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Antioxidant activity and phytochemical screening of *Nepeta nepetella* aqueous and methanolic extracts from Algeria

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ABSTRACT

Plant phenolics constitute one of the major groups of components that act as primary antioxidant free radical terminators. This paper reports the antioxidative activity of methanolic and water extract of *Nepeta nepetella*. Phytochemical screening of the crude extracts of stems, leaves and flowers revealed the presence of different kind of chemical groups such as flavonoids, tannins, alkaloids, saponins and carbohydrates. The amounts of total phenolics solvent extracts (methanol and water extract) for the three parts of plant were determined spectrometrically. From the analyses, leaves methanolic extract had the highest total phenolic content (58.11± 1.24 mg GAE/g), the highest DPPH scavenging ability with the lowest IC_{50} value (1.45 ± 0.07 mg/ml), the same tendency was observed with ferric reducing power. Concerning β-carotene bleaching assays results showed that the stems methanolic extract exhibited the highest antioxidant ability with an IC_{50} higher than standards (0.148 ± 0.003 mg/ml).

INTRODUCTION

Native plants have been reported to have antimicrobial and antioxidant properties for centuries and indigenous plants have been used in herbal medicine to cure various diseases (Tepe *et al.*, 2005). Many kinds of diseases have been treated with herbal remedies since ancient times. Herbal remedies are still being used extensively in many countries. Therefore, research on biologically active extracts and compounds from natural sources has been of great interest to scientists in an attempt to discover new sources for drugs that may be useful in combating infectious diseases. Many authors reported that it was necessary to establish a rational relationship between chemical, biological and therapeutic activities in traditional medicine (Khlebnikov *et al.*, 2007) and (Fura, 2006). In recent years, there has been a resurgence of interest in evaluating plants possessing antibacterial activities for various diseases (Roselli *et al.*, 2007). A number of studies dealing with antimicrobial screening of extracts medicinal plants have been conducted (Rangasamy *et al.*, 2007). Current research on free radicals confirms the essential role played by rich foods in antioxidants in the prevention of cardiovascular diseases and cancers (Valko *et al.*, 2007) and (Ljubuncic *et al.*, 2005). This is due to the presence of secondary metabolites which accumulate in the various parts of these plants conferring on them their pharmacological relevance (Babalola *et al.*, 2001). Generally, some of these plants especially the editable ones are eaten habitually without knowledge of their pharmacological effect. Among secondary metabolites, the polyphenol compounds play a wide range of biological effects including antibacterial, anti-inflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral, anticarcinogenic and cardioprotective and vasodilatory effects (Trigui *et al.*, 2013). A direct relationship has been found between the phenolic content and antioxidant capacity of plants (Al-Mamary *et al.*, 2002; Cowan, 1999; Robards *et al.*, 1999; Wollgast and Anklam, 2000; Vaya *et al.*, 1997). The genus *Nepeta* (Lamiaceae) comprises about 400 species, most of which grow wild in Central and Southern Europe, the North Africa and Central and Southern Asia. A lot of species of this genus are used in folk medicine for the antiseptic and astringent properties as topical remedy in children cutaneous eruptions, snakes and scorpion bites; orally, they are utilized as anti-tussive, anti-spasmodic, anti-asthmatic, febrifuge and diuretic (Lewis, 1977; Perry, 1980; Moerman, 1982; Duke and Ayensu, 1985; Bezanger-Beauquesne

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et al., 1990; Bourrel *et al.,* 1993; Herman *et al.,* 1987). Moreover, anti-bacterial, fungicidal and anti-viral activities have been attributed to nepetalactones, iridoids contained in several *Nepeta* species (Bourrel *et al.,* 1993; Sattar *et al.,* 1995).

The aim of the present investigation was to study one of genus of Nepeta who is *Nepeta nepetella* it's an endemic plant to the region of Ain Sefra, Algeria. We want to determine antioxidant capacities, phytochemical constituents, and total phenolic contents of methanolic and aqueous extracts of her leaves, flowers, and stems.

MATERIEL AND METHODS

Plant material

The aerial part (leaves, flowers and stems) of *N.nepetella* was harvested in May 2012 from Ain Sefra, Algeria. The plants collected were identified by the Vegetable Ecological Laboratory and Professor BENABADJI Noury. Plant samples were dried in the share and conserved for future use.

Preparation of extracts

Aqueous extract

10g of powder dissolved in 150 ml of water leaves distilled water were heated to reflux for 2 hours, after cold filtration, the filtrate was then evaporated to dryness under 65 ° C at reduced pressure using a rotary evaporator (Büchi Rotavapor R-200). The residues were then dissolved in 3 ml of methanol (Majhenic *et al.,* 2007).

