Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(3): 972-977
Received: 28-03-2019
Accepted: 30-04-2019

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Phytochemical screening and antioxidant activity of *Pseuderanthemum malabaricum*

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Abstract
Pseuderanthemum malabaricum which is commonly called as the Malabar false eranthemum is widely distributed in the state of Andhra Pradesh, India. Even though the plant is widely distributed there is a lack of scientific evidence for its potential phytochemicals and their capability of medicinal usage. Hence the present study is focused on the qualitative and quantitative screening of phytochemicals present in Hexane, Chloroform, Methanol, and Aqueous extracts of *P. malabaricum* and to study the antioxidant activity by analyzing the capacity to scavenge 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radicals and ferric reducing antioxidant power FRAP assay. The results showed *P. malabaricum* is the potential source of a wide variety of phytochemicals such as Alkaloids, Phenols, flavonoids, Steroids *etc*. The leaves Methanol extract showed the highest DPPH free radical scavenging activity with an IC_{50} value of 311.51 as compared with standard Ascorbic acid which showed 109.73.

Keywords: *Pseuderanthemum malabaricum*, DPPH assay, FRAP assay, phytochemical screening, medicinal plant

Introduction
Reactive Oxygen Species ROS and Reactive Nitrogen Species RNS are the byproducts of cellular metabolism which are regulated by antioxidants [1]. Failure of cellular mechanisms in mitigating damage from ROS leads to the Oxidation stress which is one among the major causative agents of Cancer. The plants are the natural gifts to the human with a rich source of phytochemicals [2]. *Pseuderanthemum malabaricum* is a plant that is widely distributed in Andhra Pradesh, India. The plant belongs to Acanthaceae family. The plant is commonly called as Malabar false eranthemum. The plant grows up to 2 feet tall and woody. The leaves are elliptic or ovate without any hairs and narrowed at both ends. The flowers having a size and shape of 4-9 inches spikes and exhibit characteristic nature of dotted spots on one of the petals. The image of the plant is represented in Image-1. Even though the plant is widely distributed and well known for its potential therapeutic applications the plant is less explored scientifically. In order to understand its potential therapeutic applications through scientific evidence, preliminary phytochemical screening was carried out followed by analyzing its DPPH scavenging assay and FRAP assay to analyze the antioxidant activity.

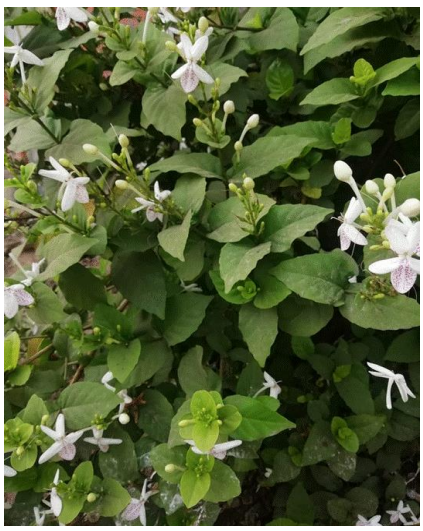


Image 1: *Pseuderanthemum malabaricum* plant with leaves and flowers

Materials and Methods

Collection of plant material

In this study, the *Pseuderanthemum malabaricum* was collected from Guntur District, Andhra Pradesh. After authenticated by taxonomist, the leaves were washed with tap water for 2-3 times thoroughly to remove dirt and debris. The leaves were shade dried and grounded to get a fine powder with the help of a mechanical blender. The powder obtained was labeled and stored for further use in a cool and dry place.

Soxhlet extraction: The 200gms of dry plant leaves powder was taken in soxhlet apparatus and run for approximately 30 cycles with different solvents such as Hexane, Chloroform, Methanol and Aqueous (Water) solvents. The temperature was maintained as per the requirement of solvent and the method of successive soxhlet extraction was applied. The obtained solvents were subjected to Rotary evaporation, labeled appropriately and used for further analysis and tests.

