AkiNik

Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

ISSN 2278-4136
ISSN 2349-8234
JPP 2014; 2 (5): 10-14

Received: 09-11-2013
Accepted: 16-11-2013

Deepa R. Hebbar
Department of Studies in Botany, University of Mysore, Manasagangotri, Mysore – 570 006, Karnataka, India.
Email: deepahebbar2011@gmail.com

Dr. M. S. Nalini
Department of Studies in Botany, University of Mysore, Manasagangotri, Mysore – 570 006, Karnataka, India.
Email: nmsomaiah@gmail.com

Correspondence:
Dr. M. S. Nalini
Assistant Professor, Department of Studies in Botany, University of Mysore, Manasagangothri, Mysore-570 006, Karnataka, India.

Evaluation of phytochemicals, total phenolics and antioxidant activities of *Schefflera* spp. (Araliaceae) from southern India

Deepa R. Hebbar, M. S. Nalini

ABSTRACT

This study is aimed to evaluate the phytoconstituents and antioxidative activities in leaves and bark of *S. racemosa* and *S. stellata*. Phytochemical screening conducted for both plant species indicated the presence of saponins, tannins, flavonoids, terpenoids, cardiac glycosides and reducing sugars. *S. racemosa* leaf extracts showed positive results for the presence of steroids. The total phenolic content was measured by the Folin-Ciocalteau assay. The total antioxidant capacity was estimated spectrophotometrically by 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), ferric reducing antioxidant power (FRAP) and reducing power assay. The aqueous extracts exhibited maximum antioxidant activity compared to the nonpolar solvents. A high phenolic content of 149.97±0.08 mg GAE/g of dry extract and potent antioxidant activity (IC_{50} value = 12.61±0.00 µg/ml) was observed in aqueous leaf extracts of *S. stellata* when compared to *S. racemosa*. High reducing ability in the FRAP assay and reducing power assay was also observed in the aqueous leaf extracts of *S. stellata.*

Keywords: Phytochemicals, Phenolic content, Antioxidant activity, *Schefflera stellata* and *Schefflera racemosa*.

1. Introduction

Schefflera is a large genus with over 650 species of polygamous or dioecious trees, tall shrubs or climbers distributed in tropical and subtropical regions. Phytochemical studies on plants in the *Schefflera* genus have revealed the presence of triterpenes, triterpenoid glycosides and saponins [1]. The ethno medicinal uses of *Schefflera* include treatment for asthma, liver diseases, rheumatism, arthritis, sprains, fracture, stomach pain, antipyretic, anti-inflammatory, analgesic, migraine and general tonic [2]. *Schefflera stellata* (Gaertn.) Harms. and *Schefflera racemosa* (Wight.) Harms. belong to the family Araliaceae. *S. stellata* is a small tree distributed in peninsular India and Sri Lanka [3]. In Kerala state of southern India, it is found in Silent Valley and Agasthyamalai Hills [1]. Ethno medicinal uses of *S. stellata* revealed that it is used to cure neurological weakness [4] and a belief that the bark ash is used to control evil spirits [5]. Sabulal *et al*. (2008) determined the antimicrobial activity of *S. stellata* from volatile oils of root, stem and leaves. *S. racemosa* is a medium sized tree, with compound leaves and flowers in lateral panicled racemes and is endemic to the Western Ghats. Plants like *Polyscias fruticosa* (L.) Harms, *Macropanax dispermus* (Blume) Kuntze, *Acanthopanax trifoliatus* (L.), *S. actinophylla* (Endl.) Harms, *S. arboricola* (Hayata) Merr. belonging to the family Araliaceae, are known to have antioxidant activity[6, 7]. Many human diseases are caused by oxidative stress that results from the imbalance between the formation and neutralization of pro-oxidants [8]. The oxidation induced by reactive oxygen species (ROS) can result in cell membrane disintegration, membrane protein damage and DNA mutation, which can further initiate or propagate the development of many diseases, such as cancer, liver injury and cardiovascular disorders [9]. Therefore, antioxidants with free radical scavenging activities may have great relevance in the prevention and therapeutics of diseases in which oxidants or free radicals are implicated [10]. In this respect, polyphenolic compounds like flavonoids and phenolic acids, commonly found in plants have been reported to have multiple biological effects, including antioxidant activity [11-14]. Currently the possible toxicity of synthetic antioxidants has been criticized. Thus the interest in natural antioxidant, especially of plant origin has greatly increased in recent years [15]. Recently, we have reported the presence of phytochemicals, phenolic content and antioxidant activities of *S. venulosa* and *S. wallichiana* collected from the Western Ghats (in press).

