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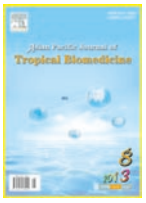

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Screening of biological activities of *Polygonum maritimum* L. from Algerian coast

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PEER REVIEW

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Comments

This work represents a moderate study but with the highest importance in the first part of antioxidant activity. These researchers have found compounds that exhibit a very interesting antioxidant activity of an original plant.
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ABSTRACT

Objective: To investigate the antioxidant and the antibacterial activities of crude extract from aerial part of *Polygonum maritimum* L. (Polygonaceae) (*P. maritimum*) and to find new actives biomolecules.

Methods: The whole plant was collected from the Rechgoune coast (West of Algeria), and methanolic crude extract of aerial parts of *P. maritimum* (PMCE) was prepared. The extract was tested against different bacterial strain and tested for his ability to neutralize free radical (DPPH) and to scavenge the H_2O_2.

Results: PMCE had a very high content of total phenol, which was (352.49±18.03) mg/g dry weight, expressed as gallic acid equivalent. PMCE exhibited excellent antioxidant activity, as measured using DPPH and H_2O_2 scavenging assays. It also showed a high antibacterial activity against gram-positive bacterial strains: *Bacillus cereus*, *Bacillus subtilis* and *Staphylococcus aureus* with an highest MIC of 120 μg/mL.

Conclusions: The antioxidant and antibacterial activity of the PMCE is probably due to phenolic compounds present in the extract. The contemporary presence of antioxidant and antibacterial activities in the PMCE suggests that this plant may be a source of bioactive substances with multifaceted activity.

KEYWORDS

Polygonum maritimum L., Phenolic compounds, Antioxidant activity, Antibacterial activity

1. Introduction

Free radical reactions, especially with the participation of oxidative radicals, have been shown to be involved in many biological processes that cause damage to lipids, proteins, membranes and nucleic acids, thus giving rise to a variety of diseases[1-6]. The harmful action of the free radicals can, however, be blocked by the antioxidant substances, which scavenge the free radicals and detoxify the organism[7].

Current research into free radicals has confirmed that the natural medicine rich in antioxidants plays the essential role in the prevention of cancers, cardiovascular diseases and the neurodegenerative diseases, including Parkinson's inflammation, as well as Alzheimer's diseases and problems resulting from the cell aging[7].

Much attention has been paid to the antioxidants, which are expected to prevent food and living systems from peroxidative damage. Incorporation of synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertbutylhydroquinone

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(TBHQ) have been added to foods can retard lipid oxidation[8]. However, the use of synthetic antioxidants in food products is under strict regulation due to the potential health hazards caused by such compounds[8,9].

Use of plants as traditional health remedies is very popular and important for 80% of the world's population in African, Asian, Latin America and Middle Eastern Countries. Their use is reported to have minimal side effects. In recent years, pharmaceutical companies have spent considerable time and money in developing therapeutics based upon natural products extracted from plants[10].

The use of medicinal plants can be traced back over five millennia in several civilizations. It is a traditional form of providing relief from illness. For years, natural products have contributed enormously to the development of the important therapeutic medicine used currently in modern medicine[7].

Phenolics are an important class of secondary plant metabolites possessing various pharmacological activities. One of the more prominent properties of the phenolics is their excellent radical scavenging ability[11]. This one is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, singlet oxygen quenchers agents, and they have also metal chelating potential[12].

Due to the continuous emergence of antibiotic-resistant strains there is continual demand for new antibiotics. In many developing countries about 80% of available drugs come from medicinal plants and in industrialized countries plants make up the raw material for processes, which synthesize pure chemical derivatives[13,14].

The genus *Polygonum* (Polygonaceae), comprising about 45 genera (300 species), is distributed worldwide, mostly in north temperate regions. They have been reported to have uses in traditional medicine, such as anti-inflammation, promoting blood circulation, dysentery, diuretic and haemorrhage[15-17].

Polygonum maritimum L. (*P. maritimum*) is a perennial herb or small shrub 20-50 cm in height that can be found in sandy coasts in Europe, America, South Africa, and the Mediterranean region[17].

The genus *Polygonum* is well known for producing a variety of plant secondary metabolites such as phenylpropanoids, acetophenones, chalcones, coumarins, flavonoids, lignans, naphtoquinones, anthraquinones, sesquiterpenoids, triterpenoids, stilbenoids and tannins described in many publications[18-25].

According to our knowledge, there are very few publications about the characterisation of the biological activities related to phenolic and flavonoid contained in the crude extract of *P. matitimum*. With respect to this, the antioxidant and the antibacterial activity of the aerial part of *P. maritimum* is presented in this study, together with the contents of phenolic and flavonoid compounds.

