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Antioxidant activity and phenolic content in methanol crude extracts from three Lamiaceae grown in southwesternAlgeria

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ABSTRACT

*The present study estimated in vitro antioxidant activities of methanolic extracts of some Algerianmedicinal plants (*Saccocalyx satureioïdes, Teucrium polium and Salvia verbenaca) (Lamiaceae) u*sing Folin–Ciocalteu, ferric-reducing/antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assays. The results showed that methanolic extract of* Teucrium polium with a total *content of polyphenols (3.81 GAE/g) and an* IC_{50}*of 5.70µg/mLwas more antioxidant. In power reduction, the antioxidant activity of T.poliumand S.satureioïdes extract's, at all the concentrations were average compared to controls used (BHA, BHT).*

Keywords: Algerian medicinal plants; Total phenolic content; Flavonoids; FRAP; DPPH.

INTRODUCTION

Active oxygen and free radicals exist in human body inthe form of superoxide anion($O_2^{\cdot-}$) hydrogen peroxide(H_2O_2) and hydroxyl radical ($^{\cdot}OH$) and so on. As normalmetabolic action going on in human body, active oxygenand free radicals are constantly formed. If they reach highlevels, oxidative stress in human body would be created, which leads to a variety of biochemical and physiologicallesions and often results in metabolic impairment and celldeath [1].Also, lipid oxidation by radicals results in food deterioration, especiallyin high fat foods [2].

The interest inantioxidants has been increasing because of their highcapacity in scavenging free radicals related to variousdiseases [3].Synthetic antioxidantssuch as butylated hydroxyanisol (BHA) andbutylatedhydroxytoluene (BHT) and so on are used as antioxidants,but they have been suspected of possessing of certain toxicity and being responsible for liver damage and carcinogenesis [4, 5,6].

Therefore, the development and isolation of natural antioxidants from natural plant has been become the focus of the research of antioxidant, and many studies have suggestedthat

consumption of certain natural antioxidants lead to areduction in oxidative stress [7] and the developmentof human major diseases [8, 9,10].

The antioxidant properties of plant extracts have been attributed to their polyphenol contents [11, 12, 13]. A number of phenolic compounds with strong antioxidant activity have beenidentified in these plants, especially those belonging to the *Lamiaceae* family, and are of interest to food manufacturersas consumers move towards functional foods with specifichealth effects[14, 15, 16].

The plant family LamiaceaeMartinov (= LabiataeAdans., the mint family) has a world-wide distribution and comprises more than 7200 species across approximately 240 genera which are classified in seven subfamilies[17].

Saccocalyx satureioïdes Coss. etDur. called Zaater or Azir El-ibel [18] by locals is a shrub present in septentrional Sahara and measures between 20 and 100 cm in height. It grows naturally on the dunes of the predesertic area [19] in the Ain sefra region of Naâma south western Algeria, with a blossoming period in March. This medicinal plant is used as an ingredient in numerous local traditional medicines and mostly in the care of diabetes [20].

Teucrium polium L. is one of 300 species of the genus *Teucrium* and found mainly in the Mediterranean and Western Irano-Turanian sphere.

Traditionally, especiallyin the Mediterranean countries, *Teucrium polium*, isused for its antispasmodic and hypoglycemic activities by thenative inhabitants and recommended by the herbalists [21]. Anti-inflammatory, anti-hypertensive, antinociceptive,anti-ulcer and anorexic effects are other activitiesreported [22, 23, 24].

Salvia, the largest genus of the family *Lamiaceae*,includes about 900 species widespread all over theworld [25].

Salvia verbenaca is a shrub present in Europe,southwesternof Asia, northern Africaand naturalizedin northern Americaand measures 8-80cm[26].

The purposes of the present study were to evaluate in vitro antioxidant activityof threeAlgerianherbal materials allowed for medicinal uses, using ferric-reducing/antioxidant power (FRAP) and DPPH radical-scavengingassays. In addition, the total content of phenolics and flavonoidsfrom plants extracts were also measured.

MATERIALS AND METHODS

2.1. Chemicals

2, 2-diphenyl-1-picrylhydrazyl (DPPH), Folin–Ciocalteu reagent, potassium ferricyanide [$K_3Fe(CN)_6$], ascorbic acid, trichloroacetic acid, butylated hydroxyanisol (BHA) were purchased from Fluka (Switzerland). $FeCl_3$was from Sigma Chemical Co (Germany).
All other chemicals and reagent used were of analytical grade.