Therefore, in addition to the earlier evaluation of phytochemicals, the present study is framed to assess the antioxidant activity from solvent extracts of leaves of *S. racemosa* and bark and leaves of *S. stellata.*

2. Materials and methods

2.1. Collection of Plant material

S. stellata was collected from Chamundi Hills, of Mysore and *S. racemosa* from forests of Kodagu, southern Karnataka. The plant parts such as leaves of *S. racemosa* and leaves and bark of *S. stellata* were excised with pliers as well as machete and placed in ziplock polythene bags, labeled, brought to the laboratory and processed for further use. A herbarium specimen of both the plants is maintained in the Herbarium collection of the Department of Botany, University of Mysore, Manasagangothri, Mysore.

2.2. Preparation of extracts

The collected plant parts were dried under shade as well as at 40 ˚C in a hot air oven prior to blending to remove the water content and powdered. The powdered materials weighing approximately 500 g were placed in polyethylene zip lock covers for further use. Fifty grams of shade dried leaf and flower powder were extracted with Soxhlet apparatus in the order of polarity (Hexane> Chloroform> Ethyl acetate>Ethanol>Methanol>Water).

2.3. Phytochemical Screening

Qualitative phytochemical analysis of the crude powder and different solvent extracts were determined using standard procedures [16, 17].

2.3.1 Tannins: One ml of the extract was stirred with one ml of Ferric chloride. A greenish black precipitate indicated the presence of tannins.

2.3.2 Alkaloids: One ml of the extract was stirred with five ml dil. Hydrochloric acid on a steam bath, filtered and One ml of Dragendorff's reagent was added. Orange red precipitate indicated the presence of alkaloids.

2.3.3 Saponins (Frothing test): 0.2 ml of the extract was mixed with five ml of distilled water, shaken for 20 min. Persistence of foam indicated the presence of saponins.

2.3.4 Cardiacglycosides (Keller-Kiliani test): Five ml of the extract was treated with two ml of glacial acetic acid containing one drop of ferric chloride solution. To this one ml of conc. Sulphuric acid was added. A brown ring in the interface indicates a deoxy sugar characteristic of cardenolides.

2.3.5 Reducing sugars: Two ml of crude plant extract was mixed with five ml of distilled water and filtered. The filtrate was boiled with 3-4 drops of Fehling's solution A and B for 2 min. An orange red precipitate indicated the presence of reducing sugars.

2.3.6 Terpenoids (Salkowski test): 0.2 g of the extract was mixed with two ml of chloroform and one ml of conc. Sulfuric acid was carefully added to form a layer. A reddish brown color in the interface indicated positive results for the presence of terpenoids.

2.3.7 Flavonoids (Ferric chloride test): 0.2 ml of the extract was added to Ferric chloride (10%) and the mixture was shaken. A wooly brownish precipitate indicated the presence of flavonoids.

2.3.8 Phlobatannins: Formation of a red precipitate when the aqueous extract of plant sample was boiled with aqueous HCl (1%) indicated the presence of phlobatannins.

2.3.9 Anthraquinones: 0.5 g of the extract was boiled with HCl (10%) and filtered. Few drops of ammonia (10%) were added to the mixture and heated. Formation of rose-pink color indicated the presence of anthraquinones.

2.3.10 Steroids: Two ml of sample, acetic anhydride and a few drops of sodium hydroxide (10%) were mixed. A green ring indicated the presence of steroids.

2.4. Determination of antioxidant activity

2.4.1 Estimation of total phenolic content

The total phenolic content of plant extracts was estimated by Folin-Ciocalteau (FC) method as per the procedure of Volluri *et al.* (2011) with some modifications. Different concentrations of the plant extracts (50-250 µg/ml) and the standard gallic acid (5-25 µg/ml) were taken in test tubes and one ml of FC reagent was added, after 3-5 min two ml of sodium carbonate (20%; w/v) was added and the mixture was allowed to stand for 30-45 min under dark. After the prescribed period of incubation the absorbance was taken at 765 nm in a spectrophotometer (T-60 UV-visible spectrophotometer, TTL-Technologies). The concentration of total phenolics was expressed in terms of gallic acid equivalence (mg GAE/g).

