



RESEARCH ARTICLE

TOTAL PHENOLIC CONTENTS, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF
LIMONIASTRUM GUYONIANUM (PLOMBAGINACEAE) EXTRACTS

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ARTICLE INFO

Article History:

Received 10th September, 2016
Received in revised form
24th October, 2016
Accepted 16th November, 2016
Published online 30th December, 2016

Key words:

Limoniastrum guyonianum,
Antioxidant activity,
Free radicals,
Antibacterial, Polyphenols
and Flavonoids.

ABSTRACT

This work was carried out to study the antioxidant capacity and antibacterial activity of *Limoniastrum guyonianum* (Plombaginaceae) extracts, traditionally used in folk medicine in Algeria. The aerial part were submitted to extraction by water and methanol to give methanolic (Met.E) and aqueous (Aq.E) extracts with that yields are 10% and 17,5% respectively. Quantitative analysis of polyphenols and flavonoids content showed that the Met. E had the highest amount (285.37µg EAG/mgE and 44.11µg EQ/mg E, respectively). The antioxidant activity of extracts was evaluated by 2,2'-diphenyl-1-picrylhydrazyl (DPPH) and ABTS free radical-scavenging test. Results showed that the Met. E exhibited the highest antiradical activity against DPPH and ABTS free radical with EC₅₀=0,099 mg/ml, and EC₅₀=0.024mg/ml, respectively. Moreover, the antibacterial activity was determined on the five bacterial strains, using disk diffusion method. The Met. E showed inhibition zone averages only on *M. luteus*, and *S. aureus* (12 and 13 mm respectively) and with MIC is 25 µg/ml. Our results showed that the methanolic extract of *L. guyonianum* reported a considerable antioxidant and antibacterial activities which may be due to their richness on polyphenols and flavonoids.

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Citation: Abderrahim BENSLAMA, Safia AFISSA, Asma SAIDI and Abdenassar HARRAR, 2016. "Total phenolic contents, antioxidant and antibacterial activities of *Limoniastrum guyonianum* (Plombaginaceae) extracts", *International Journal of Current Research*, 8, (12), 42675-42679.

INTRODUCTION

Herbal medicine represents one of the most important fields of traditional medicine all over the world and the uses of herbal remedies for various medical conditions have been popularly growing. There is increasing trend in correlating phytochemical constituents of plants with its pharmacological activities. Leaves, flowers, stems, roots, seeds, fruit and bark can all be constituents of herbal medicines. The medicinal values of these plants lie in their phytochemical components which produce definite physiological actions on the human body. While 25 to 50% of current pharmaceuticals are derived from plants, none are used as antimicrobials. Traditional healers have long used plants to prevent or cure infectious condition (Cowan, 1999). The most important of these components are alkaloids, tannins, flavonoid and phenolic compounds. Phytochemicals are extensively found at different levels in various medicinal plants and used in herbal medicine to treat diverse ailments such as cough, malaria, wounds, toothache and rheumatism diseases (Winston, 1999; Exarchou et al., 2002; Giliani and Rahman, 2005). Oxidative stress

occurs when the balance between the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) amount of antioxidants is destroyed in living cell, which cause the damage to cell components such as proteins, lipids and nucleic acids and eventually leads to cell death. ROS such as hydrogen peroxide and hypochlorous acid and free radicals such as hydroxyl radical and superoxide anion are produced as normal products of cellular metabolism. Rapid production of free radicals can lead to oxidative damage to biomolecules and may cause disorders and pathophysiology such as cancer, diabetes, inflammatory disease, asthma, cardiovascular diseases, neurodegenerative diseases, and premature aging (Halliwell, 1991 and Afanas'ev, 2010). Phenolics or polyphenols are secondary plant metabolites that are ubiquitously present in plants and plant products; they have several biological and pharmacological effects such as anti-inflammatory, antispasmodic, antiallergic, antidiabetic, antimicrobial and antiviral remedies. In addition, many of the phenolics have been shown to contain high levels of antioxidant and antimicrobial activities, that are mostly ascribed to their antioxidant capacity, free radical-scavenging power and chelation of redox-active metal ions (Robak and Gryglewski, 1988; Morel et al., 1993; Hanasaki et al., 1994). The main factor responsible for the delayed research on polyphenols is the variety and the complexity to be due to their chemical structure (Paganga and Rice-Evans, 1997). The aim of

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this consisted to valorization of medicinal and aromatic plants of the Algeria flora, in order to evaluate the antioxidant capacity and antibacterial effect of *Limoniastrum guyonianum*, Algerian Sahara plants used in Algerian traditional medicine, which has been used traditionally in folk medicine of Algeria for treatment of various diseases.