2. Material and methods

2.1. Plant materials

The whole plant of *P. maritimum* were collected from the Rechgoun coast (Region of Ain Temouchent, West of Algeria), in May 2011 and dried away from direct sunlight. Dried plant material was then crushed into a mortar and stored at very low temperature until further use.

2.2. Sample preparation

A powder (10 g) of the aerial part of *P. maritimum* was extracted by 100 mL of methanol-water (8:2, v/v) for 3 h under reflux. The extracts were then filtered and concentrated under reduced pressure at 60 °C using a rotary evaporator (Büchi Rotavapor R-200) to obtain the *P. maritimum* crude extract (PMCE). The last one was kept in dark and stored at 4 °C.

2.3. Total phenolic content

Total phenolic was estimated by the Folin-Ciocalteu method[26]. 0.1 mL of sample was mixed with 2 mL of sodium carbonate (2%) freshly prepared, the whole was vigorously mixed on a vortex. After 5 min, 100 μL of Folin-Ciocalteu reagent (1N) were added to the mixture, all was left for 30 min at room temperature and the reading of absorbance (SPECORD 200 Plus) is performed against a blank at 750 nm. A calibration curve was performed in parallel under the same operating conditions using gallic acid as a positive control. The results are expressed as mg gallic acid equivalent per gramme of dry extract (mg GAE/g).

2.4. Total flavonoid content

The total flavonoid content was determined by a colorimetric method as described in the literature[27]. Each sample (500 μL) was mixed with 2 mL of distilled water and subsequently with 150 μL of a $NaNO_2$ solution (15%). After 6 min, 150 μL of aluminum chloride ($AlCl_3$) solution (10%) was added and allowed to stand for 6 min. Then, 2 mL of NaOH solution (4%) was added to the mixture. Immediately, distilled water was added to bring the final volume to 5 mL and the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was then

determined at 510 nm versus prepared water blank. Results were expressed as catechin equivalent per gramme of dry extract (mg CEQ/g).

2.5. Antioxidant activity

2.5.1. Determination of the scavenging effect on DPPH radicals

A methanolic solution (50 μL) of each sample or positive control at different concentrations was added to 1.95 mL of DPPH solution (6×10^{-5} mol/L in methanol)[28]. The studied compounds were tested with methanol as control, BHA, ascorbic acid and quercetin as antioxidant references. The absorbance at 515 nm was determined after 30 min of incubation at ambient temperature. The absorbance (A) of the control and samples was measured, and the DPPH scavenging activity (SA), in percentage, was determined as follow:

$SA\ \% = [(A_{control}-A_{sample})/A_{control}] \times 100$

EC_{50} value (μg/mL) is the concentration at which that scavenging activity was 50%.

2.5.2. Determination of the scavenging effect on hydrogen peroxide

A solution of hydrogen peroxide (20 mmol/L) was prepared in phosphate buffered saline (PBS, 0.1 mol/L, pH 7.4). 1 mL of sample or standards in methanol was added to 2 mL of hydrogen peroxide solution in PBS. The absorbance was measured at 230 nm, after 10 min against a blank solution that contained extracts in PBS without hydrogen peroxide[29].

The percentage of H_2O_2 scavenging of examined extracts was calculated as:

$\%\ \text{of scavenged}\ H_2O_2 = [(A_{control}-A_{sample})/A_{control}] \times 100$

where the control is the phosphate buffer with H_2O_2.

2.6. Antimicrobial activity

2.6.1. Microbial strains

The PMCE was tested against the following bacteria: *Pseudomonas aeruginosa* (−) ATCC 27853, *Escherichia coli* (−) ATCC 25922, *Proteus mirabilis* (−) ATCC 35659, *Enterococcus faecalis* (+) ATCC 49452, *Acinetobacter baumannii* (−) ATCC 19606, *Bacillus cereus* (+) ATCC 10876, *Bacillus subtilis* (+) ATCC 6633, *Staphylococcus aureus* (+) ATCC 25923 and *Citrobacter freundii* (−).

2.6.2. Evaluation of the antibacterial activity

The *in vitro* antibacterial activity of the examined extract was assessed the determination of the minimum inhibitory concentration (MIC) by the microdilution method, according to recommendations of the Clinical and Laboratory Standards Institute[30,31].

2.6.3. Determinations of the MIC: broth microdilution method

The inocula of bacteria were prepared and adjusted to 0.5 McFarland standard turbidity. PMCE dissolved in 5% dimethyl sulfoxide (DMSO) were first diluted to the highest concentration to be tested and then serial twofold dilutions were made in a concentration range from 16 μL/mL to 0.016 μL/mL in sterile tubes containing Mueller-Hinton Broth.

