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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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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: Medicinal plants; Phenolic content; flavonoid content ; Antioxidant; DPPH.

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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 (Ouraiïni et al., 2005). 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 (Muthu et al., 2006). In Algeria, the empirical use of plants continues to maintain a high popularity [Benmerabet and Abed, 1982 ; Boulos, 1983]. 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 ... (Ali-Delille, 2013). The evaluation of free radical scavenging activity of plant extracts have been extensively performed by DPPH (1,1-diphenyl-2-picrylhydrazyl) method (Brand-Williams et al., 1995). DPPH is a purple colored radical that, after 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 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 (Table1). *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.

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.

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

Family; Species	Local name	Traditional uses	References
Asteraceae			
<i>Artemisia campestris</i>	Dgouft	Antidiabetic and antihypertensive	Boudjelal et al., 2013
<i>Anvillea radiata</i>	Noug	diabetes and Indigestion	Djellouli et al., 2013
<i>Cotula Cinerea</i>	Gartoufa	Asthma, Cough and Allergy	Djellouli et al., 2013
<i>Anthemis arvensis</i>	Babounj	anthelmintic and analgesic rules	Sarri et al., 2014
<i>Artemisia absinthum</i>	Chehaïba	Antidiabetic and diuretic	Meddour et al., 2015
<i>Artemisia herba alba</i>	Chih	Antidiabetic and eczema	Sarri et al., 2014

Lamiaceae			
<i>Teucrium polium</i>	Jiaida	Antidiabetic and antidiarrhoea	Hamoudi et al., 2013
<i>Mentha pulegium</i>	Feliou	Antihypertensive and antispasmodic	Derwich et al., 2010
<i>Lavandula officinalis</i>	Khozama	Antispasmodic and diuretic	Barkat et Laib 2012
<i>Thymus algeriensis</i>	djertil	Diuretic, mucolytic and analgesic	Giweli et al., 2013
<i>Salvia officinalis</i>	Siwak elnabi	antihydrotic and spasmolytic,	Pop et al., 2015
<i>Rosmarinus officinalis</i>	Iklil eljabel	Hypotensive, anemia and liver disease	Touafek et al., 2004
<i>Mentha spicata</i>	Na-naa	Carminative, Stomachic and Cooling	Znini et al., 2011
<i>Thymus capitatus</i>	Zaatar	anthelmintic, antispasmodic and tonic	Akrout et al., 2010
<i>Ocimum basilicum</i>	Hbaq	Hypertension	Sari et al., 2012
<i>Marrubium vulgare L.</i>	Temeriouit	diarrhea, fever, rheum, and headache	Sarri et al., 2014
<i>Ajuja iva</i>	Chendgoura	Diabetes and hypertension	Moussaoui et al., 2014
Amaranthaceae			
<i>Haloxylon scoparium</i>	Remth	Antidiabetic and Poulrice for mould	Bakchiche et al., 2013
Chenopodiaceae			
<i>Atriplex halimus L</i>	Gtaf	Hypertension, diabetes and diuretic,	Dehliz et al., 2016
Cupressaceae			
<i>Juniperus phoenicea</i>	Aaràar	Diarrhea, rheumatism and diabetes.	Elmhdwi et al., 2015
Ericaceae			
<i>Arbutus unedo</i>	Lindj	Astringent and diuretic	Bakchiche et al., 2013
Rhamnaceae			
<i>Zizyphus lotus</i>	Sedra	Diabetes, Analgesic and antiseptic	Dehliz et al., 2016

2.2. Determination of total Phenolic Content

The total phenolic content is estimated using Folin-Ciocalteu's method (Slinkard and Singleton 1977), 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).

2.3. Determination of total Flavonoid Content

Total flavonoid contents were determined by the method of (Ahn et al., 2007). Each sample (1.5 ml) was added to 1.5 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).

2.4. Determination of DPPH radical scavenging capacity

The DPPH assay was performed according to the method described by Brand-Williams et al., 1995) 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 : $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/ standard. The inhibition curves were prepared and IC_{50} values were obtained.

2.5 Statistical analysis

Total phenolic content, favonoid content and Antioxidant activity reported as the mean \pm 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 favonoid 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 favonoid content was found to be 1.48 ± 0.01 mg RE/g DW in *A. absinthum* extract 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 favonoid content exhibited the greatest antioxidant activity.

The total phenolics content of these plant extracts are compared to the plant extracts of some previously studied plants (Djeridane et al., 2006 ; Djidel et al., 2009 ; Bakchiche et al., 2013 ; Khacheba et al., 2014 ; Krinat et al.,

2014). 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. arborescens* (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* ($7,78 \pm 0,0041$), *M. pulegium* ($16,34 \pm 0,011$), *M. spicata* ($19,65 \pm 0,001$), *O. basilicum* ($13,1 \pm 0,021$) *T. polium* ($8,29 \pm 0,0064$), *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 μ g of dry weight of the extract per mL of the assay mixture. IC_{50} value represents the concentration of test extract where the inhibition of test activity reached 50 %. The IC_{50} 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 IC_{50} value of 20 μ g/ml as opposed to the IC_{50} value of Ascorbic acid 16 μ g/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 IC_{50} values 37 ± 1.25 , 51 ± 0.42 , 65 ± 0.85 , 70 ± 1.42 , 78 ± 0.88 μ g/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 significant 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 antioxidants of high value.

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 ($R^2=0.37$) was observed between flavonoid content and IC₅₀ values when all plant materials were included in the calculation (Figure 2).