MATERIALS

L. guyonianum (Plombaginaceae) was widely distributed in the south of Algeria. Locally named "El-zaitta", which have been used traditionally in Algerian folk medicine. The plant materials were collected in November 2015, from Biskra (south of Algeria), authenticated by Dr. SalemkourNoura, the center of scientific and technical research on arid regions (C.R.S.T.R.A), Biskra, Algeria. The aerial part of plant is dry at room temperature in the dark then crushed up in stock until use. 2,2-azino-bis(3-ethyl-benzothiazoline-6-sulphonate (ABTS), 2,2-diphenyl-2-picryl-hydrazil (DPPH), aluminum chloride (AlCl_3), sodium carbonate (Na_2CO_3), ascorbic acid, Folin-Ciocalteu reagent, gallic acid, quercetin, rutin and 2, 6 di-tert-butyl-4-methyl phenol (BHT). these products were purchased from Sigma Chemicals, Sigma-Aldrich and Fluka (Germany).

METHODS

Preparation of extract

The extractions were carried from the aerial part plants by methanol and distilled water according to the method of (Motamed and Naghibi, 2010). The extraction of powdered plant material was carried out by maceration process for 24 h. The powdered plant material (50 g) was soaked in 500 ml of methanol or distilled water. The extract was filtered using a Buchner funnel and Whatman No. 1 filter paper to obtain aqueous extract. The same procedure to obtain the methanolic extract. The extracts were stored at room temperature until use.

Determination of total phenolic and flavonoids contents

The total phenolic contents of the extracts were measured using the Folin-Ciocalteu colorimetric method described by Li and their collaborators (2007), with gallic acid as standard. Basically, 0.2 ml of extract was mixed with 1 ml of Folin-Ciocalteu's phenol reagent (10- fold diluted). After 4 min, 0.8 ml of saturated sodium carbonate Na_2CO_3 (7.5%) solution was added and the mixture was allowed to stand for 2 h. Absorbance was measured at 765 nm. The amount of total polyphenols in different extracts were expressed as μg of gallic acid equivalent (GAE)/ mg extract. Flavonoids were quantified using aluminum chloride reagent (AlCl_3) method described by (Baharun et al., 2003). The flavonoids content was expressed as quercetin equivalents (QE). Briefly, 1 ml of extract, dissolved in corresponding solvent was added to 1 ml of AlCl_3 (2% in methanol). The absorbance was measured at 430 nm, after incubation at room temperature for 10 min.

DPPH radical scavenging assay

The free radical scavenging activity of extracts was evaluated by 2,2-diphenyl-2-picryl-hydrazil (DPPH) scavenging assay (Cheelet et al., 2005). Briefly, 50 μl of solution at different doses containing the compound to be tested were added to 1250 μl of solution of DPPH (2.4 mg in 100 ml methanol). Absorbance

was measured at 517 nm that was determined after allowed to stand at room temperature for 30 min. The absorbance of the control and samples was measured, and the DPPH scavenging activity in percentage was determined was calculated according to the following formula: Scavenging effect% = $[(A_c - A_s) / A_c] \times 100$

Where A_c : control absorbance and A_s : absorbance in presence of sample (extract). The data are presented as mean of triplicate and the concentration required for a 50% (EC_{50}) reduction of DPPH radical was determined graphically.

ABTS radical scavenging assay

The ABTS radical scavenging activity of extracts was carried according (Huang et al., 2011). For the development of ABTS radicals potassium persulfate (2.45 mM) solution was mixed with ABTS (7 mM) and incubated overnight in the dark to get a dark colored solution. The standard solution of ABTS was diluted by the addition of methanol to have an absorbance of 0.70 (± 0.02) at 734 nm. An aliquot of 100 μl of extract or fraction was mixed with 1.9 ml of ABTS and absorbance was recorded after 30 minute. Ascorbic acid, BHT and quercetin used as standards. Total antioxidant capacity was calculated according to the following formula: Scavenging effect % = $[(A_c - A_s) / A_c] \times 100$

Where A_c : control absorbance and A_s : absorbance in presence of sample (extract). The data are presented as mean of triplicate and the concentration required for a 50% (EC_{50}) reduction of ABTS radical was determined graphically.