2.2. Plant materials

The aerial parts of *Saccocalyx satureioïdes*, *Teucrium polium* and *Salvia verbenaca*were collectedin April, 2007were analyzed. Thereshly-cut plants were dried in a dry and shady place atambient temperature.

Plants were identified at the laboratory of Ecology and Management of Natural Ecosystems of the University of Tlemcen (Algeria).

2.3. Preparation of the extracts

A powder (20g) of the aerial part of each plant was extracted by 100 mL of methanol-water (7:3) at 80 °C for 3 h under reflux [27]. The extracts were then filtered and concentrated under reduced pressure at 60° C using a rotary evaporator (BuchiRotavapor R- 200) to obtain the methanolic crude extract. The last one was kept in dark and stored at 4°C.

2.4. Determination of total phenolics content

Total phenolics contents of the extracts were determinedspectrophotometrically according to the Folin-Ciocalteucolorimetric method [28], calibratingagainst acid gallic standards and expressing the results as mgacid gallicequivalents (GAE)/g extract. Data presented areaverage of three measurements.

2.5. Estimation of total flavonoids content

The determination of flavonoids was performed according to the colorimetric assay of [29]. Distilled water (4 ml) was added to 1 ml of DPF extract. Then, 5% sodium nitrite solution (0.3 ml) was added, followed by 10% aluminum chloride solution (0.3 ml). Test tubes were incubated at ambient temperature for 5 min, and then 2 ml of 1 M sodium hydroxide were added to the mixture. Immediately, the volume of reaction mixture was made to 10 ml with distilled water. The mixture was thoroughly vortexed and the absorbance of the pink color developed was determined at 510 nm. A calibration curve was prepared with catechin and the results were expressed as mg catechin equivalents (CEQ)/100 g sample.

2.6. Antioxidantactivity

2.6.1. FRAP assay

The reducing power of the aerial part of the different spices was determined according to the method of Yang et al.[30] the methanolic crude extracts, BHA and BHT were used at different concentrations (0.1, 0.2, 0.3, 0.4 and 0.5mg/mL). One milliliter of each sample was mixed with phosphate buffer (2.5mL, 0.2mol/L, pH6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$(2.5mL, 30mmol/L). The mixture was incubated at 50 °C for 20 min. A 2.5mL TCA (0.6 mol/L) was added to the mixture, which was then centrifuged for 10 min at 3000 g. the supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5mL, 6mmol/L), and the absorbance was measured at 700 nm in a spectrophotometer (Jenway 6400).

2.6.2. DPPH radical assay

The DPPH free radical scavenging activity of each sample was determined using the Ultra spec[6405UV/Vis]Spectrophotometer according to the method described by Leong and Shui [31]. Briefly, a 0.1mM solution of DPPH in methanol was prepared. The initial absorbance of the DPPH in methanol was measured at 515 nm and did not change throughout the period assay. An aliquot (40µl) of an extract (with appropriate dilution, if necessary) was added to 3ml of methanolic DPPH solution. The DPPH radicals scavenging activity was calculated according to the following equation:

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

IC_{50} wasobtained graphically from nonlinear regression analysis.

Statisticalanalysis
Data are presented as the mean ± standard deviation (SD) of each triplicate test.

RESULTS AND DISCUSSION

3.1. Total phenolics and flavonoids content

It has been reported that phenolics compounds are considered to be the most important antioxidative plant components [32]. And the antioxidant activity of plant materials was well correlated with the content of their phenolics compounds [33].

The phenol contents of methanolic extracts of *S.satureioïdes, T. polium and S.verbenaca* samples werereportedas mggallic acid equivalents per gram of dry extract (GAE/g) and depicted in Table1. As can be seen, the levels of total phenols of *S.satureioïdes and T. polium*were more significant than thatof *S.verbenaca.*

Djeridane et *al.*[34] reported a total phenol content of*T. polium*of 4.91mgGAE/g. Total flavonoids content was measured using aluminum chloride colorimetric method and results are presented in table 1.

On these plants,there are very few publications that are made regarding the levels of polyphenols and flavonoids.

Table 1: Total phenolic[a] and total flavonoid[b] of methanolic crude extractsof *S.satureioïdes, T. polium and S.verbenaca*

plant	Total phenolic	Total flavonoid
S.satureioïdes	3.405 ± 20.41	2.9± 2.53
T. polium	3.810 ± 15.75	3.2± 5.86
S.verbenaca	0.845 ± 9.33	1.11± 3.47

Each value represents the mean ±SD (n = 3).
[a]Total phenolic content was expressed as mg gallic acid equivalent/g dried extract.
[b]Total flavonoid content was expressed as mg catechin equivalent/g dried extract.