Methanolic extract

A sample of 2.5 g of powder sheets was macerated in 25 ml of absolute methanol in magnetic stirring for 30 min. The extract then was stored at 4 °C for 24 h, filtered and the solvent evaporated. Dry under reduced pressure at 50 °C at pressure using an evaporator rotary (Büchi Rotavapor R- 200).The resulting solutions were evaporated under vacuum at 50 °C. The residues were then dissolved in 3 ml of methanol (Falleh *et al.,* 2008).

Phytochemical analysis

Phytochemical analysis of methanol and aqueous extracts of *Nepeta nepetella* leaves, flowers and stems were conducted by standard qualitative analytical methods for identification of diverse phytocompounds such as carbohydrates (Deb, 2001), saponins (Kokate, 1999), phenolics (Mace, 1963; Raaman, 2006) and flavonoids (Harborne, 1983; Shinoda, 1928).

Determination of total phenolic contents

The total phenolic in leaves and stems methanolic extracts content was determined by spectrometry using ''Folin-Ciocalteu'' reagent assay (Singleton and Rossi., 1965). A volume of 200 ml of the extract was mixed with 1 ml of Folin- Ciocalteu reagent diluted 10 times with water and 0.8 ml of a 7.5% sodium carbonate solution in a test tube. After stirring and 30 min later, the absorbance was measured at 765 nm by using a Jenway 6405 UV-vis spectrophotometer. Gallic acid was used as a standard for the calibration curve. The total phenolic content was expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW).

DPPH Free-Radical-Scavenging Assay

The hydrogen atom or electron donation abilities of some pure compounds were measured by the bleaching of a purple colored methanol solution of the stable 2,2-diphenyl-2 picrylhydrazyl (DPPH) radical (Sanchez-Moreno *et al.,* 1998). Fifty microliter of various concentrations of the extracts in methanol were added to 1950 ml of a 0.025 g/l methanol solution DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 515 nm. DPPH free radical scavenging activity in percentage (%) was calculated using the following formula:

DPPH scavenging activity (%) = $(A_{blank} - A_{sample} / A_{blank}) \times 100$

Where a blank is the absorbance of the control reaction (containing all reagents except the test compound), a sample is the absorbance of the test compound.

Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted of inhibition percentage against extract concentrations. The ascorbic acid (AA) methanol solution and tert-butyl-4-hydroxyanisole (BHA) methanol solution were used as positive control.

Reducing power assay

Reducing power of the extracts was determined as described by Oyaizu (1986). Different concentrations of plant extract solutions were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and 2.5ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20min. After incubation, 2.5ml of 10% trichloroacetic acid was added and the mixture was then centrifuged at 3000 rpm for 10mn. The upper layer of solution (2.5ml) was mixed with distilled water (2.5ml) and 0.1% ferric chloride (0.5ml). The absorbance of the mixture was measured at 700 nm. A higher absorbance indicates a higher reducing power. The assays were carried out in triplicate and the results are expressed as mean ± standard deviation. The extract concentration providing absorbance of 0.5 (IC_{50}) was calculated from the graph of absorbance at 700 nm against extract concentration, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (TROLOX) and tert-butyl-4-hydroxyanisole (BHA) were used as standards.

β-Carotene bleaching inhibition capacity

The capacity of extracts to inhibit the β-carotene bleaching was determined according to Koleva *et al.,* (2002) with minor modifications. Two mg of β -carotene were dissolved in 20 ml chloroform, and 4 ml of this solution were added to linoleic acid (40 mg) and Tween 40 (400 mg). Chloroform was evaporated under vacuum at 40 °C and 100 ml of oxygenated water were added. An emulsion was obtained by vigorously shaken, an aliquot (150 ml) of which was distributed in 96 well microtitre plate and methanolic solutions of the test samples (10 ml) were added.

Twice replicates were prepared for each extract concentration. The microplate was incubated at 50 °C for 120 min, and the absorbance was measured at 470 nm using a EAR 400 microtitre reader (Multiskan MS, Labsystems). Readings were performed both immediately (t 0 min) and after 120 min of incubation. The antioxidant activity of the extracts was evaluated in terms of bleaching inhibition of the β carotene using the following formula:
β carotene bleaching inhibition (%) = $[(S - A_{120})/(A_0 - A_{120}) \times 100]$
Where A_0 and A_{120} are the absorbances of the control at 0 and 120 min, respectively, and S the sample absorbance at 120 min. The results were expressed as IC_{50} value (mg/ml).

