Ethnopharmacology, Antibacterial and Antioxidant Activities, Phytochemical Screening of Bioactive Extracts From the Aerial Parts of Fagonia *Longispina*

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ABSTRACT

Fagonia Longispina (Zygophyllaceae) is a plant traditionally used in folk medicine, mainly as a popular remedy for the treatment of various skin lesions. Additionally, the aerial plant is claimed to be a remedy for cancer in its early stages [1, 2] and for the treatment of various other digestive diseases in the southeast of Algeria (saoura region of Bechar) Northern Africa. [3-4]. Aerial parts of this plant were screened for the principal classes of secondary metabolites, such as anthraquinones, terpenes, saponins, alkaloids, coumarins, flavonoids and tannins.

The tested extracts of aerial part of Fagonia longispina extracts exhibited antimicrobial activity, which may support some uses of the plant in traditional medicine. In this study, we demonstrate that the antioxidant activity of the aerial parts of fagonia longispina have the most efficient free radical scavenger by the lowest IC_{50} value.

Keywords: Fagonia longispina, bioactive extract, antioxidant activity, phytochemical screening

INTRODUCTION

South Algeria with its rich floral resources and ethnobotanical history is an ideal place to screen plants for biological activity and as a source of new pharmacological compounds. *Fagonia longispina* (family *Zygophyllaceae*) is a small spiny shrub widely distributed in the south west of Algeria and south east of morocco[1-5]. Plants belonging to the genus Fagonia are often used in folk medicine, mainly as a popular remedy for the treatment of various skin lesions. Additionally, the aerial plant is claimed to be a remedy for cancer in its early stages and for the treatment of various other diseases of digestive and blood vascular system. The medicinal properties of the plant were attributed due to its variety of active phytochemical constituents. Although the plant have received a great interest for the phytochemical investigation since many years. The entire plants of various *Fagonia* species were investigated mainly for the presence of major types of phytochemical compounds. Chemical constituents in the extracts of *fagonia Longispina.* foliage were identified by gas chromatography-mass spectrometry and their relative concentrations are determined[1].

PLANT MATERIALS AND EXTRACTION

Aerial parts of Fagonia longispina were collected in March 2010 from boukais (region of Bechar) Algeria and identified by the National Agency of Nature Protection (ANN), Bechar, Algeria.

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The aerial parts of Fagonia lonjispina were individually ground to a fine powder. Extraction using soxhlet apparatus; reflux for 6 hours was performed. The residue was evaporated in vacuo apparatus, and a phytochemical screening for the detection of the main phytoconstituents was carried out [6, 7].

BOTANICAL DESCRIPTION

Fagonia longispina is a tree which belongs to Zygophyllaceae family; it reaches between 10 to 20 cm in height. It is a Low plant whose branches lying on the ground radiate starting from the center. All the plant furnished with sparse hairs which bind sand. Plant provided long stipulate fine, the leaves with three leaflets are not very thick, the median leaflet being broader than the two side ones. The petiole is rather long, especially on the first leaves. Purplished flowers of sharp color. fagonia longispina is common plant known under the vernacular name "Atlihia" and used as a common herbal drug in the south-western Algeria[4].

Figure 1. General morphology of fagonia longispina

STUDY AREA

The district of Bechar (Saoura region of Bechar) is an area of 162400 km^2, it's situated between the high trays and the big Sahara, in the South East of Algeria Northern Africa. It's 1100 km far from the capital Algiers.

Climate is of Sahara continental, its whole dry, cold season starts from December to February and lasts May, June, July and August constitute a hot season it can reach the 45 ºC [8, 9].

POPULATION

The majority of the population consists of four important origins: dewimenâ, ouled djerir, cheraga and ksouri. A total population is 178721(March 2012). The language of the inhabitants is Arabic. The people's main source of living in this region is farming [1, 8, 9].