Phytochemical analysis

Preliminary phytochemical screening (Qualitative analysis)

Hexane, Chloroform, Methanol and Aqueous extracts were obtained after successive soxhlet extraction from the leaves of *P. malabaricum*. They was subjected to preliminary phytochemical analysis to test the presence of various phytochemicals such as Alkaloids, Phenolics, Flavonoids, Anthraquinones, Steroids, Tannins, Saponins, Anthocyanins, Leucoanthocyanins, Coumarins and Reducing Sugars through standard tests and protocols [3].

Detection of Alkaloids

50mg of solvent free extract was dissolved in few mL of dilute Hydrochloric acid and stirred well. The resultant solution was filtered and used for the analysis.

Mayer's Test: To the few mL of the filtrate Mayer's reagent (Potassium Mercuric Iodide) was added. Formation of a creamy color precipitate indicates the presence of alkaloids.

Wagner's Test: To the few mL of filtrate, Wagner's reagent (Iodine in Potassium Iodide) was added and mixed well. Reddish brown precipitate formation indicates the presence of alkaloids.

Dragendroff's Test: To the few mL of filtrate, Dragendroff's reagent (solution of Potassium Bismuth Iodide) was added. Yellow precipitate formation indicates the presence of alkaloids.

Hager's Test: To the few mL of filtrate, Hager's reagent (saturated Picric acid solution) was added. A characteristic formation of yellow precipitate indicates the presence of alkaloids.

Detection of phenols

Ferric chloride test: The filtered solution of the extract was treated with three drops of freshly prepared 1% Ferric Chloride and Potassium Ferro cyanide. Formation of bluish-green colour indicates the presence of phenols.

Detection of flavonoids

Alkaline reagent test: To the extract few drops of Sodium Hydroxide solution was added and mixed well. Formation of yellow colour is observed, which disappears after addition of dilute HCl, indicates the presence of flavonoids.

Lead acetate test: To the extract few drops of Lead Acetate solution was added and mixed well. A yellow colour precipitate was observed, which indicates the presence of flavonoids.

Detection of anthraquinones

Free anthraquinones test (Borntrager's test): 100 mg of the extract was treated with 10mL of Benzene, shaken vigorously, filtered and added with 5mL of 10% Ammonia solution to the filtrate. The mixture was shaken well and the formation of a pink, red, or violet colour in the ammonia (lower) phase indicates the presence of free anthraquinones.

Detection of phytosterols

Salkowski's Test: The extract was dissolved in 2mL of Chloroform in a test tube. Concentrated Sulfuric acid was carefully added along the wall of the test tube to form a lower layer. A reddish brown color at the interface indicates the presence of a steroid ring (*i.e*. the aglycone portion of the glycoside).

Detection of tannins

Ferric chloride test: 10mg of the extract was dissolved in double distilled water and the resultant solution was clarified by filtration to which 10% Ferric Chloride solution was added to the clear filtrate. This was observed for a change in color to bluish black.

Lead acetate test: The extract was dissolved in double distilled water and to that 10% Lead Acetate solution was added. Appearance of yellow precipitate indicates the presence of tannins.

Potassium dichromate test: The extract was dissolved in water and to it few drops of strong Potassium dichromate solution was added. Formation of yellow colour precipitate indicates presence of phenolic compounds and tannins.

Detection of saponins

Froth test: The extract was diluted with distilled water to 20mL and this was shaken in a graduated cylinder for 15minutes. Formation of 1cm layer of "honey comb" froth indicates the presence of saponins.

Detection of anthocyanins: To the extract, 2mL of 2 N HCl and Ammonia was added and mixed well. Initial appearance of pink-red colour turning into blue-violet colour on incubation indicates the presence of anthocyanins.

Detection of leucoanthocyanins: To the extract 5mL of Isoamyl Alcohol was added and mixed well. Appearance of red upper layer colour indicates the presence of leucoanthocyanins.

