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DPPH Free Radical Scavenging Activity of Ethanolic Extracts of Twenty Two Medicinal Species from South Algeria (Laghouat Region)

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Research Article

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Abstract

Free radicals scavenging Activity, total phenolic and flavonoids contents of Twenty two ethanolic extracts, from the botanical families Asteraceae, Lamiaceae, Amaranthaceae, Chenopodiaceae, Cupressaceae, Ericaceae and Rhamnaceae, collected from the Laghouat region (Algeria Sahara) were investigated. The 2,2-diphenyl-1-picrylhydrazyl radical assay was used to determine the antioxidant activity of the plant extracts, while the Folin–Ciocalteu method was used to determine the total phenolic content and flavonoids using AlCl3 method. The antioxidant capacity expressed as IC50 values ranged from 20 µg/ml for *O. basilicum* to 650 ± 8.60 µg/ml for *A. iva*. The total phenolic content ranged from 2.72 to 87.11 mg/g of dry weight of extract, expressed as gallic acid equivalents. The total flavonoid concentrations varied from 1.48 to 12.59 mg/g, expressed as rutin equivalents. The results of this study showed that there is no significant correlation between antioxidant activity and phenolic content of the studied plant materials and phenolic content could not be a good indicator of antioxidant capacity.

Keywords: FTIR Spectroscopy; Irbesartan; Angiotensin Receptor Antagonists; Chemometric Methods; Drug Analysis.

Introduction

Medicinal plants are a very important natural resource whose valuation requires a perfect knowledge of the properties to develop. The medicinal activities of herbal depend on the presence of various bioactive agents belonging to different chemical classes [1]. In many African and Asian countries, herbal medicine continues to be widely used. All these figures show that people are turning back to traditional medicine and medicinal plants mainly [2]. In Algeria, the empirical use of plants continues to maintain a high popularity [3,4]. The Algerian people are sometimes preying of quackery ignorant and dangerous for patients. Many plants are known for their therapeutic properties, especially for their antiseptic, antibacterial and antioxidant effect, as Rosemary, Sage, Thyme, Chamomile, Eucalyptus, Grenadier, etc [5]. The evaluation of free radical scavenging activity of plant extracts have been extensively performed by DPPH

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(1,1-diphenyl-2-picrylhydrazyl) method [6]. DPPH is a purple colored radical that, at er being reduced by an antioxidant turns into a yellow product. The aim of this study was to gather information about medicinal plants used traditionally in the region of Laghouat and we determined the antioxidant activity, total phenol and flavonoid contents of selected medicinal plants.

Materials and Methods

Chemicals

Folin-Ciocalteu's phenol reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), ethyl alcohol, methanol, sodium carbonate, aluminium chloride, gallic acid, rutin, ascorbic acid and butylated hydroxy toluene (BHT) were purchased from Sigma Aldrich-Fluka (Germany).

Plant Samples

The medicinal plants were selected based on information regarding their traditional uses in Algerian folk medicine (Table 1). Asteraceae and Lamiaceae were the most representative families with 6 and 11 tested species respectively. Plant materials were collected between March and April 2016 in the area of the Algerian Saharan Atlas (Laghouat region). Collected plants parts were different following the species. Fresh plant samples were cleaned and air-dried in darkness at room temperature. Dried plant parts were then powdered and stored in the dark at a dry place until further use.

