Int. J. Chem. Sci.: 13(4), 2015, 1883-1892
ISSN 0972-768X
www.sadgurupublications.com

EXTRACTION AND DETERMINATION OF ANTIOXIDANTS, POLYPHENOLS, FLAVONOIDS AND ANTIOXIDANT ACTIVITY IN SOME PLANTS

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ABSTRACT

The methanolic extracts of the leaves and stem of some plants (*Calotropis procera*, *Juniperus procera*, *Lantana camara* and *Olea europaea*) were screened for polyphenols, flavoides and their free radical scavenging properties using ascorbic acid as standard antioxidant. Free radical scavenging activity was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. Generally leaves of *Calotropis procera* and *Lantana Camara*, which are the highest records of polyphenol, 943.90 and 917.60 mg/100 mL of polyphenols, respectively while leaves of *Calotropis procera* and *Juniperus procera* are highest in flavonoids, which contain 55.60 and 53.43 mg/100 g, respectively. The leaves of *Juniperus procera* and *Olea europaea* showed the highest antioxidant activity, 12.66 and 11 μg/mL, respectively, where as methanolic extract from the stem of *Lantana Camara* and *Calotropis procera* showed weak antioxidant activity, with values of 6.0 and 4.33 μg/mL. The study reveals that the use of the leaves of these plants would exert several beneficial effects by virtue of their antioxidant activity.

Key words: Antioxidants, Polyphenol, Flavonoid, Medicinal plants.

INTRODUCTION

A large number of indigenous plant species are being used as a source of herbal therapies in Middle east. Number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials. Antioxidant is any substance, which when present even at low concentrations, compared with those of an oxidizable substrate, significantly delays or prevents oxidation of substrate. The term 'oxidizable substrate' includes almost everything found in the living cells including proteins, lipids, DNA and carbohydrates[1].

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Medicinal plants are the richest bio-resource of drugs of traditional medicines, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical termediates and chemical entities for synthetic drugs[2,3]. Plant extracts and their constituents as a natural source of antioxidants have been extensively reviewed. This includes different plant organs such as seeds (soybean, peanut, cottonseed, mustard, rapeseed, rice and sesame seed), fruits (grape, citrus, black, pepper and olive), leaves (tea, rosemary, thyme and oregano) and others (sweet potato, onion and oat seedling)[4]. Plant extracts containing low molecular mass compounds have been successively used in phytotherapy since ancient times, as reactive oxygen species are involved in several diseases. It has been demonstrated that many naturally occurring species possess notable activity as radical scavengers and lipid per oxidation inhibitors[5]. In addition to plant extracts, numerous naturally occurring compounds are useful as antioxidant, ranging from alpha tocopherol and beta carotene to plant antioxidants such as phenolic compounds (tannins, flavonoids, anthrocyanins, chalcones, xanthones, liganans, depsides, and depsidones), terpenes (sesquterpens and diterpines), alkaloids, and organic sulfur compounds[6]. A large number of experiments have been carried out concerning the antioxidant activity of several plant extracts and powders[7-9]. The results of these experiments reveal that, the activity is due to several secondary metabolites especially phenolic compounds, e.g., flavonoids, tannins, etc. Research objectives of this study is extraction and determination of falvonoids, polyphenols concentration and antioxidant activity from different natural plants using DPPH reagent.

EXPERIMENTAL

Preparation of crude plant extracts

Test plants were collected locally. About 800 g of dried leaves and stems was (plant material) ground soaked in 2.5 L of 98% methanol for 8-10 days, stirring every hr using a sterile glass rod. The final extract was passed through No. 1 Whatman filter paper (Whatman Ltd., England). The filtrate obtained was concentrated under vacuum on a rotary evaporator at 40ºC and stored at 4ºC for further use. The crude extract was obtained by dissolving a known amount of the dry extract in 98% methanol to obtain a stock solution of 40 mg mL^{-1} concentration. All the plant extracts were qualitatively tested for the presence of chemical constituents (Phenols, flavonoids and antioxidant activity).

Antioxidant activity

Antioxidant activity of different extracts were tested by the DPPH assay method.