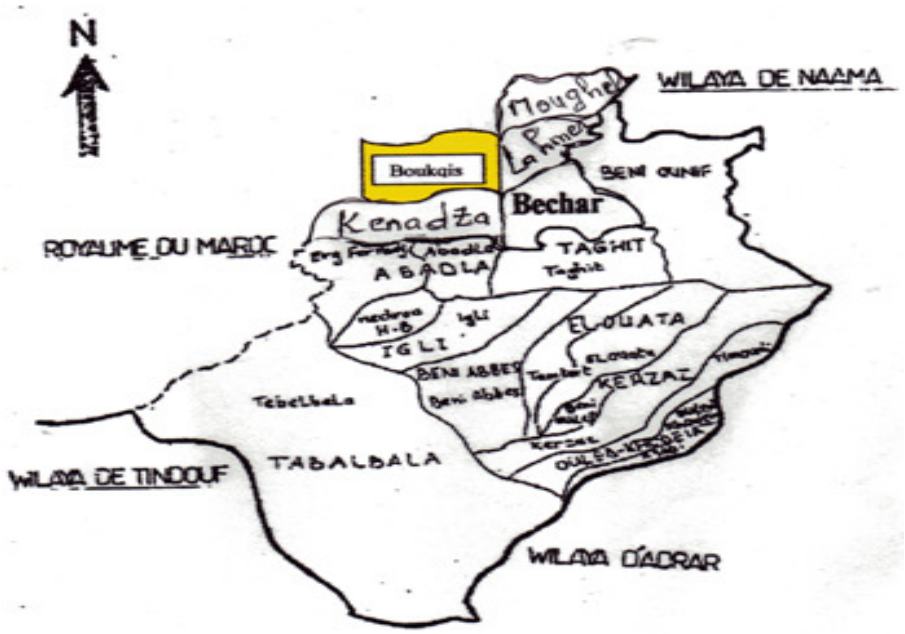

Figure 2. The location of Bechar district, Algeria

ETHNOPHARMACOLOGY SCREENING AND METHODOLOGY

The Fagonia longispina is a bare desert plant .The nomads have used it as a food for their livestock especially when it is green[1, 8, 9]. The fagonia has popularity in folk medicine for the treatment of internal and external disease. In the framework of research on medicinal plants in the South of Algeria and through the results obtained, we achieved an ethnopharmacological survey which showed that this plant is not known by its scientific names but by its vernacular ones, which are "chouika" or "Atlihia". From the study and the investigations conducted for three months in Bechar region, which included many people especially the older ones, as well as people working in the field of forestry and sellers of medicinal herbs. Through this research, we noticed that this plant is used as anti-inflammatory especially for the treatment of arthritis and urinary tract inflammation (as diuretic).

The plant is also used by nomads as an insect repellent and its extract as anthelmintic. We noted also that the plant is largely used for cancer prevention, especially in its early stages of the disease, added to other plants.

These observations were proved by another scientific research in which we confirmed the existence of bioactive chemicals with antimicrobial, antioxidant, anticancer activities in this species[1, 21].

RESULTS AND DISCUSSION

The fagonia is used as a preventive syrup for cancer (90%) and for the treatment of inflammation of the urinary tract(40%)and it is useful in the treatment of the diseases of the cold, including rheumatoid and arthritis (45%) and as insect repellents (35%) and its extract is used as an anthelmintic (35%) . The plant is used to get rid of the effects of sensitivity (30%) The ways of preparing medicines may differ from one disease to another. We can also have multiple popular recipes for the same disease, especially the quantity needed by treatment. Often in traditional medicine the water is used as solvent .

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The patient may take two glasses a day, morning and night. Statistical information on the plant medicinal value authenticity used by the patients and elderly People are shown in the following histogram (figure 3).