2.4.2 DPPH Free Radical scavenging activity:

Radical scavenging activity by DPPH method was evaluated according to the procedure of Pannangpetch *et al*. (2007). Aliquots of standard (5-25 µg/ml) and plant extracts (20-100 µg/ml) were taken and the volume was made up to 250 µl using distilled water or methanol. To this one ml of DPPH was added and the tubes were kept under dark for 10 min. The incubated mixture was read at the absorbance of 517 nm using a spectrophotometer. Per cent radical scavenging was calculated based on the extent of reduction in the color.

Per cent radical scavenging activity = $A_c - A_s / A_c \times 100$

Where A_c = absorbance of the control; A_s = absorbance of the sample.

2.4.3 Total antioxidant power by Ferric reducing antioxidant power (FRAP) assay

Total antioxidant activity by FRAP assay was determined by the modified method of Benzie and Strain (1996). The stock solutions of Acetate buffer (300 mM), 2,4,6-tripyridyl-S-triazine(TPTZ, 10 mM) in HCl (40 mM) and $FeCl_3.6H_2O$ (20 mM) were prepared. From this stock, a fresh working solution was prepared by adding 25 ml of acetate buffer, 2.5 ml of TPTZ and 2.5 ml of $FeCl_3.6H_2O$. The temperature of the solution was raised to 37 °C before use. Different concentration of plant extracts (20-100 µg/ml) as well as the standard ascorbic acid (5-25 µg/ml) was taken in test tubes and the volume was made up to 3000 µl with a freshly prepared FRAP solution and incubated for 30 min under dark condition. The absorbance was measured at 593 nm using a spectrophotometer. Based on the concentration of plant extracts, the color changes from light brown to various shades of blue. All tests were performed in triplicates. The results are expressed in µM Fe (II) /g dry mass.

2.4.4 Determination of reducing power

The reducing power of the bark extracts were evaluated according to the procedure of Yen and Chen (1995) with some modifications. The concentration of plant extracts and the standard ascorbic acid were prepared as mentioned earlier. Different concentrations of standard (5-25 µg/ml) and plant extracts (20-100 µg/ml) were taken in test tubes and the volume was made up to 500 µl by using phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide (1%). The tubes were kept in incubation at 50 °C for 20 min of incubation. After this period, 2.5 ml of trichloroacetic acid (10%) was added and centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride (0.1%) was added. The absorbance was measured at 700 nm against a blank sample. The reduction of yellow color to the various shades of green and blue depends on the reducing power capacity of each tested extract. Increased absorbance of the reaction mixture indicated increased reducing power. Butylated Hydroxytoluene (BHT) was used as the standard.

2.5. Statistical analysis: All the experiments were done in triplicates. Statistical analysis was done using SPSS program (16.0 version). One way ANOVA and post hoc tests were conducted and Probability (*P*) value less than 0.05 was considered as significantly different.

3. Results and Discussion

3.1. Phytochemical analysis

In the present study, the phytochemical screening of *S. racemosa* leaf extracts showed positive results for saponins, tannins, flavonoids, cardiacglycosides, steroids, reducing sugars and terpenoids (Table 1), whereas *S. stellata* leaf and bark extracts showed the presence of saponins, tannins, flavonoids, terpenoids, cardiacglycosides and reducing sugars mostly in polar and semi polar solvents (Table 2). The term 'phytochemicals' refers to a wide variety of compounds made by plants, and is mainly used to describe those compounds that may affect human health. Secondary metabolites are the classes of compounds which are known to show curative activity against several human ailments and therefore could explain the use of traditional medicinal plant for the treatment of some illnesses.

Table 1: Phytochemical constituents of *S. racemosa* leaf in solvent extracts.

Solvents / Tests	Hexane	Chloroform	Ethyl acetate	Ethanol	Methanol	Aqueous
Saponins	-	-	-	-	+	+
Tannins	-	-	-	-	+	+
Flavonoids	-	-	-	-	+	+
Terpenoids	-	-	+	-	-	+
Alkaloids	-	-	-	-	-	+
Steroids	-	-	-	-	-	+
Phlobatannins	-	-	-	-	-	-
Cardiacglycosides	-	-	-	-	+	-
Anthraquinones	-	-	-	-	-	-
Reducing Sugars	-	+	-	-	-	-

Note: + = present, - = absent

Table 2: Phytochemical constituents of *S. stellata* plant parts in solvent extracts.