Statistical analysis

All evaluations of antioxidant activity were performed in twice. Data were expressed as means ± standard deviation (S.D.).

RESULTS AND DISCUSSION

Phytochemical analysis

Preliminary phytochemical screening of the aqueous and methanolic extracts of *Nepeta nepetella* leaves, flowers and stems revealed the presence of various bioactive components. It revealed the presence of tanins, carbohydrtates, saponins, phenols and flavonoids in the aqueous extract. Alkaloids, carbohydrates, and saponins were absent in the methanol extract. The methanol extract was found to contain phenols and flavonoids as major phyto-components.

Total phenolics content

The totals phenols contents of extracts are shown in Table 1. The results obtained in our extracts show that the total phenolics have varied from 13.18±1.04 to 58.11± 1.24 mg GAE/g (Table 1). The extracts having extracted the highest total phenolics are: methanolic extract flowers (58.11± 1.24 mg GAE/g), methanolic extract stems (41.725±0.38 mg GAE/g) and aqueous extract leaves (41.65±2.18 mg GAE/g). The lowest contents of total phenolics were obtained with the methanolic extract flowers (21.42±0.96 mg GAE/g), followed by aqueous extract stems (14.98±1.07 mg GAE/g), and finally aqueous extract flowers with (13.18±1.04 mg GAE/g).

Antioxidant activity

In the present study, three commonly used antioxidant evaluation methods such as DPPH radical scavenging activity, reducing power assay and β-Carotene bleaching inhibition capacity were chosen to determine the antioxidant potential of the methanolic and aqueous extracts of leaves, flowers and stems of *Nepeta nepetella*.

DPPH Free-Radical-Scavenging Assay

Free radical assay is one of the most widely used methods and has become routine in establishing the antioxidant activity of herbal extracts and photochemical. Hydrogen donating ability is an index of primary antioxidants. DPPH is known to abstract labile hydrogen and the ability to scavenge the DPPH radical is related to the inhibition of lipid peroxidation (Matsubara *et al.,* 1991)

IC_{50} value was determined from plotted graph of scavenging activity against the different concentrations of *N.nepetella* extracts, ascorbic acid, and BHA. The scavenging activity was expressed by the percentage of DPPH reduction after 30 min of reaction. The measurements were duplicate and their scavenging effects were calculated based on the percentage of DPPH scavenged (Blois *et al.,* 1958; Singh *et al.,* 2008). The obtained results are summarized in table 2.

The results show that methanolic extract leaves (1.45 ± 0.07 mg/ml), methanolic extract flowers (2.75±0.03 mg/ml), were the most potent of all studied extracts of *N.nepetella*. For methanolic extract stems (7.37±0.17mg/ml), aqueous extract leaves (10.435±0.61mmg/ml), aqueous extract flowers (17.7±0.14 mg/ml) and aqueous extract stems (20.16±0.23 mg/ml).

These capacities of all extracts were less than that ascorbic acid (0.12±0.08 mg/ml) and BHA (0.09±0.03 mg/ml).

Reducing antioxidant power assay (FRAP)

The antioxidant capacity of leaf extracts was evaluated by FRAP assay because it also showed high reproducibility (Thaipong *et al.,* 2006). Reducing power is to measure the reductive ability of antioxidant and it is evaluated by the transformation of Fe^{3+} to Fe^{2+} by donating an electron, Therefore, the Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm.

However, the activity of antioxidants has been assigned to various mechanisms such as prevention of chain initiation, binding of transition-metal ion catalysts, decomposition of peroxides, and prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (Diplock, 1997; Yildirim *et al.,* 2000).

Reducing power of methanolic and aqueous extracts of *Nepeta nepetella* and standards (BHA and TROLOX) using the potassium ferricyanide reduction method were described in Fig 1. Methanolic extracts of *N.nepetella* were more potent on reducing power compared to aqueous extracts. However, the reduction power of BHA and TROLOX was relatively more pronounced than that of the different extracts *N.nepetella*.

Table. 1: Total phenolics contents of *Nepeta nepetella* methanolic and aqueous extracts of leaves, flowers and stems.

Parameter	Leaves		Flowers		Stems	
Phenols contents*	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol
	41.65±2.18	58.11± 1.24	13.18±1.04	21.42±0.96	14.98±1.07	41.725±0.38

*Expressed as mg GAE/g of dry plant material.
The data are displayed with mean ± standard deviation of twice replications.
Mean values followed by different superscript in a column are significantly different ($p<0.05$).