Detection of coumarins: To the extract, 3mL of 10% NaOH was added and mixed well. Formation of yellow colour indicates the presence of coumarins.

Detection of reducing sugars: The extract was dissolved in double distilled water and filtered. The filtrate obtained was used to test for the presence of carbohydrates.

Keller kiliani test (For deoxy sugars in cardiac glycosides): 50mg of each extract was dissolved in 2mL of chloroform. H_2SO_4 was added to form a layer and formation of a coloured ring at interphase was noted. Brown ring at interphase is characteristic of deoxy sugars in cardenolides.

Fehling's test: Filtrates of the plant extract was hydrolyzed with dilute HCl, neutralized with alkali followed by heating with Fehling's A & B solutions. A red precipitate indicates the presence of reducing sugars.

Quantitative estimation of phyto constituents

Determination of total phenol content

The amount of total phenol content, in different solvent extracts of *P. malabaricum* was determined by Folin-Ciocalteu's reagent method. 0.5mL of extract and 0.1mL (0.5 N) Folin- Ciocalteu's reagent was mixed and the mixture was incubated at room temperature for 15minutes. Then, 2.5mL saturated sodium carbonate solution was added, mixed well and further incubated for 30minutes at room temperature. The absorbance was measured at 760nm using double beam UV Visible Spectrophotometer. Gallic acid was used as a positive control. Total phenol values are expressed in terms of Gallic acid equivalent (mg/g of extracted compounds) [4].

Determination of total flavonoid content

The amount of flavonoid content in different solvent extracts of *P. malabaricum* was determined by the aluminium chloride colourimetric method [5]. To 1mL of sample at a concentration of 1mg/mL, 0.5mL of aluminium chloride (1.2%) and 0.5mL of potassium acetate (120mM) was added and incubated at room temperature for 30 min. The absorbance was measured at 415nm. Rutin was used as a positive control. The flavonoid content is estimated in terms of rutin equivalent (mg/g of the extracted compound).

Antioxidant activity

DPPH (2, 2-diphenyl-l-picryl hydrazyl) radical scavenging assay

The antioxidant activity of the plant extracts was estimated using the DPPH radical scavenging activity. A stock solution of Hexane, Acetone, Methanol, water extracts of *P. malabaricum* and standard ascorbic acid were prepared in the concentration of 100mg/100mL (1mg/mL). From each stock solution 1mL, 2mL, 3mL, 4mL and 5mL was taken in five test tubes respectively. With same solvent the contents were diluted to10mL to obtain a working concentration of 100µg/mL, 200µg/mL, 300µg/mL, 400µg/mL and 500µg/mL respectively. 2 mL of DPPH solution (0.004% w/v) was added in each of these test tubes. The reaction mixture was incubated in the dark for 15minutes and thereafter the optical density was recorded at 517nm against the blank. For the control, 1 mL of DPPH solution in Ethanol was mixed with 10mL of Ethanol and the optical density of the solution was measured after 30 minutes of incubation. The assay was carried out in triplicate [6].

The capability of scavenging DPPH radical was calculated using the following formula

$$\text{DPPH Scavenged (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of test})}{(\text{Absorbance of control})} \text{X100}$$

The results obtained were converted to IC_{50} values which denote the concentration of the sample, which is required to scavenge 50% of DPPH free radicals.

Ferric ion reducing antioxidant power (FRAP assay)

The required reagents such as Acetate buffer 300mM pH 3.6, TPTZ (2, 4, 6-tripyridyl-*s*-triazine) 10mM in 40mM HCl and 20mM $FeCl_3.6H_2O$ were prepared and used for the tests. The working FRAP reagent was prepared by mixing all the above three solvents in the ratio of 10:1:1 during the course of its use. The sample of the extract of 0.1mL was taken as a test sample which was mixed with the 3mL of working FRAP reagent and mixed well. The tubes were placed at 37 ^{0}C in a water bath for 15 minutes and then the absorbance was measured at 593nm. Ascorbic Acid standard in a concentration of 1000 µM was used as the standard.

$$\text{FRAP scavenging activity (\%)} = \left[\frac{(A0-As)}{A0}\right] X100$$

Where, A_0 is the absorbance of the control and As is the absorbance of the plant sample,

The concentration of working extract is 1mg/mL.