Solvents / Tests	Hexane		Chloroform		Ethyl acetate		Ethanol		Methanol		Aqueous	
	L	B	L	B	L	B	L	B	L	B	L	B
Saponins	+	-	+	-	+	+	+	+	+	+	+	+
Tannins	-	-	-	-	-	+	+	+	+	+	+	+
Flavonoids	-	-	-	-	-	+	+	+	+	+	+	+
Terpenoids	-	-	-	+	-	+	+	+	-	+	+	+
Alkaloids	-	-	-	-	-	-	-	-	-	-	-	-
Steroids	-	-	-	-	-	-	-	-	-	-	-	-
Phlobatannins	-	-	-	-	-	-	-	-	-	-	-	-
Cardiacglycosides	+	-	-	-	-	-	+	-	-	+	+	+
Anthraquinones	-	-	-	-	-	-	-	-	-	-	-	-
Reducing Sugars	-	-	-	-	-	-	+	-	-	-	+	-

Note: + = present, - = absent, L = leaf and B = bark

Table 3: Total phenolic content and antioxidant assays of *S. racemosa* leaf in solvent extracts. *

	TPC	DPPH	FRAP	Reducing Power
	(mg GAE/g extract)	(IC_{50} = µg/ml)	(µM Fe (II) /g extract)	(OD at 700nm)
Leaf extracts				
Hexane	0.56±0.03	150.03±0.03	0.41±0.03	0.08±0.04
Chloroform	0.87±0.03	145.03±0.03	0.77±0.03	0.09±0.04
Ethyl acetate	6.98±0.04	110.00±0.00	1.19±0.06	0.09±0.04
Ethanol	1.81±0.03	137.07±0.06	2.35±0.03	0.11±0.04
Methanol	22.05±0.02	118.03±0.05	92.40±0.05	0.43±0.03
Aqueous	67.74±0.03	24.03±0.03	193.10±0.03	1.32±0.03

* All values are expressed as mean ± standard error mean (SEM) (n=3).

There are chemical compounds (phenolic compounds, alkaloids, terpenoids, steroids, quinones, saponins, etc.) with complex structures and with more restricted distribution than primary metabolites [18]. The wide molecular diversity of secondary metabolites throughout the plant kingdom represents an extremely rich biogenic resource for the discovery of novel drugs and for developing innovative drugs.

Table 4: Total phenolic content and antioxidant assays of *S. stellata* in solvent extracts.*

	TPC	DPPH	FRAP	Reducing Power
	(mg GAE/g extract)	(IC_{50} = µg/ml)	(µM Fe (II) /g extract)	(OD at 700nm)
Leaf extract				
Hexane	1.33±0.05	159.90±0.05	0.41±0.03	0.08±0.07
Chloroform	1.74±0.03	157.03±0.03	6.19±0.03	0.09±0.07
Ethyl acetate	2.28±0.05	149.17±0.03	2.35±0.03	0.09±0.07
Ethanol	28.28±0.05	43.06±0.03	92.40±0.05	0.49±0.07
Methanol	2.03±0.05	107.10±0.05	3.24±0.03	0.09±0.07
Aqueous	149.97±0.08	12.61±0.00	193.33±0.03	1.51±0.05
Bark extract				
Hexane	1.72±0.05	161.21±0.00	0.77±0.03	0.09±0.04
Chloroform	1.15±0.01	159.03±0.03	1.19±0.06	0.08±0.04
Ethyl acetate	15.31±0.03	28.06±0.03	81.40±0.05	0.30±0.07
Ethanol	10.62±0.03	45.80±0.00	34.10±0.00	0.17±0.07
Methanol	5.99±0.03	36.51±0.00	50.76±0.03	0.20±0.06
Aqueous	115.83±0.08	13.50±0.00	121.43±0.03	0.98±0.10

* All values are expressed as mean ± standard error mean (SEM) (n=3).