Antibacterial activity

The antibacterial activity of extracts was performed by disk diffusion method (Balouiriet et al., 2016). The selected bacterial strains included *Staphylococcus aureus* ATCC 25923, *Micrococcus luteus* NRRL B-4375, *Bacillus subtilis* NRRL NRS-744, *Pseudomonas aeruginosa* ATCC27853, *Klebsiella pneumonia* NRRL B-4420. A 100 μl of each bacterial suspension was spread on a Mueller-Hinton agar plate. Sterile paper disk (5 mm diameter) were impregnated with 20 μl of each extract dissolved in DMSO at 200 mg/ml. After incubation at 37 °C for 24 h, the zone of inhibition surrounding the disk was measured. Additionally, and for comparative purposes, standard ampicillin were included in the test as positive controls. For data analysis, the antimicrobial activity was expressed as inhibition diameter zones in millimeters (mm). To compare the sensitivity of the bacterial strains to the extracts, the extracts that exhibited antibacterial activity in disk diffusion assay were submitted the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) test according to method described by Gulluce et al. (2003). The inoculums of the bacterial strains were prepared from 18-24 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. Each extract sample that was dissolved in DMSO to initial concentration of extract of 1000 $\mu\text{g}/\text{ml}$. Then, a six-fold serial dilution was made in order to obtain concentration ranges of 15.62-1000 $\mu\text{g}/\text{ml}$. The 96-well plates were prepared by dispensing into each well 400 μl of nutrient broth and 50 μl of the inoculum. A 50 μl from each extract dilution was added into the wells. The last well containing 1400 μl of nutrient broth, 50 μl of the inoculum and 50 μl of DMSO on each strip was used as the negative control. The plates were incubated at 37 °C for 18-24

h. The MIC was defined as the lowest concentration of the compounds to inhibit the growth of the microorganisms. The MBC values were interpreted as the highest dilution (lowest concentration) of the sample, which showed clear fluid with no development of turbidity and without visible growth.

Statistical analysis

Experimental results were expressed as mean±SD of triplicate. The data were analysed by Student's *t*-test to determine statistical significance. *p*-values and *p*<0.05 was considered as indicative of significance. The EC₅₀ values was calculated from linear regression analysis. All statistical analysis and graphing of data were performed using Graph pad prism 6 software.

RESULTS AND DISCUSSION

Extraction and Total phenolic and flavonoid contents

Extract was dried with rapport of 1/10 (w/v) under reduced pressure giving different yields (Table 1). The total phenolic and flavonoids contents in the extracts was determined using spectrophotometric method using Folin-Ciocalteu and aluminum chloride methods, respectively. The content of total phenolic was expressed in terms of µg GAE/ mg E, when the content of flavonoids was expressed in terms of µg QE/ mg E. The results showed that the highest amounts of phenolic compounds and flavonoids recorded in Met.E (Table 1). The total phenolic contents in plant extracts depend on the type of extract, i.e. the polarity of solvent used in extraction. High solubility of phenols in polar solvents provides high concentration of these compounds in the extracts obtained using polar solvents for the extraction. The concentration of flavonoids in plant extracts depends on the polarity of solvents used in the extract preparation (Zhou and Yu, 2004, Do *et al.*, 2014).

Table 1. Total polyphenol and flavonoids contents of extracts

Extract	Yield (%)	Total phenolic contents (µg GAE/mg E)	Flavonoids (contents µg QE/mg E)
Met.E	10	285.38	44.11
Aq.E	17.5	52.63	12.61

The results of Meddour *et al.* (2013) have shown that the polyphenol contents of the methanol extract that is greater than the aqueous extract although the study is carried out on the *Capparis spinosa* L. Therefore, many studies demonstrated that polar solvents give higher yields than apolar solvents, since the polar solvents have the ability to spread within the plant powder, reaching the vegetable matrix and therefore recover the possible metabolites. While non-polar solvents which are immiscible with water, does not have the ability to extract the maximum amount of bioactive molecules because of the presence of the water contained in the plant tissue (Ghedadba *et al.*, 2014).