Family; Species	Local name	Traditional uses	References
Asteraceae			
Artemisia campestris	Dgouft	Anti diabetic and anti hypertensive	Boudjelal, et al. [7]
Anvillea radiata	Nougd	diabetes and Indigestion	Djellouli, et al. [8]
Cotula Cinerea	Gartoufa	Asthma, Cough and Allergy	Djellouli, et al. [8]
Anthemis arvensis	Babounj	antihelmintic and analgesic rules	Sarri, et al. [9]
Artemisia absinthum	Chehaîba	Antidiabetic and diuretic	Meddour, et al. [10]
Artemisia herba alba	Chih	Antidiabetic and eczema	Sarri, et al. [9]
Lamiaceae			
Teucrium polium	Jiaida	Antidiabetic and antidiarrhoea	Hamoudi, et al. [11]
Mentha pulegium	Feliou	Antihypertensive and antispasmodic	Derwich, et al. [12]
Lavandula officinalis	Khozama	Antispasmodic and diuretic	Barkat, et al. [13]
Thymus algeriensis	djertil	Diuretic, mucolytic and analgesic	Giweli, et al. [14]
Salvia officinalis	Siwak elnabi	antihydrotic and spasmolytic,	Pop, et al. [15]
Rosmarinus officinalis	Iklil eljabel	Hypotensive, anemia and liver disease	Touafek, et al. [16]
Mentha spicata	Na-naa	Carminative, Stomachic and Cooling	Znini, et al. [17]
Thymus capitatus	Zaatar	anthelmintic, antispasmodic and tonic	Akrout, et al. [18]
Ocimum basilicum	Hbaq	Hypertension	Sari, et al. [19]
Marrubium vulgare L.	Temeriouit	diarrhoea, fever, rheum, and headache	Sarri, et al. [9]
Ajuja iva	Chendgoura	Diabetes and hypertension	Moussaoui, et al. [20]
Amaranthaceae			
Haloxylon scoparium	Remth	Antidiabetic and Poultice for mould	Bakchiche, et al. [21]
Chenopodiaceae			
Atriplex halimus L	Gtaf	Hypertension, diabetes and diuretic,	Dehliz, et al. [22]

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Cupressaceae			
Juniperus phoenicea	Aaràar	Diarrhea, rheumatism and diabetes.	Elmhdwi, et al. [23]
Ericaceae			
Arbutus unedo	Lindj	Astrigent and diuretic	Bakchiche, et al. [21]
Rhamnaceae			
Zizyphus lotus	Sedra	Diabetes, Analgesic and antiseptic	Dehliz, et al. [22]

Table 1: List of plants used in the study with their traditional uses.

Extraction Procedure

One gram of each powdered plant material was soaked in 10 mL of ethanol at room temperature overnight. The solvents were decanted and residues macerated two more days with the same solvent. The pooled solvents were combined and filtered. The obtained extracts were used for antioxidant activity, total phenols and flavonoids determination.

Determination of total Phenolic Content

The total phenolic content is estimated using Folin-Ciocalteu's method [24], with some modifications. 200 µl of sample was dissolved in 1000 µl of Folin-Ciocalteu reagent (1/10 dilution). The solution were mixed and incubated at room temperature during 5 mn. Then, 800 µl of saturated sodium carbonate (7.5%) was added. The final mixture was mixed and then incubated for one hour in the dark at room temperature. The absorbance of all samples was measured at 760 nm and the results are expressed in milligrams of gallic acid equivalents per gram dried weight (mg GAE/g DW).

Determination of total Flavonoid Content

Total flavonoid contents were determined by the method of Ahn MR, et al. [25]. Each sample (1.5 ml) was added to1.5 ml of aluminum chloride (AlCl3) solution (2%) and allowed to stand for 15 at room temperature. The absorbance of the mixture was determined at 430 nm against the same mixture without the sample as a blank. Total flavonoid content was expressed as Rutin equivalent per gram dried weight (mg RE /g DW).

Determination of DPPH Radical Scavenging Capacity

The DPPH assay was performed according to the method described by Brand-Williams B, et al. [26] with modifications. A 50 µl aliquot of extract was mixed with 1950 µl of ethanolic solution of DPPH in concentration of 60 µM. The reaction mixture was shaken vigorously and incubated 30 min at room temperature. Then the absorbance at 517 nm was taken against a blank (DPPH solution without extract). The decrease in absorbance indicates the free radical scavenging effect of the tested sample. The scavenging activity was estimated based on the percentage of DPPH radical scavenged according to the following formula: [(A0–A1)/A0] x100, where A0 is the absorbance of the control, and A1 is the absorbance of the extract/ standard. The inhibition curves were prepared and IC50 values were obtained.