3.2. Total phenolic content and antioxidant activity

The total phenolic content expressed as GAE (gallic acid equivalence) in mg/g of dry plant extract varied from 1.15±0.01mg GAE/g extract to 149.97±0.08 mg GAE/g extract in *S. stellata* (Table 4) and from 0.56±0.03 mg GAE/g extract to 67.74±0.03 mg GAE/g extract in *S. racemosa* (Table 3). The results from this study, presented in table 3 and 4, strongly suggest that phenolic compounds are important components of this plant, and some of their pharmacological effects could be attributed to the presence of these valuable constituents. The phenolic compounds have been reported to be significantly associated with the antioxidant activity of plant and food extracts mainly because of their redox properties, allowing them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, hydroxyl radical quenchers and metal chelaters [19].

The DPPH radical is stable and free. It is commonly used as a substrate to evaluate *in vitro* antioxidant activity of extracts of fruits, vegetables and medicinal plants [20]. Antioxidants can scavenge the radical by hydrogen donation, which causes a decrease of DPPH absorbance at 517 nm. The concentration of sample at which the inhibition percentage reaches 50% is defined as the IC_{50} value. Thus, IC_{50} values are negatively related to the antioxidant activity, the lower IC_{50} value indicates highest antioxidant activity of the tested sample. Out of the different solvent extracts of two plant species, aqueous leaf (IC_{50} value = 12.61±0.00 µg/ml) and bark extracts (IC_{50} value = 13.50±0.00 µg/ml) of *S. stellata* showed potent antioxidant activity when compared to the standard antioxidant, ascorbic acid (IC_{50} value = 11.5±0.00 µg/ml). Low antioxidant IC_{50} value was seen in hexane bark extracts (IC_{50} value = 161.21±0.00 µg/ml) of *S. stellata*. However, *S. racemosa* showed comparatively less antioxidant activity (IC_{50} value = 24.03±0.03 µg/ml).

The antioxidant potential of both the plant species was ascertained from FRAP assay based on their ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II). TPTZ-Fe (II) has an intensive blue color and can be monitored at 593 nm [21]. The reducing ability of various solvent extracts was examined and the maximum reducing ability at 100 µg/ml was seen in aqueous leaf extracts of *S. stellata* (193.33±0.03 µM Fe (II) /g of extract) and also in the aqueous leaf extracts of *S. racemosa* (193.10±0.03 µM Fe (II) /g of extract) whereas minimum activity was seen in hexane leaf extracts of both the plant species (0.41±0.03 µM Fe (II) /g of extract).

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity [22]. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation process. They can act as primary as well as secondary antioxidants [23]. In this assay, the yellow color of the test solution changes to various shades of green and blue depending on the reducing power of each compound. The presence of reducers cause the conversion of the Fe^{3+}/ferricyanide complex present in the extracts to the ferrous form. By measuring the formation of Pearl's Prussian blue color at 700 nm, it is possible to determine the concentration of Fe^{3+} ion. In the present study, the reductive effect of *S. racemosa* and *S. stellata* extracts increased with increase in extract concentration. High absorbance at 700 nm indicates a high reducing power. Maximum reducing power was seen in the aqueous leaf extracts of *S. stellata* (1.51±0.05 O.D) and the aqueous leaf extracts of *S. racemosa* (1.32±0.03 O.D) (Tables 3 and 4) when compared to standard (3.08±0.15 O.D) whereas low reducing power was seen in the hexane leaf extract of *S. racemosa* (0.08±0.04 O.D) and *S. stellata.* (0.08±0.07 O.D).

4. Conclusion

The results of the present study have indicated that the aqueous extract of *S. stellata* contains high amount of phenolic compounds and is associated with good antioxidant activity in comparison to *S. racemosa*. The high scavenging property of *S. stellata* may be due to hydroxy groups present in the phenolic compounds. Hence *S. stellata* plant may be a potential source of natural antioxidants.

5. Acknowledgement

Thanks are due to University Grants Commission (UGC) for financial assistance in the form of Faculty Improvement Program

(FIP) awarded to Mrs. Deepa R. Hebbar (No. FIP/11th plan/KAMY004 TF 08). The authors are also thankful to the University of Mysore, Manasagangotri, for providing research facilities.