DPPH radical scavenging assay

DPPH radicals are widely used in the model system to investigate the scavenging activities of several natural compounds. When DPPH radical is scavenged, the color of the reaction mixture changed from purple to yellow with decreasing of absorbance at wavelength 517 nm. The antioxidant activity of different extracts was determined using

DPPH radical scavenging test then antiradical activity was expressed by effective concentration (EC₅₀). DPPH radical scavenging activity of the extracts is shown in Fig. 1. The results showed that the Met. E exhibited the highest scavenging activity against DPPH radical (EC₅₀=0.099±0.0011 mg/ml) more than aqueous extract (EC₅₀=0.34±0.0023 mg/ml). In fact, the scavenger effects of extracts were not very far from used standards BHT, quercetin, rutin and gallic acid. The result of (Villano *et al.*, 2007) improved that that the efficiency scavenging activity could be linked not only on the polyphenols and flavonoids contents of the extract, but also to the nature structure of these compounds.

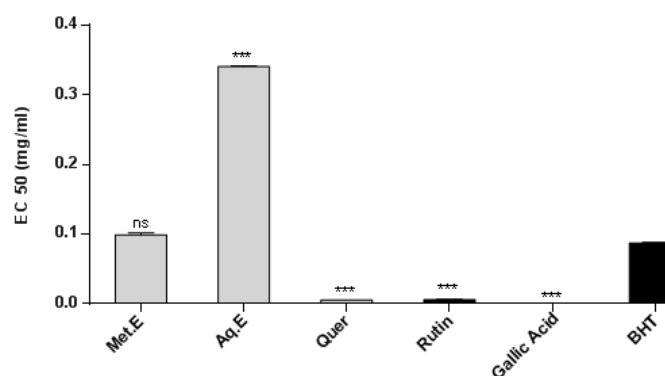


Fig. 1. EC₅₀ values in free radical scavenging activity of the extracts against DPPH free radical. Values were means ± SD of triplicate (ns: *p* > 0.05, *:*p* ≤ 0.05, **: *p* ≤ 0.01, *: *p* ≤ 0.001)**

ABTS radical scavenging

ABTS radical scavenging ability of different extract was determined by the method of ABTS radical cation decolorization assay described by (Re *et al.*, 1999). The extract tested were able to scavenge the ABTS radical cation with different EC₅₀ values (Fig. 2). The Met. E of *L. guyaniana* exhibited the highest scavenging potential when reacted with ABTS radicals as compared to the Aq.E. The best ABTS⁺ scavenging activity were shown by the methanolic extract (EC₅₀=0.024±0.001mg/ml). The activity Met. E can be explained by their higher contents of total phenols. The antioxidant capacity of samples follows the same order as that of the levels of polyphenols which make a height correlation between the ABTS⁺ scavenging activity of extracts and their polyphenols contents. In fact, polyphenols compounds undoubtedly play an important role in free radical scavenging ability and could be considered the most effective antioxidants (Craft *et al.*, 2012). Radical scavenging activities are very important due to the deleterious role of free radicals in foods and in biological systems. Diverse methods are currently used to assess the antioxidant activity of plant phenolic compounds. Chemical assays are based on the ability to scavenge synthetic free radicals, using a variety of radical-generating systems and methods (Huang *et al.*, 2005). Several phenolic compounds such as resveratrol, catechin, caffeic acid and chlorogenic acid all possess several readily oxidizable O₂⁻ substitutions and can act as potent antioxidants reducing ROS to less reactive molecules (Hu *et al.*, 1995). Robak and Gryglewski (1988) reported that flavonoids are effective antioxidants mainly because they scavenge superoxide anions. DPPH, hydroxyl radical scavenging activity, and superoxide anion radical scavenging activity have been used to measure antioxidant activity and these results should correlate with those of total phenolic and flavonoid content (Zheng and Wang, 2001).

Phenolic compounds are called antioxidants because of their ability to scavenge free radicals and active oxygen species such as singlet oxygen, superoxide free radicals and hydroxyl radicals. Furthermore, a positive and significant correlation existed between antioxidant activity and total phenolic content which revealed that phenolic compounds were the dominant antioxidant (Maksimović *et al.*, 2005).