Results and Discussion

The preliminary phytochemical screening of *P. malabaricum* revealed that the leaves are the richest source of various phytochemicals which are diverse in nature. The Alkaloids and flavonoids were detected in all the extracts except Hexane. There were no traces of certain phytochemicals in any of the extracts which include Anthocyanins, Coumarins and Anthraquinones. On the other hand, the Tannins, Saponins, Steroids and Reducing Sugars were detected in all the four extracts. The results of Phytochemicals analysis were represented in table-1. The various phytoconstituents such as glycosides, tannins, flavonoids, phenolic compounds, alkaloids, proteins and vitamins etc., contribute to the biological activity of a plant. Natural antioxidants such as Vitamin C and Vitamin E directly confer antioxidant activity. Majority of the phytoconstituents were known to act synergistically. Hence, they have to be quantified in the plant extract [7].

The present study also focused on the quantitative analysis of certain phytochemicals such as estimation of Total Phenol content and Flavonoid content. The Phenol content increased with increased in conc. of extract used and the highest phenol content was found to be in Methanol extract with a conc. of 500 µg/ml. and was found to be 78.46 followed by Aqueous extract having the conc. of 74.28. The results of total phenol content were represented in Table-2 and Graph-1. The Flavonoids quantity was highest in Methanol extract with 38.22 followed by Chloroform which is having 34.42. The results of total flavonoid content were represented in Table-3 and Graph-2.

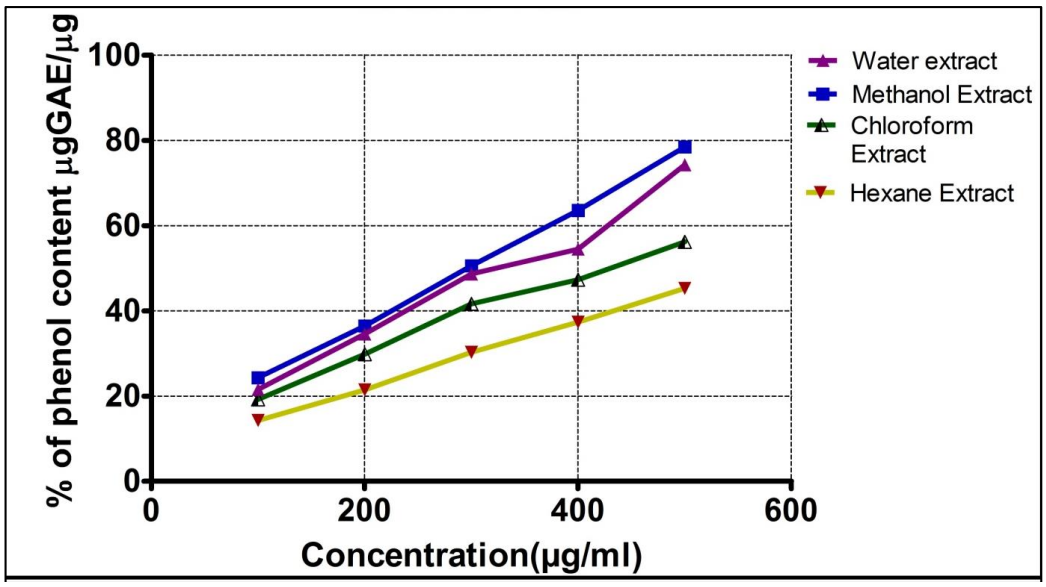
The antioxidant activities of extracts from the leaves of *P. malabaricum* were assayed by analyzing the scavenging of DPPH and FRAP Assay. The IC_{50} values were calculated and compared with the standards. Ascorbic acid was taken as the standard for DPPH assay and FRAP assay. The Methanol extract exhibited the highest antioxidant activity as evidenced by the results having an IC_{50} value of 311.51 and Ascorbic acid having 109.73. The FRAP assay also exhibited the highest activity with 469.04 and control exhibited IC_{50} of 158.49. The results of the FRAP assay were represented in Table-5 and Graph-4. Plants are the potential sources of antioxidants that play a pivotal role in the health of human beings [8].