Statistical Analysis

Total phenolic content, flavonoid content and Antioxidant activity reported as the mean ± Standard Deviation (SD). Significant differences for multiple comparisons were determined using one way Analysis Of Variance (ANOVA). Tukey's multiple range tests was used to assess the significant differences with the SPSS statistical analysis package (version 15.0; SPSS Inc., Chicago, IL, USA). Difference at $P<0.05$ were considered statistically significant.

Results and Discussions

Total Phenolic and Flavonoid Content

The total phenolic content was found to be 2.72 ± 0.12 mg GAE/ g DW in *A. iva* extract and 87.11± 0.71 mg GAE/g DW in *A. unedo* extract. The total flavonoid content was found to be 1.48 ± 0.01 mg RE/g DW in *A. absinthum* extracts and 12.59 ± 0.06 mg RE/ g DW in *A. campestris* extract (Table 2). Considering the broad range of variation of the results, the phenolic contents were also categorized into three groups: high (> 50 mg), moderate (20-50 mg) and low (< 20 mg). The extracts of *A. unedo, M. spicata, J. phoenicea, A. campestris* and *O. basilicum* have high value of phenolic and flavonoid content exhibited the greatest antioxidant activity.

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Plant extracts	Total phenolics (mg GAE/g DW)	Total flavonoids (mg RE/ g DW)	DPPH IC_{50} (µg/ml)
A. campestris	57.82±0.12^{c}	12.59±0.06^{a}	37±1.25^{n}
A. radiata	9.45±0.3^{l}	6.57±0.1^{cd}	177±3.22^{f}
C. Cinerea	10.98±0.92^{kl}	5.07±0.02^{efg}	494±5.55^{b}
A. arvensis	9.69±0.15^{l}	3.56±0.03^{hij}	242±2.22^{e}
A. absinthum	12.62±0.09^{jk}	1.48±0.01^{l}	145±1.18^{i}
A. herba alba	22.41±0.08^{i}	2.39±0.13^{jkl}	103±1.22^{jk}
T. polium	14.21±0.27^{j}	4.95±0.09^{fg}	171±1. 32^{fgh}
M. pulegium	13,43±0.89^{j}	5,60±0.22^{def}	161±1.45^{gh}
L. officinalis	7,01±0.83^{M}	2,72±0.01^{ijk}	65 ±0.85^{lm}
T. algeriensis	10,95±0.85^{kl}	3,67±0.05^{hi}	111±1.12^{jk}
S. officinalis	23,50±0.47^{hi}	5,63±0.16^{def}	70±1.42^{l}
R. officinalis	39,54±0.95^{f}	6,12±0.09^{cdef}	176±2.35^{fg}
M. spicata	64,18±0.36^{b}	10,75±0.2^{b}	98±1.24^{k}
T. capitatus	41,99±0.21^{e}	6,98±0.06^{c}	78±0.88^{l}
O. basilicum	50,86±0.92^{d}	9,62±0.32^{b}	20±0.71^{o}
M. vulgare	6,05±0.05^{M}	4.19±0.22^{gh}	381±5. 26^{d}
A. iva	2.72±0.12^{N}	2.15±0.01^{kl}	650±8.60^{a}
H. scoparium	25.84±0.24^{h}	3.15±0.59^{hijk}	114±1.50^{j}
A. halimus L	3.51±0.3^{N}	2.03±0.01^{kl}	156±2. 85^{hi}
J. phoenicea	63.46±0.71^{b}	9.59±0.63^{b}	98±1.45^{k}
A. unedo	87.11±0.71^{a}	9.23±0.06^{cde}	51±0.42^{mn}
Z. lotus	31.19±0.86^{g}	3.96±0.02^{ghi}	464±4.45^{c}
Ascorbique acid	-	-	16±1.22^{o}

Table 2: Total phenolic content, flavonoid content and free radical scavenging (IC_{50}).