DPPH assay method

DPPH assay was based on the measurement of the scavenging ability of antioxidant towards the stable DPPH. (2,2-diphenyl-1-picryl hydrzyl) radical. It is a discoloration assay. Antioxidant activity was evaluated by the addition of the antioxidant to a DPPH solution in ethanol or methanol and the decrease in the absorbance was measured at 517 nm. Preliminary qualitative analysis was made for the presence of flavonoids and polyphenol. The methanol extract was used for antioxidant studies.

Antioxidant assay

The antioxidant activity of plant extract was determined by a *in vitro* method such as, the DPPH free radical scavenging assay method. The extract was dissolved in DMSO at the concentration of 2 mg mL. All the assays were carried out in triplicate and average value was considered.

Preparation of test and standard solution

The extract and standard, ascorbic acid (2 mg each) was separately dissolved in 5 mL of DMSO. The solutions were serially diluted with freshly distilled DMSO to obtain the lower dilutions.

DPPH radical scavenging activity

The free radical scavenging activity of the extract was measured in terms of hydrogen donating or radical scavenging ability using the stable free radical DPPH. 0.1 mm solution of DPPH in methanol was prepared and 1.0 mL of this solution was added to 3.0 mL of extract solution in water at different concentrations (50-250 μg mL^{-1}). It was incubated at 37°C for 30 min and the absorbance was measured at 517 nm UV-Visible Spectrophotometer (Systronics UV-Visible Spectophotometer 117, INDIA) against the corresponding blank solution. The assay was performed in triplicate. Ascorbic acid was taken as standard. Percentage inhibition of DPPH free radical was calculated based on the control reading, which contains DPPH and distilled water using the equation.

$$\% \text{ Inhibition} = [\text{Absorbance of control} - \text{Absorbance of test sample}/\text{Absorbance of control}] \times 100 \quad \ldots(1)$$

Determination of polyphenols

Total polyphenols were determined according to purssion blue spectrophotometric method[10], where tannic acid was used instead of catechin and total polyphenols was

calculated as tannic equivalent. Sixty mg of plant was shaken manually for 1 min with 3 mL of methanol in a test tube. The mixture was filtered, and then quickly rinsed with additional 3 mL of methanol. The content was poured at once into a funnel. The filtrate was mixed with 50 mL of distilled water and analyzed within one hr. 3 mL of 0.1 M $FeCl_3$ in 0.1 M HCl were added to 1 mL of filtrate followed immediately by timed addition of 3 mL of freshly prepared $K_3Fe(CN)_6$. The absorpance was read on a spectronic 21-Bausch & Lamb UV Spectrophotometer after 10 min from the addition of the 3 mL of 0.1 M $FeCl_3$ and 3 mL of 0.008 M $K_3Fe(CN)_6$ at 720 nm. Tannic acid was used to make the standard curve following the same steps in the sample procedure (Fig. 3.2).

The total polyphenols content was calculated as follows :

$$\text{Polyphenols (\%)} = \frac{C \times 56 \times 100}{60} \qquad \ldots(2)$$

Where: C = Concentration corresponding to absorbance

56 = Volume of extract and

60 = Sample weight

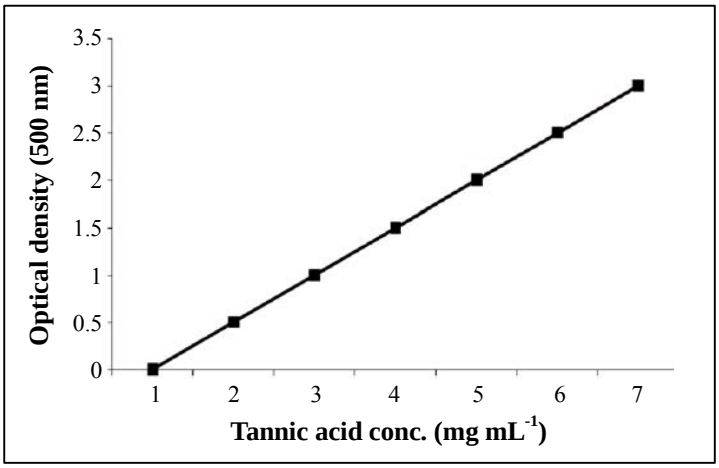

Fig. 1: Standard curve of polyphenols

Determination of total flavonoids

The determination of flavonoids was performed according to the colorimetric assay of Kim et al.[11] 4 mL distilled water was added to 1 mL of plant 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 (Fig. 3.1) was prepared with catechin and the results were expressed as mg catechin equivalents (CE mg/100 g) sample.