Table 1: Comparative analysis of phytochemicals from different extracts of *P. malabaricum*

S. No	Name of the Tests	Hexane extract	Chloroform extract	Methanol extract	Aqueous extract
01	**Alkaloids**				
	Mayer's Test	Negative	Positive	Positive	Positive
	Dragon Test	Negative	Positive	Positive	Positive
	Wagner's Test	Negative	Positive	Positive	Positive
	Hager's Test	Negative	Positive	Negative	Negative
02	**Phenolics**				
	$FeCl_2$ Test	Positive	Positive	Positive	Positive
03	**Flavonoids**				
	Lead Acetate Test	Negative	Positive	Positive	Positive
	NaOH Test	Negative	Positive	Positive	Positive
04	**Anthraquinone Test**				
	Borntrager's Test	Negative	Negative	Negative	Negative
05	**Steroids**				
	Salkowski's Test	Positive	Positive	Positive	Positive
06	**Tannins**				
	$FeCl_2$ Test	Negative	Negative	Positive	Positive
	Lead acetate Test	Negative	Negative	Positive	Positive
	Pot. dichromate Test	Positive	Positive	Positive	Positive
07	**Saponins**				
	Vigorous Shaking Test (***Froth Test***)	Positive	Positive	Positive	Positive
08	**Anthocyanins**				
	Ammonia-HCl Test	Negative	Negative	Negative	Negative
09	**Leuco- Anthocyanin**				
	Iso Amyl Alcohol Test	Negative	Negative	Negative	Negative
10	**Coumarins**				
	NaOH Test	Negative	Negative	Negative	Negative
11	**Reducing Sugars**				
	Keller-Kiliani Test	Positive	Positive	Positive	Positive
	Fehling's Test	Positive	Positive	Positive	Positive

Table 2: Total phenol content of *P. malabaricum*

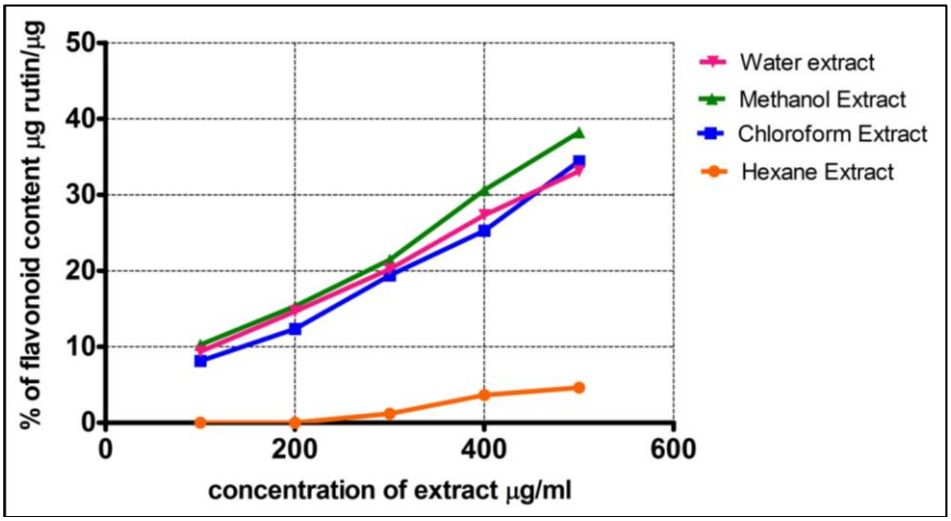
Concentration of extracts (µg/ml)	% of Phenol content µg GAE/µg			
	Hexane extract	Chloroform extract	Methanol extract	Aqueous extract
100	14.28	19.24	24.30	21.52
200	21.42	29.82	36.44	34.56
300	30.30	41.64	50.56	48.64
400	37.34	47.32	63.58	54.46
500	45.28	56.18	78.46	74.28



