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Phytochemical screening and quantitative estimation of total flavonoids of *Ocimum sanctum* in different solvent extract

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Abstract
Ocimum sanctum (Tulsi) is important and widely used medicinal plant. It is an aromatic plant of the lamiaceae family. The aim of present study is to estimate the phytochemical components and total flavanoid content assay of Chloroform, methanol and ethanolic solvent extract from basil leaf and stem. The basil leaf and stem was extracted by maceration process using ethanol, chloroform, and methanol. The Chloroform, ethanol and methanol extract were screened of phytochemical content including identification of flavonoid, alkaloid, polyphenols, glycosides, tannin, saponin etc. Estimation of total flavonoids content was based on aluminium chloride method in the sample extract by spectrophotometrically. Phytochemical screening test showed that the presence of diterpines, saponins, proteins, flavonoids, amino acids, carbohydrates, alkaloids in leaves and stem parts when extracted with methanolic and ethanolic solvents. Chloroform extract of basil leaf and stem does not show the presence of any phytochemicals. Higher flavonoid component were present in methanolic extract of Ocimum *sanctum* leaves than ethanolic solvent extract. In this study, *Ocimum sanctum* has phytochemicals properties in the leaves and stem which are used in curing the ailments and higher flavonoid content indicated the natural antioxidant activity signifying their medicinal importance and potent source in pharma industries. We conclude that *Ocimum sanctum* is highly useful medicinal plant. However, there is necessary to explore natural plant sources with their medicinal value used in medical field.

Keywords: Phytochemical, Ocimum sanctum, flavonoid content

Introduction
The value of medicinal plants in drug discovery is known to us well and the human being used them for various purposes from the beginning of the human history [1]. Over the years, medicinal plants have been useful sources of several active compounds of recuperative value and it is used as a substitute medicine for treating numerous diseases [2]. Medicinal plants are very important to the health of individuals and communities. These plants have some phytochemical components with their medicinal value that produce a physiological action on the human body. In Ayurveda, *Ocimum sanctum* (Tulsi) has been used for its therapeutic values and described as antiasthmatic and antikaphic drugs [3]. Medicinal palnts having therapeutic or pharmalogical properties, can effect on the human body. There are 260,000 species of plant present in plantae kingdom. In which 230,000 species are flowering herbs, vine, shrubs, vegetable, flower, fruits and legume comes under the *Magnoliphyta* phyla [4].
The present study is based on lamiaceae family because it is best known family for therapeutic properties and use as a source of culinary herbs [5]. *O. sanctum* is known as Tulsi and Holi basil. It is a most important herbs used for this study. This herb is found on tropical and semitropical area of India. *O. sanctum* can grow in each and every part of india. It is a 30-75 cm high erect herbs, Leaves are 2.5 – 5 cm long and 1.6 – 3.2 cm broad, elliptical. Inflorescence is verticillate and flowers are in racemes 15-20 cm long in close whorls. Odour and taste are aromatic and sharp [6]. This herbs and its application are using day by day in Indian Traditional medicine system. *O. sanctum* has variety of properties such as antibiotic, antioxidant, antiatherogenic, anti-inflammatory, antiulcer, analgesic, immunomodulatory, antipyretic and chemopreventive properties [7]. Flavonoids component is widely distributed groups of phenolics plants; it is most common phytochemicals present in food material. Quercetin and rutin are the main flavonoid component abundant in food [8,9]. Medicinal plants has many bioactive phytochemical component can be beneficial for human health. Flavanoids, alkaloids, phenolics and tannins are most common phytochemical components present in plants [10].

Plants produced these phytochemical components has a property to protect itself and the human body from disease causing agent. It is bioactive chemicals that have disease protective and preventive properties. Phytochemicals is a natural compound present in every part of the plant such as root, stem leaf, flower and fruits. Phenolics compounds are mostly distributed group of phytochemicals. It is derivatives of benzene with one or more hydroxyl groups associated with the aromatic ring [11]. Naturally these plants are available in whole worlds. These plants have many properties that are beneficial to human being and complete society in the medicinal and pharmacological field. Alkaloids, tannins, terpenoids, flavonoids, phenols are active compounds which show the physiological action on human body [12]. Hence, the objective of this study is to determine the phytochemical constituents present in leaves and stem extracts of *O. sanctum* and flavonoids component in the plant sources.