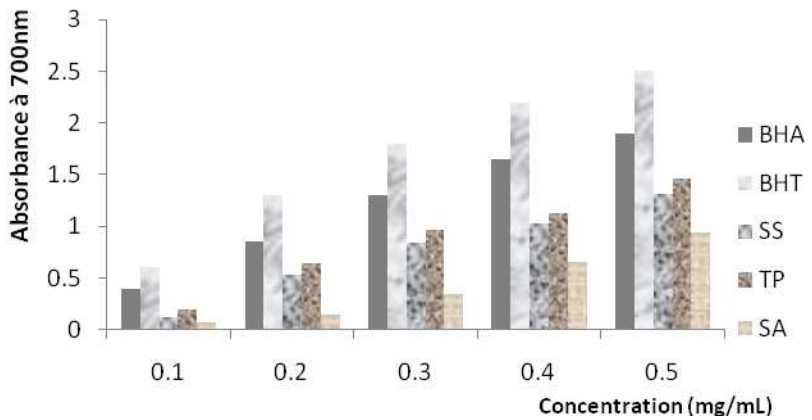

Figure 1. Reducing power activity of methanolic crude extracts of *S.satureioïdes, T. polium and S.verbenaca.*
SS: S.satureioides, TP: T.polium, SA: S.verbenaca.

3.2. Ferric reducing antioxidant power assay

The total antioxidant capacities of the plant extracts was determined by FRAP method, a simple, speedy, inexpensive,and reproducible method, which can be applied tothe assay of antioxidants in plasma or botanicals [35, 36].

The principle of the assay is based on the reduction of ferric 2, 4, 6-tripyridyl-S-triazine [Fe(III)-TPTZ] to the ferrous 2,4,6-tripyridyl-S-triazine [Fe(II)-TPTZ] complex by a reductant at low pH. This complex has an intense blue colour that can be monitored at 595 nm. The antioxidant efficiency of the samples was calculated with reference to thereaction signal given by a Fe^{2+} solution of known concentration.

Increase in absorbance of the reaction indicated the higher reducing power of the test samples.

Figure 1 shows the reductive capability of methanolic extracts of the spicesstudied compared to BHA and BHT as standards. The antioxidant activity of *T.polium*and *S.satureioïdes* extract's, at all the concentrations were average compared to controls used (BHA, BHT).

Ferric reducing antioxidant power assayof *S.satureioïdes, T. polium and S.verbenaca*is studied for the first time.

3.3. Determination of the scavenging effect on DPPH radicals

Antioxidants are able to reduce the stable DPPH radical to yellow-colored and the antioxidant power is indicated by the degree of discoloration which could be determined by measuring of a decrease in the absorbance at 515 nm.

The role of antioxidants is their interaction with oxidative free radicals. The essence of DPPH method is that the antioxidants react with the stable free radical i.e., a, adiphenyl-b-picrylhydrazyl (deep violet colour) andconvert it to a, a-biphenyl-b-picrylhydrazine with discoloration.The degree of discoloration indicatesthe scavenging potentials of the sample antioxidant.A concentration-dependent assay was carried out with the methanolic extracts of*S.satureioïdes, T. polium, S.verbenaca, and ascorbic acid*and the results are presented in Fig.2.In our present study, the decreasing order of antioxidantactivity among the plants extractswas found to besimilar to the phenolic contents of the extracts.

The free radical scavenging activity is also expressed by the antioxidant concentration required for a 50% DPPH redaction (IC_{50}) (table 2).

Table 2. The IC_{50}value of methanolic crude extracts of *S.satureioïdes, T. polium, S.verbenaca,* and ascorbic acid

Plant extract	IC_{50}
S.satureioïdes	7.17±0.23
T. polium	5.70±0.56
S.verbenaca	9.79±0.47
Ascorbic acid	0.27±0.12

Each value represents the mean ±SD (n = 3).
IC_{50}values were expressed as µg.mL^{-1}

CONCLUSION

Methanolic extracts, of *S.satureioïdes, T. polium, S.verbenaca,* were examined by DPPHand FRAP methods fortheir free radical and reductionpower respectively. The total phenolic contents and total flavonoid contents were measured also. Results showed that these plants can be a source of polyphenols and flavonoids,confirmtheir antioxidants activities and underline their potential either as natural preservatives or in pharmaceutical applications.

The present results encourage additional and more in-depth studies on the phenolic composition of these plant extracts and assessment of antioxidant activity of each compound separately. Some phenolic compounds remain to be identified and further biological tests should be conducted.

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