The 96 well-sterile microplate were prepared by dispensing into each well 100 μL of the dilution crude extract. These dilutions were inoculated with 100 μL of a solution containing 10^6 CFU/mL. The microplate was incubated at 35-37 °C for 24 h. The MIC was considered as the lowest concentration of the essential oil that will inhibit the growth of the microorganism being tested as detected by lack of visual turbidity, matching with a negative control.

2.7. Statistical analysis

Data were reported as means ±standard deviation (SD) of three parallel measurements.

3. Results

3.1. Total phenolic and flavonoid contents

Regarding the levels of phenolic and flavonoid contents. The results, as presented in Table 1, show that the PMCE contained high phenolic and flavonoid contents. (Table 1).

Table 1
Total phenolic, total flavonoid contents and the EC_{50} values of PMCE, BHA, ascorbic acid and quercetin.

	DPPH scavenging (μg/mL)*	Yield (%)	Total phenolica	Total flavonoidb
PMCE	7.71 ±1.88	14.34 ±2.44	352.49 ±18.03	147.26 ±4.67
BHA	2.61 ±0.13	–	–	–
Ascorbic acid	2.48 ±0.09	–	–	–
Quercetin	2.59 ±0.15	–	–	–

Each value represents the mean±SD (n=3). Total phenolic content was expressed as mg gallic acid equivalents/g dried extract; total flavonoid content was expressed as mg catechin equivalents/g dried extract. EC_{50} values were expressed as final concentrations.

3.2. Antioxidant activity

3.2.1. DPPH radical scavenging

The PMCE was tested for its antioxidant scavenging effects on DPPH radical and it activity was compared to different positive control: the synthetic antioxidant BHA (butylated

hydroanisol), ascorbic acid and quercetin.

The results obtained are given in Figure 1. From these results, it is demonstrated that all the tested extracts showed a non-linear dose-dependant activity.

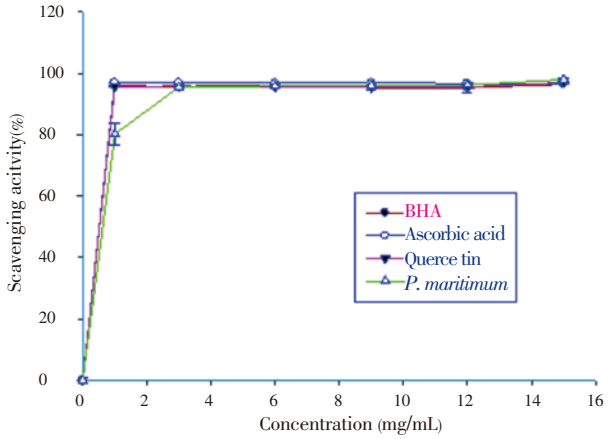

Figure 1. Scavenging activity of PMCE on the DPPH radical scavenging.

The free radical scavenging activity is also expressed by the antioxidant concentration required for a 50% DPPH reduction (EC_{50}) (Table 1).

3.2.2. Hydrogen peroxide radical scavenging

As shown in Table 2, scavenging activity of hydrogen peroxide in PMCE at 100 μg/mL and BHA, α-tocopherol (100 μg/mL) as reference compound was different, we remarked that the extract exhibit a high activity twice more efficient than controls used, that result showed clearly the capacity of this plant to neutralize free radical since the reaction with phenolic compounds present in this plant.

Table 2
Hydrogen peroxide scavenging activity.

	H2O2 scavengingActivity (%)
PMCE	44.22 ±9.93
BHA	24.13 ±7.32
α-Tocopherol	32.44 ±5.87

Each value represents the mean ±SD (n = 3).

3.3. Antibacterial activity

As can be seen in Table 3, PMCE exhibit a remarkable antibacterial activity. We remarked also that gram-positive strains were the most sensitive (*Bacillus cereus*, *Bacillus subtilis* and *Staphylococcus aureus*: MIC=120 μg/mL). Moderate activity of extract was remarked against *P. aeruginosa* and *E. coli* with an MIC = 64.35 and 16.08 mg/mL, respectively.

Table 3
Minimum Inhibitory Concentrations (MIC) of PMCE.