In the column different lettres mean siginficant differences by the Tukey's multiple range test (P<0.05)

The total phenolics content of these plant extracts are compared to the plant extracts of some previously studied plants [11,21,27-30]. Total phenolics of some previously studied plant extracts was found as *M. vulgare* (1.36±0.07 mg GAE/gdw), *A.iva* (3.16±0.016), *A. arboresens* (3.42±0.50), *A. arvensis* (3.94±0.05), A. herba halba (4.93±0.0036), *H. scoparium* (26.71±0.15), *S. officinalis* (7,78±0,0041), *M. pulegium* (16,34±0,011), *M. spicata* (19,65±0,001), *O. basilicum* (13,1±0,021) *T. polium* (8,29±0,0064), *A. campestris* (18.96±0.0079), *J. phoenicea* (46.61±4.59), *T. algeriensis* (18.73±4.59), *A. unedo* (104.98±4.59), and *Z. lotus* (36.30±4.59). The result showed that the plant extracts studied in this work showed the potent sources of secondary metabolites and could be used as the sources to isolate the active ingredient.

DPPH Radical-Scavenging Activity

DPPH is a free radical, stable at room temperature, which produces a violet solution in ethanol. It is reduced in the presence of an antioxidant molecule, giving rise to uncoloured ethanol solutions. The use of DPPH provides an easy and rapid way to evaluate antioxidants. The dosage of extract is expressed in µg of dry weight of the extract per mL of the assay mixture. IC50 value represents the concentration of test extract where the inhibition of test activity reached 50 %. The IC50 values of all the 22 different plant extracts have been furnished in the Table 2. Highest scavenging was observed with *O. basilicum* extract with an IC50 value of 20 µg/ml as opposed to the IC50 value of Ascorbic acid 16 µg/ml, which is a well known antioxidant Scavenging of DPPH radical was found to rise with increasing concentration of the extracts (Figure 1). Plant extracts of *A. campestris*, *A. unedo*, *L. officinalis*, *S. officinalis* and *T. capitatus*, ranked as the top five most active plant extracts, exhibited strong activity on scavenging DPPH radicals with the determined IC50 values 37 ± 1.25, 51 ± 0.42, 65 ± 0.85, 70 ± 1.42, 78 ± 0.88 µg/mL, respectively. Another plant extracts of *T. algeriensis*, *H. scoparium*, *M. pulegium*, *T. polium*, *R. officinalis*, *A. radiata*, *A. arvensis*, *M. vulgare* and *Z. lotus* also possessed signii cant activity and their IC50 values were between 100-500 µg/mL. Little antioxidant activity (>500µg/mL) was observed for the rest of extracts. Based on these results of investigation, it could be concluded that *O. basilicum* is a rich source of phenolic compounds as natural antioxidans of high value.