Graph 1: Total Phenol content of *P. malabaricum*

Table 3: Total Flavonoid content of *P. malabaricum*

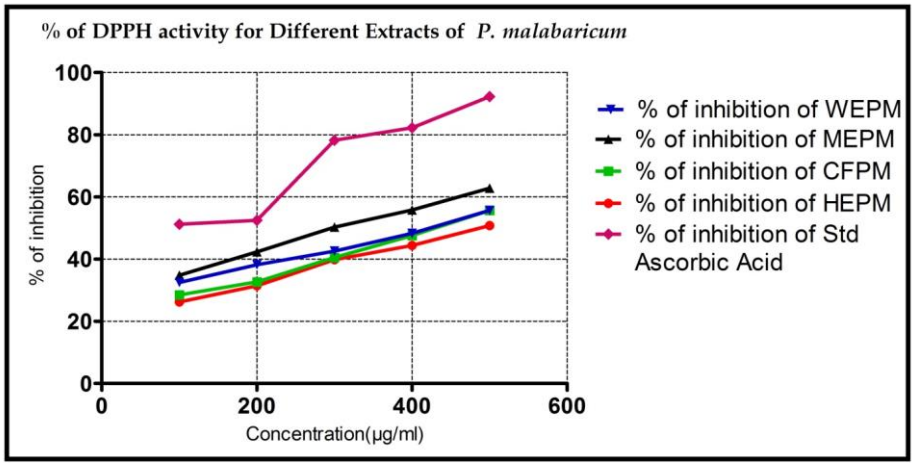
	% of Flavonoid content µg Rutin/µg			
Conc. of extracts (µg/ml)	**Hexane extract**	**Chloroform extract**	**Methanol extract**	**Aqueous extract**
100	0.00	8.14	10.28	9.38
200	0.00	12.36	15.34	14.64
300	1.20	19.40	21.46	20.22
400	3.64	25.28	30.64	27.34
500	4.64	34.42	38.22	33.10



Graph 2: Total Flavonoid content of *P. malabaricum*

Table 4: DPPH radical scavenging antioxidant activity of *P. malabaricum*

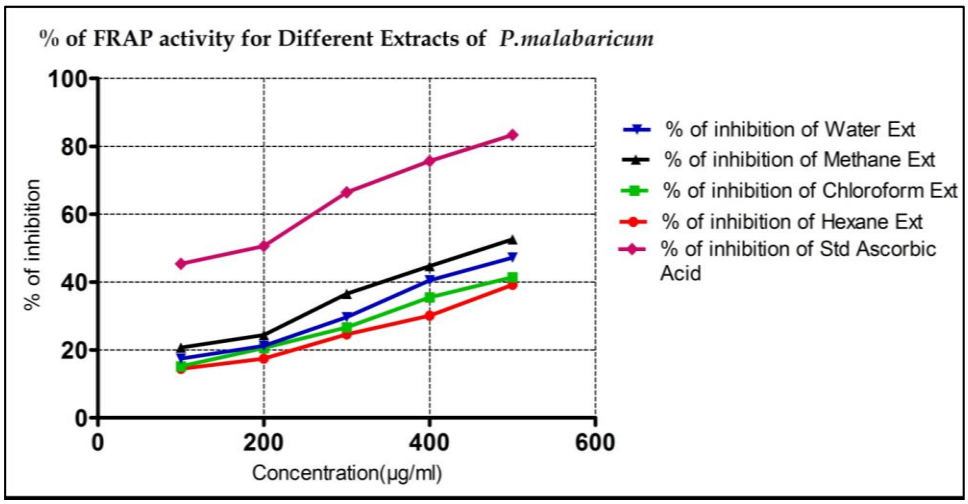
Conc (µg/ml)	**Hexane extract**	**IC 50**	**Chloroform extract**	**IC 50**	**Methanol Extract**	**IC 50**	**Aqueous extract**	**IC 50**	**Standard % of inhibition (Ascorbic acid)**	**IC 50**
100	26.2		28.5		34.8		32.5		51.22	
200	31.4		32.7		42.3		38.2		52.48	
300	39.8	484.57	40.5	429.87	50.3	311.51	42.6	415.75	78.22	109.73
400	44.4		47.6		55.8		48.3		82.24	
500	50.8		55.7		62.8		55.7		92.26	