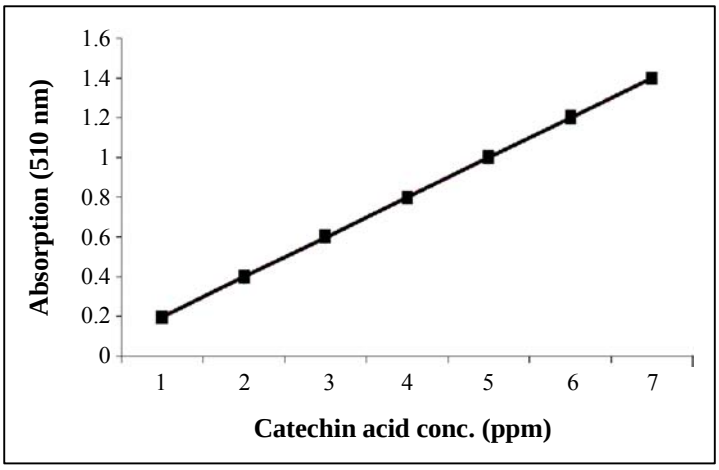

Fig. 2: Standard curve of flavonoids

Statistical analysis

The Duncan multiple range test was used to analysed the data (SAS).

RESULTS AND DISCUSSION

Natural antioxidants that are present in herbs and spices are responsible for inhibiting or preventing the deleterious consequences of oxidative stress. Plants parts contain free radical scavengers like polyphenols, flavonoids and phenolic compounds. In the present paper, polyphenol and falvonoides concentration have been determined of four different plants (*Calotropis procera*, *Juniperus procera*, *Lantana camara* and *Olea europaea*). The concentration of polyphenols and flavonoids are presented in Tables 1 and 2, respectively. the total flavonoids and polyphenolic contents in the leaves in all plants were more than in the stems. In case of polyphenols, the results of the analysis of variance showed high significant ($p = 0.001$) differences. The Duncan multiple range test proved that leaves of

Calotropis procera and *Lantana Camara*, which are the highest records of 943.90 and 917.60 mg/100 mL, respectively do not differ from each other, but they are significantly different ($p = 0.001$) from all other samples. The total content of flavonoids and phenolics are influenced by the interaction between varieties and parts of plants.

Table 1: Polyphenol determination of leaves and stems of different natural plants (mg/100 g)

Plant		
Calotropis procera	Leaves	943.90a
	Stem	245.34g
Juniperus procera	Leaves	896.52b
	Stem	556.07d
Lantana Camara	Leaves	917.60ab
	Stem	328.56f
Olea europaea	Leaves	934.43c
	Stem	549.61d

Table 2: Flavonoids determination of leaves and stems of different natural plants (mg/100 g)

Plant		
Calotropis procera	Leaves	55.6a
	Stem	31.9bc
Juniperus procera	Leaves	53.43a
	Stem	6.96e
Lantana Camara	Leaves	3.29e
	Stem	8.03e
Olea europaea	Leaves	27cd
	Stem	24.34d

In fact, many medicinal plants contain large amount of antioxidants such as polyphenols. Phenolic compounds are considered to be the most important antioxidants and are widely distributed among various plant species. These phenols play important roles in plants such as protection against herbivores and pathogens, cementing material joining phenolic polymers to cell wall polysaccharides, regulation of cell growth and cell division. The scavenging properties of *C. procera* flowers are often associated with their flavonoid, phenol and tannin, which have the ability to form stable radicals[12].