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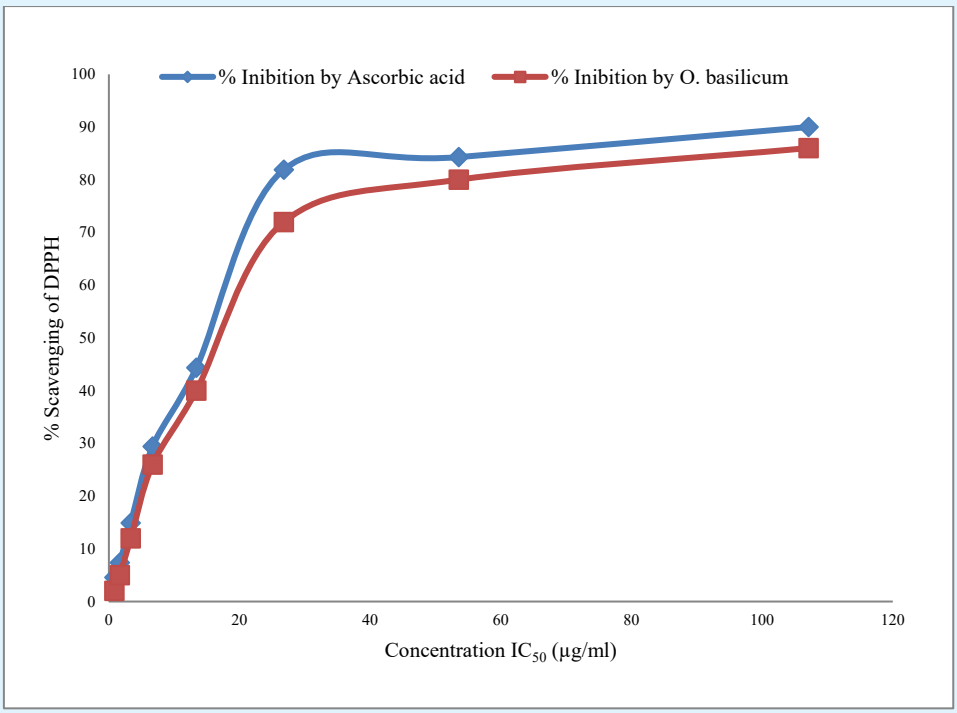

Figure 1: DPPH radical scavenging activity of *O. basilicum* extract and Ascorbic acid.

Relationship between Flavonoid Content and Antioxidant Activity

Attempts to correlate the level of flavonoid content of these medicinal plants with their antioxidant activity were not successful. No significant correlation (R2=0.37) was observed between flavonoid content and IC50 values when all plant materials were included in the calculation (Figure 2).

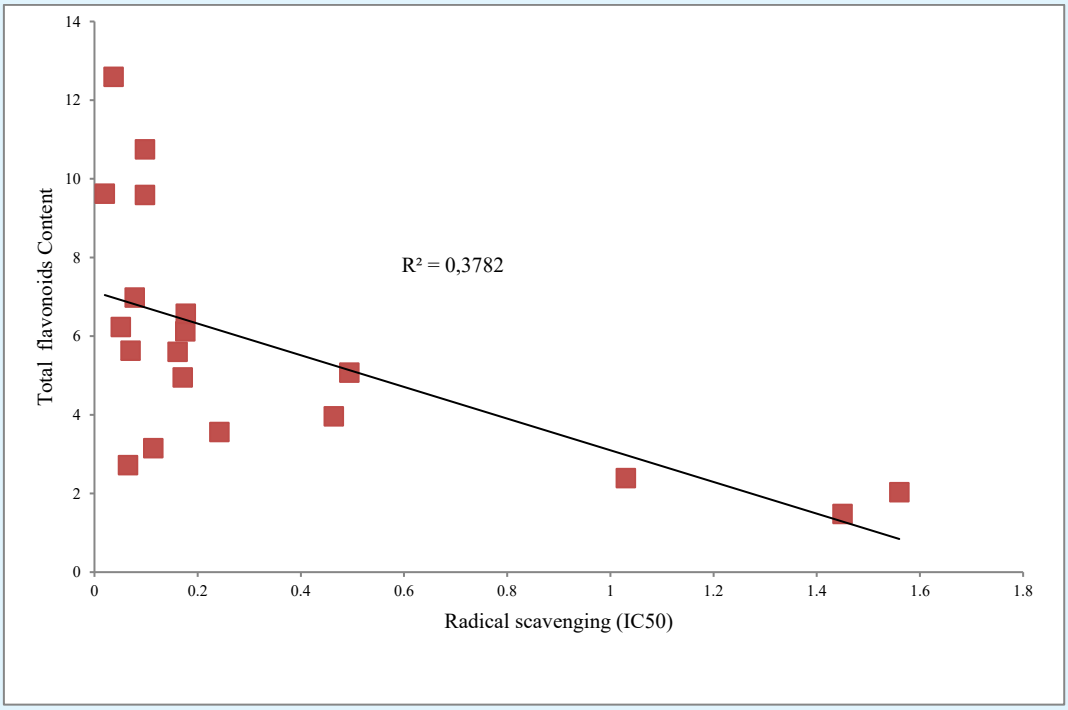

Figure 2: Correlation of flavonoid content and IC_{50} radical scavenging.