Material and Methods

Collection of plant Material

Plant material (leaves and stem) of *Ocimum sanctum* were collected from ruler area of chitrakoot (M.P.), in the april month of 2011. These herbs were authenticated by Dr. Rajesh Garg, Department of Botany, Gov. autonomous college, Satna (M.P.).

Extraction procedure

Following procedure was adopted for the preparation of chloroform; methanol and ethanol extracts from the shade dried and powdered plant herbs [13].

Defatting of plant material

Powdered material of *Ocimum sanctum* was dried in shade at 37^0C. Shade dried powdered material used for extraction process with individual solvents by maceration process.

Extraction by maceration process

100 g. of *Ocimum sanctum* dried material were exhaustively extracted with chloroform, methanol and ethanol using maceration for 24 hrs. The extract was evaporated above their boiling points. Finally the percentage yields were calculated of the dried extracts.

Determination of Percentage yield

The yield of extract of plant was calculated by using formula:

$$\text{Percentage yield} = \frac{\text{Weight of extract x 100}}{\text{Weight of powdered drug taken}}$$

Phytochemical Qualitative analysis

The plant extracts obtained by using different solvent extraction process and it is subjected to different phytochemical tests to identify the plant constituents by using standard following methods [14, 15].

Preparation of Test Solution

The test solution was prepared by taking 1 g of the extract in 25 ml of methanol.

A. Test for Carbohydrates

Various tests used for carbohydrates:

a) Molisch's test: Sample of plant extract was taken in a test tube. Then 20% alcoholic solution and concentrated sulphuric acid, which is freshly prepared is added in to test tube along the sides. This test developed reddish violet and purple colour at junction between two liquids if carbohydrates present in the sample extracts.

b) Benedict's test: Taken a test tube, which contain small amount of plant extracts sample. In a test tube added small quantity of benedict's solution and mix properly. Then boiled this sample mixture for two minutes and cool it. If carbohydrates present in the sample, it formed red precipitate.

c) Barfoed's test: The barfoed's solution added to 0.5 ml of solution under examination, heated to boil. If carbohydrates present in the sample extracts, it formed red precipitate of copper oxide.

B. Test for Alkaloids

a) Dragendorff's test: Taken a few mg of extracts sample and dissolved in 5ml water. Then 2 M hydrochloric acid added until an acid reaction developed. In this mixture, 1ml of dragendorff's reagent (potassium bismuth iodine solutions) was added. If alkaloids present in sample extracts, it formed orange red precipitate.

b) Wagner's test: Acidify the plant extract sample with hydrochloric acid (1.5% v/v) and added a few drop of Wagner's reagent (iodine potassium iodide solution) in the test tube. It formed reddish brown precipitates which indicate the presence of alkaloids.

c) Mayer's test: 2ml of plant extracts sample was taken and 2 - 3 drops of Mayer's reagent was added (potassium mercuric iodine solution) in the test tube. If alkaloids present in the sample, it formed dull white precipitate.

C. Test for Glycosides

a) Legal's test: Taken a extracts sample and dissolved in pyridine then added sodium nitroprusside solution. Make this solution completely alkaline. Presence of glycosides produced pink red colour.

b) Baljet's test: Taken a plant extracts sample in the test tube and added sodium picrate solution. Presence of glycosides produced yellow to orange colour.

c) Borntrager's test: The test solution of plant extract was added in few ml of dilute sulphuric acid solution. This solution was filtered. Then Chloroform and ether was added in to filtrate and shaken well. In this solution ammonia was added and separated the organic layer. Organic layer showed pink, red or violet colour due to the presence of glycosides.

D. Test of Saponins

a) 1ml of alcoholic sample extract was taken and diluted with 20ml of distilled water. This solution was shaked for 15 min in graduated cylinder. If saponins present in the extracts, it generate foam layer of 1cm.

E. Test for Flavonoids

a) Shinoda test: Taken the alcoholic sample extract in the test tube and 5-10 drops of hydrochloric acid added in the sample. Then small pieces of magnesium added in tubes. Reddish pink or brown colour was indicated the presence of flavonoids.

b) Alkaline reagent test: Plant extracts sample was mixed with 2ml of 2% NaOH solution. It produced yellow colour. In this solution, 2 drops of diluted acids was added. If flavonoids present in the extracts, yellow colour changed into colourless.