The analysis of variance showed the same level of significance ($p = 0.001$) in the experiment for the flavonoids parameter; however, separating the treatment by Duncan's method, leaves of *Calotropis procera* and leaves of *Juniperus procera*, which contain 55.60 and 53.43 mg/100 g, respectively, do not differ significantly from each other, $p = 0.001$. Flavonoides are important components of this plant, and some of its pharmacological effects could be attributed to the presence of these valuable constituents. Previous studies have shown that some flavonoids components such as quercetin had anticancer activities and were able to inhibit cancer cell growth[13-15]. Antioxidant activity was determined in vitro using DPPH reagent. Phenolic compounds are known as powerful chain breaking antioxidants. Phenolic acids, flavonoids and tannins are the most commonly found polyphenolic compounds in plant extracts[16]. The antioxidant activity of phenolics plays an important role in the absorption or neutralization of free radicals[17].

Among the eight extracts and standard tested for the *in vitro* antioxidant activity using the DPPH method, it was observed that methanolic extracts of the leaves had higher activity than that of the stems (Table 3). The leaves of *Juniperus procera* and *Olea europaea* showed the highest antioxidant activity, 12.66 and 11 µg/mL, respectively (Table 3). The methanolic extract from the stem of *Lantana Camara* and *Calotropis procera* showed weak antioxidant activity, with values of 6 and 4.33 µg mL^{-1}, respectively. The phytochemical tests indicated the presence of alkaloids, glycosides, tannins, and flavonoids in the crude methanolic extract. Several of such compounds are known to possess potent antioxidant activity[18,19]. Some of these constituents have already been isolated from these plants. Hence, the observed antioxidant activity may be due to the presence of any of these constituents[20]. The antioxidant activity is presented in the Fig. 3, which showed the percentage inhibition of the leaves extract, which ranged between 60-70%, higher than that of stems extract, which was range 40-50%. It is comparable with the standard antioxidant activity of ascorbic acid (58%).

Table 3: Table *in vitro* antioxidant activity of methanolic extract of plants parts (mg/mL)

Plant		
Calotropis procera	Leaves	6.67c
	Stem	4.33c
Juniperus procera	Leaves	12.66c
	Stem	8.33c
Lantana Camara	Leaves	8.33c
	Stem	6.00c
Olea europaea	Leaves	11.00d
	Stem	10.61d
Ascorbic acid	8.2	

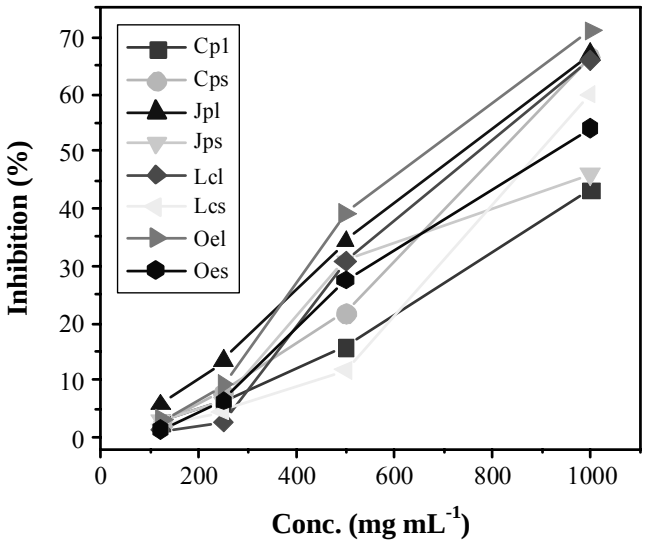

Fig. 3: DPPH free radical scavenging activity of standard ascorbic acid and methanolic plant extracts

Cpl = *Calotropis procera* leaves, Cps = *Calotropis procera* stem, Jpl = *Juniperus procera* leaves, Jps - *Juniperus procera* stem, Lcl = *Lantana camara* leaves, Lcs = *Lantana camara* stems Oel = *Olea europaea* leaves, Oes = *Olea europaea* stem

The free radical scavenging activity of methanolic extract was confirmed in the present investigation. However, these chemical constituents present in the extract are responsible for this activity[20]. This property may be due to its antioxidant activity. This study reveals that the use of the leaves of these plants would exert several beneficial effects by virtue of their antioxidant activity.

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Revised : 13.11.2015 *Accepted : 16.11.2015*