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Conclusion

The main objective of our study was to determine the total phenolics compounds as well as to evaluate the antioxidant activity of 22 plants from Algerian Sahara, which are used in traditional medicines. Many plants contain high amounts of phenolics and exhibited high antioxidant capacity. The DPPH radical scavenging assay shows that *Ocimum basilicum* shows a very good scavenging activity among all the plants. The results obtained showed that this plant is very important from medicinal point of view, and it needs further phytochemical exploitation to isolate phytochemical constituents showing antioxidant activity. The present study will help the researchers as basic data for future research in exploiting the hidden potential of this important plant which has not been explored so far.

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References

1. Ouraïni D, Agoumi A, Ismaili-Alaoui M, Alaoui K, Cherrah Y, et al. (2005) Study of the activity of essential oils from aromatic plants antifungal properties in the different stages of development of dermatophytes . Phytotherapy 4: 147-157.

2. Muthu C, Ayyanar M, Raja N, Ignacimuthu S (2006) Medicinal plants used by traditional healers in Kancheepuram District of Tamil Nadu, India. J Ethnobiol Ethnomed 2: 43.

3. Ben Merabet K, Abed A (1982) Quelques aspects de la pharmacopée traditionnelle algérienne. Le Pharmacien du Maghreb 2: 18-28.

4. Boulos L (1983) Medicinal plants of North Africa. Ed Reference Publications Inc Michigan 286.

5. Ali-Delille L (2013) Les plantes médicinales d'Algérie. BERTI Edition Alger Algeria.

6. Brand-Williams B, Cuvelier ME, Berset C (1995) Use of a free radical method to evaluate antioxidant activity. LWT, Food Science and Technology 28: 25-30.

7. Boudjelal A, Henchiri C, Sari M, Sarri D, Hendel N, et al. (2013) Herbalists and wild medicinal plants in M'Sila (North Algeria): Anethnopharmacology survey J Ethnopharmacol 148(2): 395-402.

8. Djellouli M, Moussaoui A, Benmehdi H, Ziane L, Belabbes A, et al. (2013) Ethnopharmacological study and phytochemical screening of three plants (Asteraceae family) from the region of south west Algeria. Asian J Nat Appl Sci 2(2): 59-65.

9. Sarri M, Mouyet FZ, Benzianea M, Cheriet A (2014) Traditional use of medicinal plants in a city at steppic character (M'sila, Algeria). Journal of Pharmacy & Pharmacognosy Research 2(2): 31-35.

10. Meddour R, Meddour OS (2015) Medicinal plants and their traditional uses in kabylia (Tizi- ouzou, Algeria). Arabian Journal of Medicinal and Aromatic Plants 1(2): 137-151.

11. Hammoudi R, Hajj Mahammed M, Ramdane F, Ouldelhadj MD (2013) Antimicrobial activity of phenolic extract from *Teucrium polium geyrii* (Lamiaceae) plant. Pharmacognosy Communications 3(3): 35-38.

12. Derwich E, Benziane Z, Boukir A (2010) GC/MS analysis and antibacterial activity of the essential oil of Mentha pulegium grown in Morocco. Research Journal of Agriculture and Biological Sciences 6(3): 191-198.

13. Barkat M, Laib I (2012) Antioxidant activity of the essential oil from the flowers of Lavandula stoechas J Pharmacognosy Phytother 4(7): 96-101.