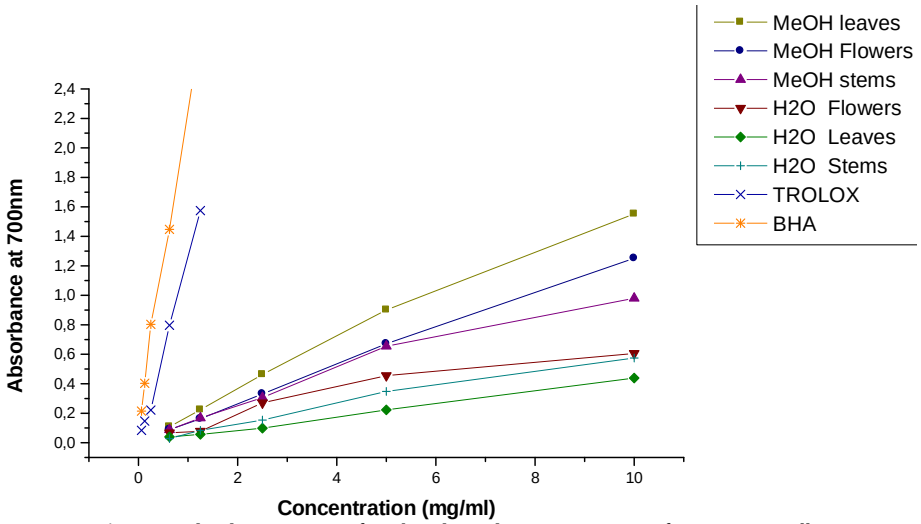

Fig. 1: Total reducing power of methanolic and aqueous extracts of *Nepeta nepetella*.

Table. 2: IC_{50} (mg/ml) values of different methanolic and aqueous extracts of *N.nepetella*.

Extract	DPPH IC_{50} (mg/ml)
Methanol leaves	1.45 ± 0.07
Aqueous leaves	10.435±0.61
Methanol flowers	2.75±0.03
Aqueous flowers	17.7±0.14
Methanol stems	7.37±0.17
Aqueous stems	20.16±0.23
Ascorbic acid	0.12±0.08
BHA	0.09±0.03

The data are displayed with mean ± standard deviation of twice replications.
Mean values followed by different superscript in a column are significantly different ($p<0.05$).

Table. 3: IC_{50} (mg/ml) values of β carotene-linoleic acid assay of methanolic and aqueous extracts of *N.nepetella*.

Extract	β carotene IC_{50}(mg/ml)
Methanol leaves	0.247 ± 0.003
Aqueous leaves	8.02 ±0.15
Methanol flowers	0.195 ± 0.007
Aqueous flowers	24.815 ± 0.26
Methanol stems	0.148 ± 0.003
Aqueous stems	24.815 ± 0.26
Gallic acid	0.435 ± 0.003
Trolox	0.242 ± 0.0028

The data are displayed with mean ± standard deviation of twice replications.
Mean values followed by different superscript in a column are significantly different ($p<0.05$).

β-Carotene –linoleic acid assay

In this model system, β-Carotene undergoes rapid discoloration in the absence of an antioxidant, which results in a reduction in absorbance of the test solution with reaction time. This is due to the oxidation of linoleic acid that generates free radicals that attacks the highly unsaturated β-Carotene molecules in an effort to reacquire a hydrogen atom. When this reaction occurs the b-carotene molecule loses its conjugation and, as a consequence, the characteristic orange color disappears. The presence of antioxidant avoids the destruction of the β -carotene conjugate system and the orange color is maintained. The obtained results are summarized in table 3. The results show that methanolic extract leaves (0.247 ± 0.03 mg/ml), methanolic extract flowers (0.195±0.03 mg/ml),and methanolic extract stems(0.148 ±0.003 mg/ml) were the most potent of all studied extracts of N.nepetella and are similar to high than Gallic acid (0.435±0.003 mg/ml) and Trolox (0.242±0.002 mg/ml). For aqueous extract leaves (8.02±0.15 mg/ml), aqueous extract flowers (24.815±0.26 mg/ml) and aqueous extract stems (24.815±0.26 mg/ml).

CONCLUSION

On basis of the results obtained in the present study, it was concluded that the methanolic extracts of leaves, stems and flowers of this species possess significant antioxidant activity in comparison with the aqueous extracts, the unequal distribution of phenolics compounds in different extracts may account for this fact. We can say that methanol is a better solvent for the full exploitation of the therapeutic potentials of *N.nepetella* since it exhibited higher antioxidant properties than aqueous extract for all parts of plant. Further studies are needed for the isolation and identification of bioactive compounds responsible for antioxidant activity.

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