Table 2 : Total phenolic content, flavonoid content and free radical scavenging (IC₅₀)

Plant extracts	Total phenolics (mg GAE/g DW)	Total flavonoids (mg RE/ g DW)	DPPH IC ₅₀ (µg/ml)
<i>A. campestris</i>	57.82±0.12 ^c	12.59±0.06 ^a	37±1.25 ⁿ
<i>A. radiata</i>	9.45±0.3 ^l	6.57±0.1 ^{cd}	177±3.22 ^f
<i>C. Cinerea</i>	10.98±0.92 ^{kl}	5.07±0.02 ^{efg}	494±5.55 ^b
<i>A. arvensis</i>	9.69±0.15 ^l	3.56±0.03 ^{hij}	242±2.22 ^e
<i>A. absinthum</i>	12.62±0.09 ^{jk}	1.48±0.01 ^l	145±1.18 ⁱ
<i>A. herba alba</i>	22.41±0.08 ⁱ	2.39±0.13 ^{ijkl}	103±1.22 ^{jk}
<i>T. polium</i>	14.21±0.27 ^j	4.95±0.09 ^{fg}	171±1.32 ^{gh}
<i>M. pulegium</i>	13,43±0.89 ^j	5,60±0.22 ^{def}	161±1.45 ^{gh}
<i>L. officinalis</i>	7,01±0.83 ^M	2,72±0.01 ^{ijk}	65 ±0.85 ^{lm}
<i>T. algeriensis</i>	10,95±0.85 ^{kl}	3,67±0.05 ^{hi}	111±1.12 ^{jk}
<i>S. officinalis</i>	23,50±0.47 ^{hi}	5,63±0.16 ^{def}	70±1.42 ^l
<i>R. officinalis</i>	39,54±0.95 ^f	6,12±0.09 ^{cdef}	176±2.35 ^{fg}
<i>M. spicata</i>	64,18±0.36 ^b	10,75±0.2 ^b	98±1.24 ^k
<i>T. capitatus</i>	41,99±0.21 ^e	6,98±0.06 ^c	78±0.88 ^l
<i>O. basilicum</i>	50,86±0.92 ^d	9,62±0.32 ^b	20±0.71 ^o
<i>M. vulgare</i>	6,05±0.05 ^M	4.19±0.22 ^{gh}	381±5.26 ^d
<i>A. iva</i>	2.72±0.12 ^N	2.15±0.01 ^{kl}	650±8.60^a
<i>H. scoparium</i>	25.84±0.24 ^h	3.15±0.59 ^{hijk}	114±1.50 ^j
<i>A. halimus L</i>	3.51±0.3 ^N	2.03±0.01 ^{kl}	156±2.85 ^{hi}
<i>J. phoenicea</i>	63.46±0.71 ^b	9.59±0.63 ^b	98±1.45 ^k
<i>A. unedo</i>	87.11±0.71 ^a	9.23±0.06 ^{cde}	51±0.42 ^{mn}
<i>Z. lotus</i>	31.19±0.86 ^g	3.96±0.02 ^{ghi}	464±4.45 ^c
Ascorbique acid	-	-	16±1.22 ^o

In the column different letters mean significant differences by the Tukey's multiple range test ($P<0.05$)

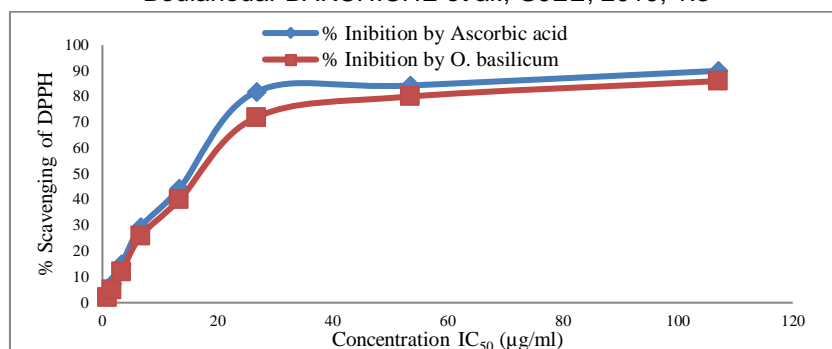


Figure 1 : DPPH radical scavenging activity of *O. basilicum* extract and Ascorbic acid

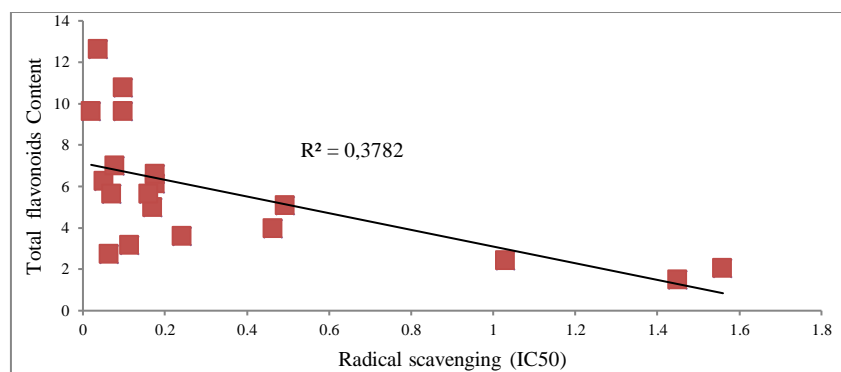


Figure 2: Correlation of flavonoid content and IC₅₀ radical scavenging

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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