Graph 3: DPPH radical scavenging antioxidant activity of *P. malabaricum*

Table 5: FRAP assay of different extracts of *P. malabaricum*

Conc. µg/ml	**Hexane**	**IC50**	**Chloroform**	**IC50**	**Methanol**	**IC50**	**Aqueous**	**IC50**	**Ascorbic Acid**	**IC50**
100	14.42		15.20		20.68		17.46		45.40	
200	17.48		20.60		24.42		21.24		50.60	
300	24.62	700.16	26.68	628.01	36.54	469.04	29.68	538.32	66.42	158.49
400	30.08		35.48		44.68		40.48		75.68	
500	39.18		41.44		52.60		47.24		83.36	



Graph 4: FRAP assay of different extracts from *P. malabaricum*

References

1. Zubair H, Azim S, Ahmad A, Khan MA, Patel GK, Singh S *et al*. Cancer Chemoprevention by Phytochemicals: Nature's Healing Touch. Molecules. 2017; 22(3):E395. doi: 10.3390/molecules22030395.
2. Wang Y, Chang CF, Chou J, Chen HL, Deng X, Harvey BK, *et al*. Dietary supplementation with blueberries, spinach, or spirulina reduces ischemic brain damage. Exp. 2005; 193:75-84.
3. Rahman Gul, Syed Umer Jan, Syed Faridullah, Samiullah Sherani, Nusrat Jahan. Preliminary Phytochemical Screening, Quantitative Analysis of Alkaloids, and Antioxidant Activity of Crude Plant Extracts from *Ephedra intermedia* Indigenous to Balochistan. Scientific World Journal. 2017; doi: 10.1155/2017/5873648
4. Kaneria M, Kanani B, Chanda S. Assessment of effect of hydroalcoholic and decoction methods on extraction of antioxidants from selected Indian medicinal plants. Asian Pac J Trop Biomed. 2012; 2(3):195-202. doi: 10.1016/S2221-1691(12)60041-0.
5. Zhang H, Li X, Wu K, Wang M, Liu P, Wang X *et al*. Antioxidant Activities and Chemical Constituents of Flavonoids from the Flower of *Paeonia ostii*. Molecules. 2016; 22(1). pii: E5. doi: 10.3390/molecules22010005.
6. Trease GE, Evans WC. Pharmacognosy. 15th Ed. London: Saunders Publishers. 2002; 42-44:221-229, 246-249, 304-306, 331-332, 391-393.
7. Vijayasekhar VE, Prasad MS, DSD. Joshi S, Narendra K, Satya AK, Sambasiva Rao KRS. Evaluation of Phytochemicals and *in vitro* Pharmacological Activity of *Acacia Sinuata* Plant Leaves. Int J Pharm Sci Res 2016; 7(6):2423-34.doi: 10.13040/IJPSR.0975-8232.7(6).2423-34.
8. El Jemli M, Kamal R, Marmouzi I, Zerrouki A, Cherrah Y, Alaoui K. Radical-Scavenging Activity and Ferric Reducing Ability of *Juniperus thurifera* (L.), *J. oxycedrus* (L.), *J. phoenicea* (L.) and *Tetraclinis articulata* (L.). Adv Pharmacol Sci. 2016; 2016:6392656. doi:10.1155/2016/6392656.