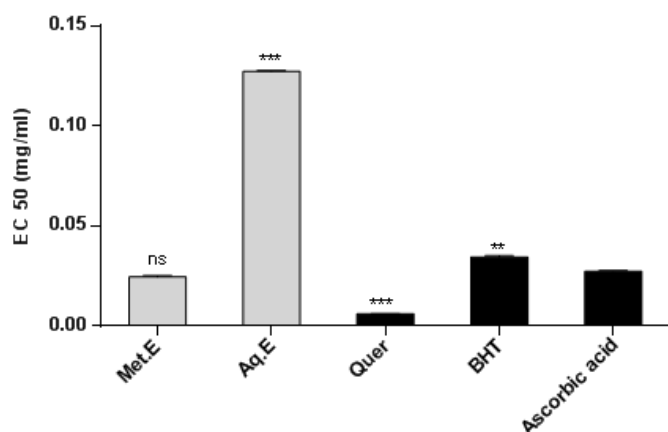


Fig.2. EC₅₀ values of scavenging activity of extracts on ABTS free radical. Values were expressed as means \pm SD of triplicate. (ns: $p > 0.05$; * $p \leq 0.05$; ***: $p \leq 0.001$)

Table 2. The antibacterial activity of *L.guyonianum* extracts

Bacterial strain	Inhibition zone (mm)			Sensitivity to Met. E	
	Met.E	Aq.E	Amp	MIC	MBC
<i>S. aureus</i> ATCC 25923	13	-	31	25 μ g/ml	-
<i>M. luteus</i> NRRL B-4375	12	-	45	-	-
<i>B. subtilis</i> NRRL NRS-744	-	-	15	-	-
<i>P. aeruginosa</i> ATCC27853	-	-	-	-	-
<i>K. pneumonia</i> NRRL B-4420	-	-	-	-	-

Antibacterial activity

The antibacterial activity of extracts was determined against a five bacterial strains using disk diffusion method. The activity obtained by measuring the inhibition zone diameters of growth of the bacteria tested. The methanolic extract of *L.guyonianum* showed antibacterial activity against *S. aureus* ATCC25923, *M. luteus* NRRL B-4375 with inhibition zones ranged from 12-23 mm (Table 2). However, The Aq. E did not present any antibacterial effect on all bacterial strain. In addition, the zones of inhibition of extracts were lower than the antibiotic ampicillin, which showed a very strong inhibition of bacterial growth exception in *B. subtilis* NRRL NRS-744.

Determination of MIC and MBC

The sensitivity of bacterial strains (*S. aureus*, *M. luteus*) to methanolic extract of *L. guyonianum* was carried and the MIC and the MBC were determined. The susceptibility of bacterial strains vary from strain to another depending on the type of extract. Similarly, the results differ depending on the concentrations of extract used (Moterya *et al.*, 2015). The Met.E showed a better inhibitory activity against *S. aureus* MIC= 25 μ g/ml but it hasn't any inhibitory activity against *M. luteus* NRRL B-4375. However; Met. E did not present any bactericidal activity on bacteria tested (Table 2). Several recent papers report the regular presence of antibacterial activity among flavonoids, this activity may involve complex mechanisms, like the inhibition of the synthesis of cell walls

and cell membranes, nucleic acids and proteins, as well as the inhibition of the metabolism of nucleic acids (Oyaizu *et al.*, 2003). Recent papers report the regular presence of antibacterial activity among flavonoids. Thus, the myricetin is active against *S. aureus* with a MIC of 6.25 μ g/ml. Also, gossypetin has an MIC of 50 μ g/ml towards *Staphylococcus epidermis* (Xu and Lee, 2003). Phenolic compounds have also been reported to be responsible for antimicrobial properties; Penna *et al.* (2001) isolated two antibacterial phenolic compounds, methylgallate (MIC=128 μ g/ml) and protocatechic acid (MIC= 128 μ g/ml) from *Sebastianiabrsiliensis*. Taking into consideration the properties of the organic solvent used for the extraction, the extract seems to contain diverse substances, ranging from nonpolar to polar compounds. Specify that the antibacterial effect can also be due to various chemical substances contained in the extract such as polyphenols, saponins, tannins, alkaloids and terpenoids (Parekh *et al.*, 2005).

Conclusion

In conclusion, in this study we consisted to valorization of medicinal and aromatic plants of the Algeria flora, in order to evaluate the antioxidant capacity and antibacterial. The results has demonstrated that the methanolic have a high antioxidant and antibacterial activities may be due to high phenolic contents in this extract. In view of the potential use of methanolic extract of *L. guyonianum* in therapeutic benefits and bioactive compounds warrant for further investigations. These results show that methanolic extract of this plant could be considered as a natural alternative source for food, pharmacology and medicine sectors.

Acknowledgments

We would like to thank by Dr. Salemkour Noura, the center of scientific and technical research on arid regions (C.R.S.T.R.A), Biskra, Algeria, for the identification of the plant material.

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