F. Test for Tannins

a) Taken the sample of plant extracts in the test tube and added ferric chloride solution. If tannin present in the sample, dark blue or greenish black colour appeared.

b) Taken the sample extracts and added potassium cyanide. It produced deep red colour, which indicate the presence of tannins.

c) Potassium dichromate was added in to sample extracts. Yellow precipitate was formed indicate the presence of tannins.

G. Test for Protein and Amino acid

a) Biuret's test: Taken 2-3 ml of sample extract and added 1 ml sodium hydroxide solutions (40%) and 2 drops of copper sulphate solution (1%) and mixed properly. Presence of proteins showed a pinkish - violet and purple - violet colour.

b) Ninhydrin's test: Plant extracts sample mixed with freshly prepared 2 drops of 0.2% ninhydrin solution and heated to boiling for 1-2 min and allowed cooling. Blue colour appearance indicate the presence of amino acids, proteins, peptides.

c) Xanthoprotein test: Extracts sample was taken in test tube and added conc. nitric acid. A white precipitate was obtained and upon heating turns to yellow and cool the solution carefully. 20% sodium hydroxide solution added in excess, which produce orange colour that indicate the presence of amino acids.

H. Test of Fats or Fixed oils

a) Using sodium hydroxide: The extract was mixed in one ml 1 % of copper sulphate solution then 10% sodium hydroxide solution was added. Blue colour appeared in the solution, which showed the presence of glycerin.

b) Saponification: plant extracts was taken and mixed with 2% sodium carbonate solution. Shaked vigorously and boiled. A clean soapy solution was formed cooled and few drops of conc. HCl was added and observed that fatty separate out and float up.

Determination of total flavonoids content (TFC)

Determination of total flavonoids content was based on aluminum chloride ($AlCl_3$) method [16]. Taken a 50 mg quercetin component and dissolved in 50 ml methanol solution and various aliquots of 5- 25µg/ml were prepared in methanol. It was used as a standard. 10mg of dried plant extracts were dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was used for the estimation of flavonoid. In the last step, taken 3 ml of plant extract or standard and add 1 ml of 2% $AlCl_3$ methanolic solution. This mixture is allowed to keep for 60 min at room temperature. Then absorbance was measured at 420 nm by using spectrophotometer.

Results

Yield of extract

The crude extracts so obtained after the maceration extraction process, extracts was further concentrated on water bath evaporation the solvents completely to obtain the actual yield of extraction. To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extracts obtained from sample using chloroform, methanol and ethanol as solvents are shown in the table. 1**.**

Table 1: Result of Percentage Yield of Different Extract

S. No.	Solvents	Yield (%)
1.	Chloroform	1.9
2.	Methanol	2.4
3.	Ethanol	3.5

Ocimum sanctum exhibited highly yield in ethanol followed by methanol and chloroform solvent extract.

Phytochemical testing

Taken a small amount of the dried extracts and subjected to the phytochemical screening test by using Kokate methods to test for flavonoids, alkaloids, glycosides, tannins, saponins, phenol and steroids separately for extracts of all samples. Then little amount of each extract is suspended into sterile distilled water and make the concentration of 1 mg/ml. The outcomes of the results are discussed in the table 2.

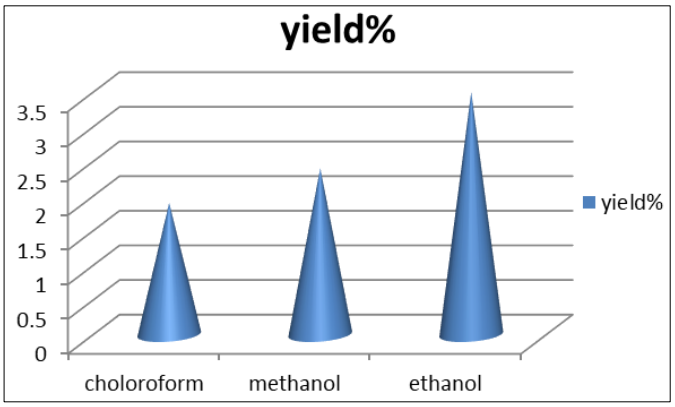

Fig 1: Yield of extract

Table 2: Result of Phytochemical Screening of *O. sanctum*

	Constituents	Chloroform		Methanolic		Ethanolic	
		Leaves	Stem	Leaves	Stem	Leaves	Stem
1.	Alkaloids	-	-	-	-	-	+
2.	Glycosides	-	-	-	-	-	-
3.	Flavanoids	-	-	+	+	-	+
4.	Phenolics	-	-	-	-	-	-
5.	Amino Acids	-	-	+	+	+	-
6.	Carbohydrate	-	-	+	-	-	-
7.	Proteins	-	-	+	+	+	-
8.	Saponins	-	-	+	-	+	-
9.	Determines	-	-	-	-	-	-