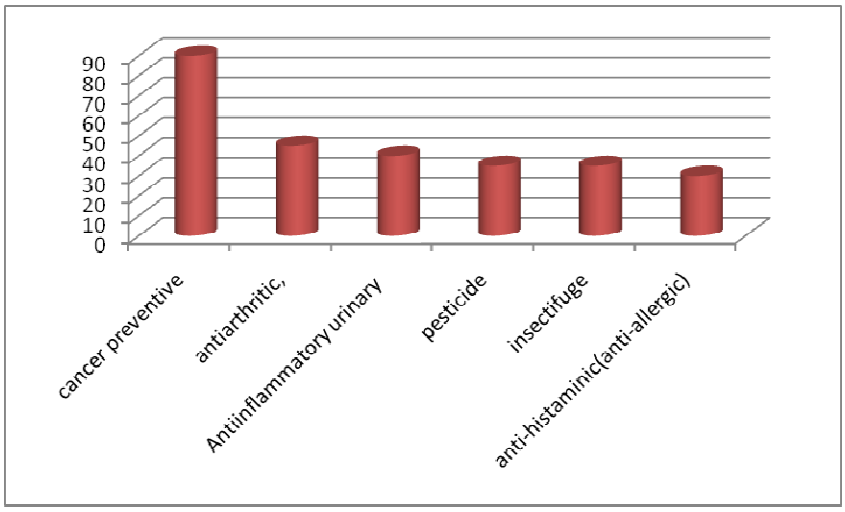

Figure 3. Statistical information of plant ethnopharmacology uses

BIOLOGICAL SCREENING

Plant Materials and Extraction

The aerial parts were separated and oven dried (overnight), the plants were grounded into powder from using the grinder. Extraction using soxhlet apparatus, reflux with H2O, MeOH, Acetone, chloform, Petroleum ether and distillation for 6 h were performed and gave12 % extraction yield[10,11] .

Microorganisms and Medium

The microorganisms used in this present study were: bacteria (Enterococcus faecalis, Bacillus spizigenil, Salmonella heindelberg, Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli).

Antimicrobial Sensitivity Tests

Sterile 6.0 mm diameter blank disc were used to impregnate of two dilutions of the extracts (Heptane, MeOH, Acetone, Chloroform, Petroleum ether and Dichloromethane). Discs were stored at –5°C prior to use. Tests were performed by the disc diffusion method. Extract impregnated discs were placed on agar and incubated either at 37°C for 24 - 48 h for bacteria or 25°C for 24 h. the antibacterial effect is then measured by the clear zones of inhibition.[12-14]

RESULTS AND DISCUSSION

With the only exception of the heptan extract, the tested extracts of aerial part of Fagonia longispina extracts exhibited antimicrobial activity, which may support some uses of the plant in traditional medicine. The results for the antimicrobial activity test of the extracts (heptan, MeOH, Acetone, Chloform, Petroleum ether and Dichloromethan) of Fagonia longispina are shown in table 1.

Table1. Antibacterial Activity of the aerial parts of Fagonia Longispina

Microorganism	*Heptan*	*Petroleum ether*	*CH_2Cl_2*	*$CHCl_3$*	*Acetone*	*MeOH*
Enterococcus faecalis ATCC 29212	n.d	7.5	7	11	11	9
Bacillus spizigenil ATCC6633	n.d	8	7.5	10	23	n.d
Salmonella heindelberg	n.d	7.5	8	13.5	11	8
Staphylococcus aureus ATCC6538	n.d	8	8	9	12	16
Klebsiella neumoniae	n.d	8.5	8.5	20	9	8
Escherichia coli ATCC25922	n.d	10	9	20	12	7.5

Phytochemical Screening

Chemical compounds that occur naturally in plants, are responsible for color and organoleptic properties. The term is generally used to refer to those chemicals that may have biological significance but are not established as essential nutrients. Scientists estimate that there may be as many different phytochemicals having the potential to affect diseases such as cancer, stroke or metabolic syndrome. Phytochemical tests are performed on different extracts prepared from the aerial parts of fagonia longispina using solvents of different polarities [8, 15-20].

Test of Flavonoids

Treat 5 ml of alcoholic extract with a few drops of concentrated HCl and 0.5 g of magnesium turnings.