Bacterial Strains	MIC (μg/mL)
Pseudomonas aeruginosa (-) ATCC 27853	64.35
Escherichia coli (-) ATCC 25922	16.08
Proteus mirabilis (-) ATCC 35659	4.02
Enterococcus faecalis (+) ATCC 49452	4.02
Citrobacter freundii (-)	2.01
Acinetobacter baumannii (-) ATCC 19606	1.00
Bacillus cereus (+) ATCC 10876	0.12
Bacillus subtilis (+) ATCC 6633	0.12
Staphylococcus aureus (+) ATCC 25923	0.12

4. Discussion

Total phenolic contents were estimated with Folin-Ciocalteu colorimetric method. This reagent is a mixture of phosphotungstic ($H_3PW_{12}O_{40}$) and phosphomolybdic acids ($H_3PMo_{12}O_{40}$). It is reduced during the oxidation of phenols in a mixture of blue oxides of tungsten and molybdenum. The colour produced, whose absorption maximum is between 700 and 750 nm, is proportional to the amount of phenolic compound present in plant extracts.

In $AlCl_3$ colorimetric method, aluminum chloride forms acid stable complex with the keto and/or the hydroxyl groups in the A or C ring of flavonoids[27].

As reported in many publications, the genus *Polygonum* is rich in phenolic compounds such as flavonoids, phenolic acid[18-25]. As shown in this study, the PMCE contained high phenolic and flavonoid contents.

The DPPH is a stable organic free radical with an absorption maximum band around 515-528 nm and it is a useful reagent for evaluation of antioxidant activity of compounds.

The model for scavenging stable DPPH free radicals can be used to evaluate the antioxidative activities in a relatively short time. The absorbance decreases as a result of a colour change from purple to yellow as the radical is scavenged by antioxidants through donation of hydrogen to form the stable DPPH-H molecule[32].

The PMCE was presented a remarkable antioxidant activity compared to positive controls; the EC_{50} was also near for those of synthetic and natural antioxidant. These results can be explained by the presence, in the tested extract, of phenolic compounds such as: quercetin, catechin, resorcinol, acetophenone, *etc*[17].

Scavenging activity of hydrogen peroxide in PMCE at 100 μg/mL and BHA, α-tocopherol (100 μg/mL) as reference compound was different, we remarked that the extract exhibit a high activity twice more efficient than controls used, that result showed clearly the capacity of this plant to neutralize free radical since the reaction with phenolic

compounds present in this plant.

In this assay, since antioxidant compounds present in the extract are good electron donors, they may accelerate the conversion of H_2O_2 to H_2O[33].

H_2O_2 is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membrane rapidly. Once inside the cell, H_2O_2 can probably react with Fe2+ and possibly Cu2+ to form hydroxyl radical and this may be the origin of many of its toxic effects. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate[21].

Our findings on antibacterial activity of PMCE could justify some ethnopharmacological uses such as against diarrhea and dysentery (local investigations) because we demonstrated a remarkable activity of this plant against some pathogens of the digestive tract. We can suggest that high level in phenolic compounds contained in PMCE is the reason in which this extract had an antibacterial activity, phenolic antimicrobial activity is well documented[14,34].

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

The use of natural products becomes one of the important researches around the world. They exhibit, in the most cases, a good compound that used as drug, preservative agents, additives, ingredients in food , cosmetic and pharmaceutical products without hazardous to consumers. For this subject, researchers are interested to find new bioactive molecules from medicinal plants. The production of secondary metabolites depends on many factors such as climate, cultivation, localization and others. This plant (*P. maritimum*) represents an endemic plant harvested from algerian coast, that gives to this research some power of its originality; and we can consider this research as new and important.

Research frontiers

This research focuses on the evaluation of the antioxidant (by DPPH and H_2O_2 scavenging assays) and the antibacterial activities (for both Gram + and - bacteria) of crude extract from aerial part of *P. maritimum* L. (Polygonaceae).

Related reports

Kazantzoglou *et al*. have isolated and identified some compounds from the dichloromethane and methanol extracts of *P. maritimum*. However, as far as I know, there is no published study on the antibacterial and antioxidant activities of this endemic plant.

Innovations and breakthroughs

The requirements of food, pharmaceutical and cosmetic industries, have led researchers to find new bioactive molecules to open a wide range of using and replacing these toxic agents in the market. This type of research is a part of preliminary studies for this purpose. The innovation in this paper is: the originality of this plant used (species and region); and the specific activities of its phenolic compounds.

Applications

We can apply this plant in many area as active agents or supplementing in food, pharmaceutical and cosmetic industries.

Peer review

This work represents a moderate study but with the highest importance in the first part of antioxidant activity. These researchers have found compounds that exhibit a very interesting antioxidant activity of an original plant. In the second, this study has been enhanced by the evaluation of the antibacterial activity against nine bacteria species. In all the cases, the originality of this plant gave us a great satisfaction on the importance of this study in a number of biological, chemical and medicine fields.

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