From the results obtained it is clear that the *Ocimum sanctum* plants shows the occurrence of flavonoids, alkaloids, glycosides, saponins, tannins, phenolics, amino acid, diterpines, were found present in leaves and stem parts when extracted with methanolic and ethanolic solvents using maceration extraction procedure. Chloroform extract of leaf and stem of *O. sanctum* does not showing the presence of any phytochemical.

Results of total flavonoid content

Total flavonoid (Quercetin) content estimation

For the estimation of TFC, firstly prepared the quercetin (flavonoid) calibration curve, which was taken as standard in different concentration (µg/ml) and measured the absorbance of quercetin at 420nm. According to this calibration curve, flavanoid content of leaf and stem was determined by using following equation: Y=0.040 X+0.009, R^2=0.999, where X is the Quercetin equivalent (QE) and Y is the absorbance.

Table 3: Preparation of Calibration Curve of Quercetin

S. No.	Concentration (µg/ml)	Absorbance (Mean) λmax=420 nm
0	0	0
1	5	0.216
2	10	0.425
3	15	0.625
4	20	0.815
5	25	1.021

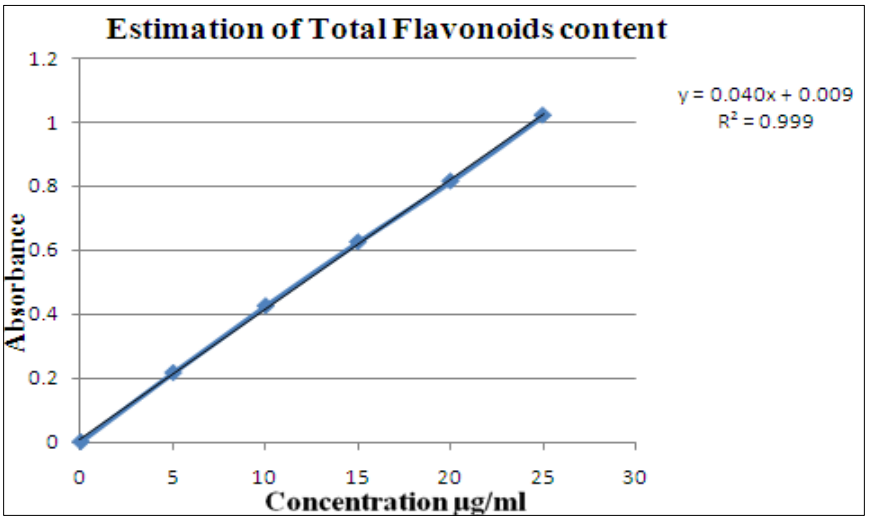

Fig 2: Calibration curve of Quercetin

Table 4: Total Flavonoid Content of *Ocimum Sanctum* Extract

Estimation	Methanolic extract		Ethanolic extract	
	Leaves	Stem	Leaves	Stem
Absorbance	1.903	0.727	-	1.227
Total Flavonoids (mg/100mg)	4.75	1.81	-	3.06

