeruginosa	-	-	-	-	-
C. albicans	9.37±0.53	10.125±0.17	11.75±0.35	11.8±0.17	13.75±0.3

Table 5. Antimicrobial activity of *Arenaria rubra* methanol extract expressed in diameters of inhibition zones

Test organism	500 µg/mL	1000 µg/mL	2000 µg/mL	4000 µg/mL	8000 µg/mL
E.coli	7.5±0	8±0	10±0	10.5±0.7	11±1.4
S.aureus	-	-	-	-	-
P.aeruginosa	-	-	-	-	-
C. albicans	9.25± 0.35	10±0	9.5±0.35	11.125±0.17	11.75±0.53

Table 6. Antimicrobial activity of *Arenaria rubra* methanol/water extract expressed in diameters of inhibition zones

Test organism	500 µg/mL	1000 µg/mL	2000 µg/mL	4000 µg/mL	8000 µg/mL
E.coli	6.5±0.7	7±1.4	8.5±0.7	10±0	10±1.41
S.aureus	-	-	-	-	-
P.aeruginosa	-	-	-	-	-
C. albicans	8±0	8.75±0.35	9.87±0.17	10±0	11.25±0.35

Conclusion

The results obtained from this study show that this plant possessed of a strong antioxidant activity while for the antimicrobial activity it is moderate.

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