14. Giweli AA, Džamić AM, Marina D Soković, Mihailo S, Ristić Petar D. Marin (2013) Chemical composition antioxidant and antimicrobial activities of essential oil of Thymus algeriensis wild-growing in Libya. Cent Eur J Biol 8(5): 504-511.

15. Pop AV, Tofană M, Sonia A, Vârban D, Nagy M, et al. (2015) Essential oil composition, phenolic content and antioxidant activity in Romanian Salvia officinalis L. J Agroalimentary Processes & Technol 21(3): 241-246.

16. Touafek O, Nacer A, Kabouche A, Kabouche Z, Bruneau C (2004) Chemical composition of the essential oil of rosmarinus officinalis cultivated in the algerian sahara. Chemistry of Natural Compounds 40(1): 28-29.

17. Znini M, Bouklah M, Majidi L, Kharchouf S, Aouniti A, et al. (2011) Chemical Composition and Inhibitory Effect of Mentha Spicata Essential Oil on the Corrosion of Steel in Molar Hydrochloric Acid. Int J Electrochem Sci 6: 691-697.

18. Akrout A, El Jani H, Amouri S, Neffati M (2010) Screening of antiradical and antibacterial activities

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of essential oils of Artemisia campestris L, Artemisia herba alba Asso and Thymus capitatus Hoff. Et Link. Recent Research in Science and Technology 2(1): 29-39.

19. Sari M, Sarri D, Hendel N, Boudjelal A (2012) Ethnobotanical study of therapeutic plants used to treat arterial hypertension in the hodna region of Algeria. Glob J Res Med Plants Indig Med 1: 411-417.

20. Moussaoui F, Alaoui T, Aoudry S (2014) Census Ethnobotanical Study of Some Plants Used in Traditional Medicine in the City of Meknes. Am J Plant Sci 5(15): 2480-2496.

21. Bakchiche B, Gherib A, Smail A, Custodia G, Graca M (2013) Antioxidant activities of eight Algerian plant extracts and two essential oils. Ind Crops Prod 46: 85-96.

22. Dehliz LW, Acheuk F, Mlik R (2016) Ethnobotanical study of some plants used in traditional medicine in the region of Oued Righ (Algerian Sahara). Journal of Medicinal Plants Studies 4(2): 204-211.

23. Elmhdwi MF, Attitalla IA, Khan BA (2015) Evaluation of Antibacterial Activity and Antioxidant Potential of Different Extracts from the Leaves of *Juniperus Phoenicea.* J Plant Pathol Microb 6: 9-15.

24. Slinkard K, Singleton VL (1977) Total phenol analyses: Automation and comparison with manual methods. American Journal of Enology and Viticulture 28: 49-55.

25. Ahn MR, Kumazawa S, Usui Y, Nakamura J, Matsuka M, Zhu F, et al. (2007) Antioxidant activity and constituents of propolis collected in various areas of China. Food Chem 101: 1400-1409.

26. Brand-Williams B, Cuvelier ME, Berset C (1995) Use of a free radical method to evaluate antioxidant activity. LWT, Food Science and Technology 28(1): 25-30.

27. Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, et al. (2006) Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chemistry 97(4): 654-660.

28. Djidel S, Khennouf S, Baghiani A, Harzallah D, Arrar L (2009) Medicinal plants used traditionally in the Algerian folk medicine for gastrointestinal disorders and hypertension: total polyphenols, flavonoids and antioxidant activity. Acta Hortic 854(6): 59-65.

29. Khacheba I, Djeridane A, Yousfi M, Twenty Traditional Algerian Plants Used in Diabetes Therapy as Strong Inhibitors of-Amylase Activity. Hindawi Publishing Corporation. International Journal of Carbohydrate Chemistry (2014) 1-12.

30. Krimat S, Dob T, Lamari L, Boumeridja S, Chabane C, et al. (2014) Antioxidant and antimicrobial activities of selected medicinal plants from Algeria. Journal of Coastal Life Medicine 2(6): 478-483.