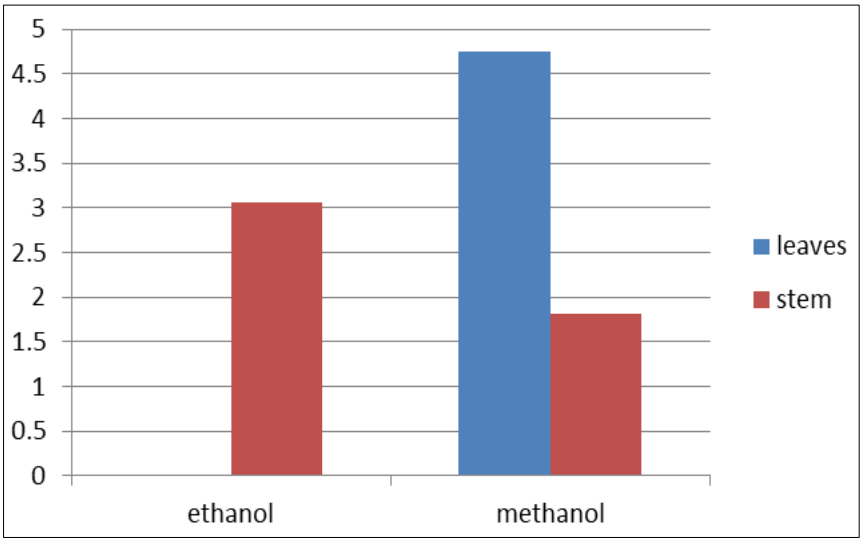

Fig 3: Total flavonoid content of different solvent extract

Discussion

The phytochemical screening and quantitative estimation of chemical components of the plants studied showed that the leaf and stem were rich in flavonoids, saponins, amino acids, proteins, diterpines. These phytochemicals has medicinal and physiological activity [17]. Phytochemicals are the chemical constituents present in plants which show physiological action on the human body [18]. Alkaloids, flavonoids, phenols, diterpines, carbohydrates, proteins, glycosides, and essential oils are some of the important bioactive phytochemicals [19]. A number of report are available that represent the phytochemical presence in plant such as flavanoids, glycosides, tannins, alkaloids phenols, proteins in medicinal plants [20, 22]. Major phytochemicals are reported in *O. sanctum* are flavonoids, glycosides, Alkaloids, proteins, tannins and phenols [23]. It has been determined that antioxidant activity present in flavonoid component and its effect on human nutrition and their health. The mechanism of action of flavanoids is through scavenging or chelating process [24]. Flavonoids are the common antioxidants present in various medicinal plnats [24, 25]. The present work was carried out to analyse the phytochemicals and flavonoids component in medicinal plant *Ocimum sanctum*. This study showed more flavonoid component present in leaves of *Ocimum senctum*. However, flavonoid component was more in methanol extract of *Ocimum sanctum* leaves and ethanol extract of *Ocimum senctum* stem. While methanol extract of *Ocimum sanctum* stem show less flavonoid content and ethanol extract of *Ocimum sanctum* leaves does not show any result. Various reports also available that indicate *O.sanctum* as a good source of flavonoids [26]. Since, phenolic and flavonoid components present in high amount in Ocimum species. Thus, widely used in traditional medicine system [27]. Flavonoids have one hydroxyl group that is substituted with aromatic ring. Flavonoids combine with metal ions and form chelate complex and can easily oxidized and donating electrons to scavenge free radicles [25, 28]. Higher flavonoid component in *O. sanctum* is correlated with increased antioxidant activity [29]. It has been reported that good amount of total flavonoid contents present in methanol extract of *Ocimum sanctum*. It has been also reported that flavonoid component and antioxidant activity has linear correlation.

Conclusion

India has a rich flora used in traditional medical treatment. These plants have medicinal properties because of their phytochemical components. These phytochemicals show therapeutic and antioxidant effect on mankind. From this current study, we concluded that the methanol leaves and stem extracts of *O. sanctum* showed high antioxidant activity and potentially phytochemical properties. Extracts of this plant is abundant in flavonoids, diterpines, alkaloids, proteins, carbohydrates, saponins. In this study, we concluded that biologically active phytochemicals present in methanol and ethanol extracts of Ocimum leaves and stem. While chloroform extract do not show the phytochemical activity. The medicinal properties of *O. sanctum* leaves and stem extracts may be due to the presence of the active biochemicals and phytochemicals. The study showed that the plants are a source of significant natural antioxidant and may be beneficial in protection against oxidative stresses. Hence, there is necessity to explore the applicability of these plant resources which are rich in phytochemical/flavonoid may have been beneficial effects of health.