Test of Tannins

In a test tube, 1ml of ethanolic solution was added to 2 ml of water and 2-3 drops of diluted solution of FeCl3and observed for a green, a blue black or a blue - green coloration, which shows the presence of tannins.

Test for Steroids

After evaporation of 10ml of the ethanolic solution, the residue was taken in 10ml of $CHCl_3$, filtrates and added to 5ml of acetic anhydride and some drops of H_2SO_4.

The mixture was agitated. The color changed from violet to green indicating the presence of steroids.

Test for Terpenoids

Amixture of 0.5ml of acetic anhydride and 0.5ml of $CHCl_3$ was added to the residue obtained after evaporation of ethanolic solution (10 ml). The filtrate was treated with Liebermann's reagent Burchardt. If a solution is violet- green appears, it indicates the presence of terpenoids.

Test of Coumarins

15ml of HCl (10%) was added to 25ml of ethanolic solution, and heated under reflux for 30 min and strain the mixture. The residue was extracted with 15 ml of etherin triplicate. Divide the filtrate into three equal parts, evaporate the first in a rotary evaporator, dissolve the residue in 1ml of water and divide the volume into two parts, treat the first with 0.5ml

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NH4OH (10%), examined under ultra-violet light, fluorescence intensity indicates the presence of coumarins. The second one was used as control.

Test of Anthracenosides

8 ml of the ethereal solution was treated by extractive reagent Bornträger. A positive test is revealed by the appearance of a color ranging from bright orange - red to purple.

Test of Anthocyanosides

The acidic aqueous solution was treated with NaOH. The presence of anthocyanins was confirmed by a red color at pH under 3 and blue color at a pH between 4 and 6.

Test of Alkaloids

The resulting residue obtained was dissolved after evaporation of 10 ml of the ethereal solution in 1.5 ml of HCl 2 % and add 1-2 drops of Mayer or Wagner reagent. The appearance of yellowish white precipitate indicates the presence of alkaloid.

Test of Saponins

2 ml of the aqueous solution was added to a little of water and then stir in a strong way. Persistent foam confirmed the presence of saponins.

Abandon the mixture for 20 minutes and classify content saponins:

1- No foam = Negative test.2- Foam less than 1 cm = weakly positive test.3- Moss 1-2 cm = positive test.4- Foam over 2 cm = very positive test.

Table 2. Results of phytochemical screening of aerial part of Fagonia Longispina

Natural Products	*Aerial part of Fagonia Longispina*
Alcaloïds	+
Saponins	+
Terpenoids	+
Tanins	+
Flavonoïds	+
Coumarins	+
Anthocyanosides	-
Steroids	+
Anthracenosides	-

'+' = Present; '-' = Absent

Qualitative phytochemical screening of plant aerial parts revealed the presence of steriods, coumarins, flavonoids, Saponins and Tanins. However, anthocyanosides and anthracenosides were absent.

Preliminary Phytochemical Screening of Various Extracts of the Aerial Part of Fagonia Longispina

Phytochemical screening was carried out by using procedure by Kokate. All the extracts were concentrated by distilling the solvent and the extracts were dried under reduced pressure. The extracts obtained from successive solvent extraction were then subjected to

various qualitative chemical tests to determine the presence of various phytoconstituents like Alkaloids, Saponins, Steroids, Coumarins, Flavonoids, Terpenoids and Tanins (Table 3).

Table 3. Results of phytochemical screening of various extracts of the aerial part of Fagonia longispina

Natural Products	*Extracts From Aerial Part of Fagonia Longispina*						
	AQ	*Petroleum ether*	*CH2CL2*	*CHCL3*	*Acetone*	*heptan*	*MeOH*
Alcaloids	+	+	+	+	+	-	+
Saponins	+	-	-	+	+	-	+
Seteroids	+	+	+	+	+	+	+
Tanins	+	+	+	+	+	-	+
terpenoids	-	+	+	+	+	+	+
Cardenolids	-	-	-	+	+	-	+
Flavonoids	+	+	+	+	+	+	+

+ : existence; - : not detected

Antioxidant Activity

Preparation of the Extract

The dried powder (100g) of the aerial parts of *Fagonia lonjispina* was extracted exhaustively for 6 hours with 60% EtOH that became (6.5g) after being evaporated[1].