6. References

1. Sabulal B, George V, Pradeep NS, Dan M. Volatile oils from the root, stem and leaves of *S. stellata* (Gaertn.) Harms (Araliaceae): Chemical characterization and antimicrobial activity. Journal of Essential Oil Research 2008; 20(1):79-82.
2. Ragasa CY, Lim K. Secondary metabolites from *Schefflera odorata* Blanco. Philippine Journal of Science 2005; 134(1):63-67.
3. Mohanan N, Sivadasan M. Flora of Agasthyamalai, Bishen Singh and Mahendra Pal Singh, Dehra Dun 2002, 302.
4. Rao JK, Reddi TVVS, Kumar OA. Ethnobotany of stem bark of certain plants of Visakhapatnam District, Andra Pradesh. Current Botany 2011; 2(5):1-6.
5. Kadavul K, Dixit AK. Ethnomedicinal studies of the woody species of Kalrayan and Shervarayan Hills, Eastern Ghats, Tamil Nadu. Indian Journal of Traditional Knowledge 2009; 8(4):592-597.
6. Chanwitheesuk A, Teerawutgulrag A, Rakariyatham N. Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand. Food Chemistry 2005; 92:491-497.
7. Moussa AM, Emam AM, Diab YM, Mahmoud ME, Mahmoud AS. Evaluation of antioxidant potential of 124 Egyptian plants with emphasis on the action of *Punica granatum* leaf extracts on rats. International Food Research Journal 2011; 18:535-542.
8. Hazra B, Santana B, Nripendranath M. Antioxidant and free radical scavenging activity of *Spondias pinnata*. Journal of Bio-organic and Medicinal Chemistry 2008; 8:63.
9. Liao KL, Yin MC. Individual and combined antioxidant effects of seven phenolic agents in human erythrocyte membrane ghosts and phosphotidylcholine liposome systems: Importance of the partition coefficient. Journal of Agricultural and Food Chemistry 2000; 48:2266-2270.
10. Soares JR, Dinis TCP, Cunha AP, Almeida LM. Antioxidant activities of some extracts of *Thymus zygis*. Free Radical Research 1997; 26:469-478.
11. Vinson JA, Dabbagh YA, Serry MM, Jang J. Plant flavonoids, especially tea flavonols are powerful antioxidants using an *in vitro* oxidation model for heart disease. Journal of Agricultural and Food Chemistry 1995; 43:2800-2802.
12. Brown JE, Rice-Evans CA. Luteolin–rich artichoke extract protects low density lipoprotein from oxidation *in vitro*. Free Radical Research 1998; 29:247-255.
13. Gil MI, Ferreres F, Tomas-Barberan FA. Effect of postharvest storage and processing on the antioxidant constituents (lavonoids and vitamin C) of fresh-cut Spinach. Journal of Agricultural and Food Chemistry 1999; 47:2213-2217.
14. Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M. Antioxidant activity of plant extracts containing phenolic compounds. Journal of Agricultural and Food Chemistry 1999; 47:3954-3962.
15. Jayaprakash GK, Rao LJ. Phenolic constituents from lichen *Parmotrema stuppeum*. Food Control 2000; 56:1018-1022.
16. Harborne JB. Phytochemical methods. Edn 2, Champion and Hall Publishers, London, 1984, 84-196.
17. Trease G.E, Evans W.C. Pharmacology. Edn 15, Saunders 2002, 214-393.
18. Zohra SF, Meriem B, Samira S, Alsayadi-Muneer MS. Phytochemical Screening and identification of some compounds from Mallow. Journal of Natural Products and Plant Resources 2012; 2(4):512-516.
19. Gupta S, Prakash J. Studies on Indian green leafy vegetables for their antioxidant activity. Plant Foods for Human Nutrition 2009; 64:39-45.
20. Krout AA, Mighri H, Krid M, Thabet F, Turki H, El-Jani H, Neffati M. Chemical composition and antioxidant activity of aqueous extracts of some wild medicinal plants in Southern Tunisia. International Journal of Life Science and Medical Science 2012; 2(1):1-4.
21. Benzie IFF, Strain JJ. Ferric reducing ability of plasma (FRAP) a measure of antioxidant power: The FRAP assay. Annals of Biochemistry 1996; 239:70-76.
22. Oktay M, Gulcin I, Kufrevioglu OI. Determination of in vitro antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. LWT - Food Science and Technology, 2003, 36:263-271.
23. Chanda S, Dave R. *In vitro* models for antioxidant activity evaluation and some medicinal plants pocessing antioxidant properties. An overview. African Journal of Microbiology Research 2009; 3(13):981-996.