References

1. Farnsworth N. The role of ethno pharrnacology in drug development. Bioactive compounds from plants. John Wiley & Sons. 2008.
2. Nostro AMP, Germano VD. Angelo Marino A, Cannatelli MA. Extraction methods and bioautography for evalution of Meditional plant antimicrobial activity. Lett. Applied Microbial. 2000; 30:379-384.
3. Chopra RN, Nayar SI, Chopra IC. Glossary of Indian Medicinal Plants. Published by CSIR, New Delhi.
4. Akinmoladun AC. EOIbukun; Emmanuel A; EMObuotor; EO Farombi. Sci. Res. Essays. 2007; 2(5):163-166.
5. Baskaran X. Ethnobotanical Leaflets, 2008: 12:1236-1239.
6. Gupta SK, Prakash J, Srivastava S. Validation of claim of Tulsi, *Ocimum sanctum* Linn. as a medicinal plant. Indian J Exp. Biol. 2002, 40(7):765-773.
7. Singh S, Taneja M, Majumdar DK. Biological activities of *Ocimum sanctum* L. fixed oil -an Overview. Indian J Exp. Biol. 2007; 45(5):403-412.
8. Harborne JB. The Flavonoids: Advances in research since 1980, Chapman and Hall Ltd, New York, 1988, 121.
9. Nakamura Y, Ishimitsu S, Tonogai Y. Effects of quercetin and rutin on serum and hepatic lipid concentrations, fecal steroid excretion and serum antioxidant properties. Journal of Health Science. 2000; 46:229-240.
10. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology. 2005; 4(7):685-688.
11. Andjelkovic M, Camp JV, Meulenaer BD, Depaemelaere G, Socaciu C, Verloo M *et al*. Iron-chelation properties of phenolic acids bearing catechol and galloyl groups. Food Chem. 2006; 98:23-31.
12. Yadav RNS, Agarwala M. Phytochemical analysis of some medicinal plants. J Phytol. 2011; 3:10-14.
13. Mukherjee PK. Quality control of herbal drugs. 2nd Ed. Business Horizons, 2007.
14. Khandelwal KR, Practical Pharmacognogy technique and experiments. 23nd Ed. Nirah Prakashan, 2005.
15. Kokate CK. Practical Pharmacognogy, 4th Ed. Vallabh Prakashan, 2011.
16. Olajuyigbe OO, Afolayan AJ. Flavanoid content and antioxidant property of the bark extract of *ziziphus mucronata willd*. Subsp. *Mucronata willd* BMC, Comp Alt med. 2011; 11:130.
17. Vimala A, Thamizharasi T, Sathish SS, Palani R, Vijayakanth P. Phytochemical studies on selective medicinal plants. In.t J Res Eng Biosci. 2013; 1:57-62.
18. Anwar F, Jamil A, Iqbal S, Sheikh MA. Antioxidant activity of various plant extracts under ambient and accelerated storage of sunflower oil. Grassay Aceites, International Journal of Fats and Oils. 2006; 57:189-97.
19. Deshpande SN, Kadam DG. Preliminary phytochemical analysis of some medicinal plants. DAV Int. J Sci. 2013; 2:61-5.
20. Kiranmai M, Mohammed I. Anti bacterial potential of different extracts of *Tagetes erecta* Linn. Int. J Pharm. 2012; 2:90-6.
21. Sharma V, Paliwal R. Preliminary phytochemical investigation and thin layer chromatography profiling of sequential extracts of *Moringa oleifera* pods. Int. J Green

Pharm. 2013; 7:41-5.
22. Nagaveni P, Kumar KS, Rathnam G. Phytochemical profile and antipyretic activity of Mangifera indica. JITPS. 2011; 2:167-73.
23. Ndong M, Uehara M, Katsumata S, Suzuki K. Effects of oral administration of *Moringa oleifera* Lam on glucose tolerance in goto-kakizaki and wistar rats. J Clin Biochem Nutr. 2007; 40:229-33.
24. Sankhalkar S. Antioxidant enzyme activity, phenolics and flavonoid content in vegetative and reproductive parts of *Moringa oleifera* Lam. Am J Pharmatech Res. 2014; 4:255-70.
25. Siddhuraju P, Becker K. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. J Agric Food Chem. 2003; 51:2144-55.
26. Alhakmani F, Kumar S, Khan SA. Estimation of total phenolic content, *in-vitro* antioxidant and anti-inflammatory activity of flowers of *Moringa oleifera*. Asian Pac J Trop Biomed. 2013; 3:623-7.
27. Wang H, Provan GJ, Helliwell K. Determination of rosmarinic acid and caffeic acid in aromatic herbs by HPLC. Food Chem. 2004; 87:307-11.
28. Sreedam CD, Kaiser H, Jahan BI, Sultana S, Md, Islam S. *In vitro* antioxidant activity of different parts of the plant diospyros discolor. Res J Agric. Biol. Sci. 2010; 6:472-5.
29. Kostyuk VA, Potapovich AI, Vladykovskaya EN, Korkina LG, Afanas'ev IB. Influence of metal ions on flavonoid protection against asbestos-induced cell injury. Arch Bio chem.