DPPH Radical Scavenging Test

The free radical scavenging activity of the ethanolic extract of *fagonia longispina* was determined by using 2, 2 Diphenyl-1-picryl hydrazyl radical (DPPH) using UV-Spectrometry at 517nm.

The DPPH solution was prepared in 95% methanol. The stock solution of the extract was also prepared in 95% methanol (2mg/2ml), which can be prepared by dissolving 2 mg of plant residue in 2 ml of methanol. Preparation of the crude extract concentrations: From the stock solution, different concentrations of the extract were prepared (0.5, 0.27, 0.125, 0.0625, 0.0312, and 0.0150). 2ml of DPPH was added to each dilution. The reading of absorbance was done after 30 min at 517nm using methanol as white. To prepare the positive control; 4 mg of ascorbic acid is dissolved in 4 ml of methanol. From the stock solution, different concentrations of the extract were prepared (0.5, 0.27, 0.135, 0.0625, 0.0312, and 0.0150). Ascorbic acid was used as standard, 95% methanol was used as blank. All the tests were performed in triplicate to avoid test error. % scavenging of the DPPH free radical was measured using following equation[22-26].

% of DPPH radical scavenging =(Absorbance of control – Absorbance of test Sample) x 100(Absorbance of control)

After the measurement and calculation of %DPPH the results are recorded in the following charts (figure 3, figure 4).

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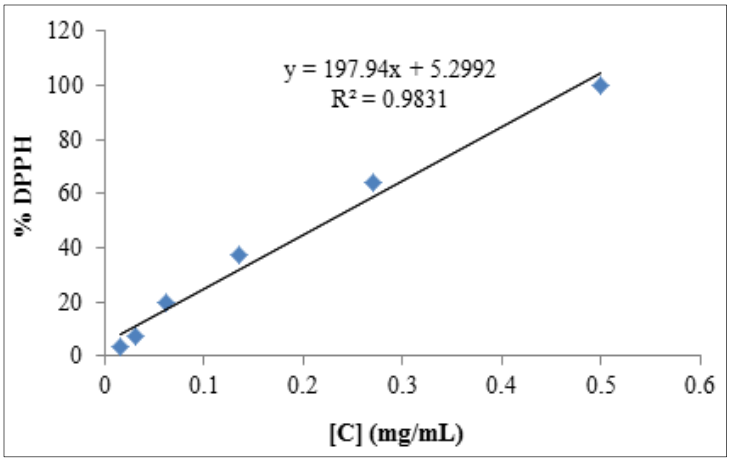

Figure 4. DPPH radicals scavenging activity of Ethanolic extracts of the aerial parts of Fagonia longispina

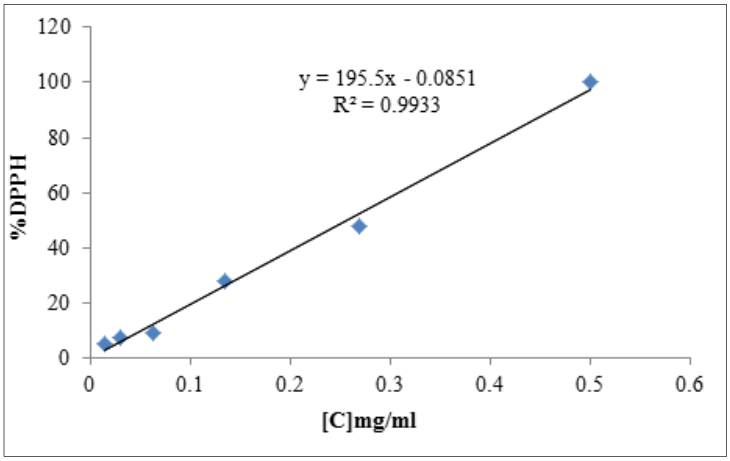

Figure 5. DPPH radicals scavenging activity of Ascorbic acid extracts of the aerial parsts of *Fagonia longispina*

The IC50 values determined in mg / ml expressing effective concentration of the antioxidant extract necessary for trapping and the 50 mole% of DPPH dissolved in methanol (Table 4).

Table 4. Antioxidant test result of expressing the 50% effective concentration in mg / ml

EEEextract /Standart	*% of DPPH radical scavenging (IC50)*
Ethanolic	0.22±0.0075
Ascorbic acid	024±0.0085

RESULT AND DISCUSSION

The DPPH test showed the ability of the test compound to act as a free radical scavenger. DPPH assay method is based on the ability of 1, 1-diphenyl-2-picrylhydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants. DPPH, a protonated radical, has characteristic absorbance maximal at 517 nm, which decreases with the scavenging of the proton radical. This property has been widely used to evaluate the free radical scavenging

effect of natural antioxidants. When DPPH radical is scavenged, the color of the reaction mixture changes from purple to yellow with decreasing of absorbance at wavelength 517nm. In this analysis, the scavenging activity of ethanolic extract was similar to that of Ascorbic acid. The DPPH radical scavenging activity of ascorbic acid and Ethanolic extract, increased in a dose-dependent manner. At a concentration of ethanolic extract from the aerial parts of fagonia *longispina* and standard Ascorbic acid showed 0.22mg/ml and 0.24 mg/ml antioxidant activity respectively by DPPH radicals scavenging assay.

In this study, we demonstrate that the antioxidant activity of the aerial parts of *fagonia longispina* have the most efficient free radical scavenger by the lowest Ic50 value.

The DPPH scavenging capacity of the ethanolic extract (IC50= 0.22±0.0075 mg/ml) is compared with the known antioxidant substances such as ascorbic acid (IC50= 0.24±0.0.0081 mg/ml) that has been shown to possess DPPH radical scavenging convergent activity.

The observation of the convergent activity was proved by our former scientific research[1] through which we confirmed the existence of bioactive chemicals: Ethyl Palmitate, Phenol2,6-bis (1,1-dimethylethyl)-4-methyl, n-Hexadecaonoic acid and 9,12,15-Octadecatrienoic acid, (Z,Z,Z). In another research[21] it was discovered that these compounds had antimicrobial, antioxidant, anticancer activities. Consequently, our present study concludes that ethanolic extract of the aerial parts of *fagonia longispina* is good source for natural antioxidants.

CONCLUSION

Fagonia longipina is a plant traditionally used as a preventive for cancer and for the treatment of inflammation, prepared by decoction in water. .Qualitative phytochemical screening of plant aerial parts revealed the presence of steriods, terpenoids, coumarins, flavonoids, Saponins and Tanins. The plant under investigation can be a potential source of useful drugs. In addition the antimicrobial antioxidant activities study of the aerial part of the plant showed that it has a biological efficiency for different extracts.

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REFERENCES

[1] Hamidi et al. (2012). MS analysis of ethanol extract from the aerial parts of Fagonia Longispina (family Zygophyllaceae). *Asian Journal of Natural & Applied Sciences, 1*(2), 136-142.

[2] Chopra et al. (1956). *Glossary of Indian Medicinal Plants.* New Delhi: CSIR

[3] Quezel, P., & Sant, S. (1962). New flora of Algeria and southern desert regions tome. 2nd Edition @ Volumes, Paris: National Centre for Scientific Research.

[4] Ozenda, P. (1983). *Flore du Shara* (2nd ed.). Paris: CNRS.

[5] Chopra, R. N., Nayar, S. L., Chopra, I. C. (1956). *Glossary of Indian Medicinal Plants*. New Delhi: CSIR.

[6] Maire, R. (1960). Flora of North Africa: Morocco, Algeria, Tunisia, Tripolitaine, *Cyrenaique and Sahara. The Knight, Paris, 6*, p. 394

[7] Killian, C. (). *Anabasis aretioides Coss,et Moq, endémique du Sud Oranai.* Travail du laboratoite de biologie saharienne de la Faculté des Sciences d'Alger à Beni Ounif n°11. p 423-436.

[8] Praveen et al. (2010). Screening of antioxidant activity, total phenolics and GC-MS study of Vitex negundo. *African journal of Biochemistry, 4*(7), 191-195.

[9] Ziane et al. (2013). Ethnopharmacology and phytochemical screening of bioactive extracts of limoniastrum feei (plombagenaceae). *Asian Journal of Natural & Applied Sciences, 2*(1), 5-9.

[10] Djellouli et al. (2013). Ethnopharmacological and phytoPhytochemical screening of three plants (Asteraceae family) from the region of south west Algeria. *Asian Journal of Natural & Applied Sciences, 2*(2), 59-65.

[11] Cruickshank, R. (1968). *Medical microbiology: a guide to diagnostic and control of infection* (11th ed.). Edinburgh and London: E. & S. Livingston Ltd.

[12] Bauer AW, Kirby WMM, Sherries JC, Truck M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol., 45*(4), 493-496.

[13] Ashraf, M., Rachid, S., Bibi, S., and Anjum, R. (2000). *Pakistan Journal of Biological Sciences.*

[14] Rahman et al. (1999). *Mannual of Bioassay Tecniues for Natural Product Research.* Amsterdam: Harward Academic Press.

[15] Reichelt, J. L., & Borowitzka, M. A. (1984). Antimicrobial activity from marine algae: Result of a large screening programme. *Hydrobiol., 1*(116/117), 158-168.

[16] Ziane et al. (2012). Phytochemical screening of bioactive extracts of limoniastrum feei, International Congress on Aromatic and Medicinal Plants CIPAMSidi Bel-Abbes, Algeria.

[17] Harborne, J. B. (1984). *Phytochemical methods* (2nd ed.). London; New York: Chapman and Hall.

[18] Belmekki, N., Bendimerad, N., Seladji, M. (2012). Phytochemical constituents of some Algerian medicinal plants. *J. Nat. Prod. Plant Resour., 2*(5), 558-562.

[19] Cavé, 1. A. (1993*). Pharmacognosie, phytochimie, plantes médicinales.* 2ème Ed. Tec. et Doc. Ed. Lavoisier, Paris.

[20] Bruneton, J. (1999). *Pharmacognosie, phytochimie, plantes médicinales.*3èmeEd. Tec. & Doc. Eds. Lavoisier. Paris.

[21] Peter, Y. M., Georgi, Y. P. (1983). Furanoid diterpenes from Teucrium polium. *Phytochemistry, 22*(12), 2791-2793.

[22] Praveen Kumar1 et al., (2010). Screening of antioxidant activity, total phenolics and GC-MS study of Vitex negundo. *African Journal of Biochemistry Research, 4*(7), 191-195.

[23] Rahman, K. (2007). Studies on free radicals, antioxidants, and co-factors. *Clin Interv Aging. June; 2*(2), 219–236.

[24] Anitha et al. (2011). Evaluation of the Antimycotic Activity of Aqueous and Ethanolic Extracts of Aesculus hippocastanum–An In Vitro Study. *Int. J. Drug Dev. & Res. July-Sep2011, 3*(3), 335-338.

[25] Bielanski, T. E., Piotrowski, Z. H. (1999). Horse-chestnut seed extract for chronic venous insufficiency. *J.Fam. Pract., 48,* 171-172.

[26] Sharma. U. S., and Kumar, A. (2011). In vitroantioxidant activity of Rubus ellipticus fruits. *J Adv Pharm Technol Res., 2*(1), 47–50.

[27] Molyneux, P. (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *J. Sci. Technol., 26*(2), 211-219.

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