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Research Article

Proportionate Phytochemical Screening and Assessment of Antioxidant Potency on Selected Species of Lamiaceae Family

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ABSTRACT

Antioxidant compounds are abundantly available in plants and play an important role in scavenging free radicals, thus providing protection to humans against oxidative DNA damage. *Mentha spicata, Plectranthus amboinicus and Ocimum sanctum* commonly called as spearmint, Indian borage and holy basil, belongs to lamiaceae family was selected in the present study because these three plant extracts have good antioxidant properties. Three solvent fractions namely acetone, aqueous and ethanol from dried leaves powder of three plants were analysed for the phytochemicals present in them which is responsible for the herb's rich medicinal heritage. Free radical scavenging activity of the herbs under study was also evaluated with DPPH using BHT as standard in which acetone fraction was found to exhibit higher activity. The total flavonoid content was found to be highest in acetone fraction of *Plectranthus amboinicus* (29.70 mg/g) and least in *Ocimum sanctum* (25 mg/g). The antioxidant activity was assumed to be from the total flavanoid content of the plant extract which was estimated using Quercetin as a standard. The present study reveals that the selected plants would exert several beneficial effects by virtue of their antioxidant activity and could be harnessed as drug formulation.

Keywords: *Mentha spicata, Plectranthus amboinicus, Ocimum sanctum,* BHT, DPPH, flavonoids

INTRODUCTION

Antioxidants are substances that protect the cells against the effects of free radicals or simply antioxidant means "against oxidation." It works to protect lipids from peroxidation by radicals. Antioxidants are effective because they give up their electrons to free radicals. When a free radical gains the electron from an antioxidant it no longer needs to attack the cell.

Free radicals are atomic or molecular species with unpaired electrons in the outermost bonding orbital and are likely to take part in chemical reactions. The increased production of free radicals in the diet and the atmosphere can cause high blood pressure, heart disease, cancer and other diseases (John McLaren,2004).

The main aim of phytochemical screening is to identify the nature of the compounds present in a given plant extract which may be responsible for the observed biological effect. They clear up residual symptoms or destroy the cause of the disease in most cases infectious microorganisms. They increase the body's resistance to disease, retard or ease the process of natural ageing. These components are responsible of a green therapeutic effect and they frequently serves as model for the synthetic of new medicine (David G *et al*., 1997). Phytochemical screening of the active principle and compounds contained in plants as a result were able to discover compounds such as Tannis, Quinones, Flavonoids, Alkaloids, Saponins, Steroids, Coumarins and Glycosides.(Soni *et al*., 2013)

Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action (Frankel *et al*., 1995). Some evidence suggests that the biological actions of these compounds are related to their antioxidant activity (Gryglewski *et al*., 1987). An easy rapid and sensitive method for the antioxidant screening of plant extracts is free radical scavenging assay using 1,1-diphenyl-2-picryl hydrazyl (DPPH) stable radical spectrophotometrically. In the presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases (Koleva *et al*., 2002)

MATERIALS AND METHODS

Plant Materials Collection

Mentha spicata, Ocimum sanctum and *Plectranthus amboinicus* were collected from Chengalpet, Kanchipuram district, Tamil Nadu. The leaves were cut separately, washed thoroughly to remove away the soil particles and was shade dried. The dried leaves were ground using a homogeniser and stored in air tight container for further analysis.

Preparation of plant extract

Mentha spicata, Plectranthus amboinicus and *Ocimum sanctum* leaves powder was subjected to successive extraction with different solvents in increasing polarity such as Acetone, ethanol and aqueous using direct extraction method. (Eloff , 1999)

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Table 1: Phytochemical Analysis Of *Mentha Spicata*

Solvent/phytochemicals	Ethanol	Acetone	Aqueous
Tannin	+	+	+
Saponin	+	_	_
Flavanoids	+	+	_
Quinones	+	+	+
Glycosides	_	+	_
Cardioglycosides	_	_	+
Terpenoids	+	+	_
Phenols	+	+	+
Coumarins	+	+	_
Steroids	+	+	_
Alkaloids	+	_	_
Anthacyanin	_	_	_
B-cyanin	+	+	_

Table 2: Phytochemical Analysis Of *Plectranthus Amboinicus*

Solvent/phytochemicals	Ethanol	Acetone	Aqueous
Tannin	_	+	+
Saponin	_	_	_
Flavanoids	+	+	+
Quinones	+	+	_
Glycosides	_	_	+
Cardioglycosides	_	_	_
Terpenoids	+	+	+
Phenols	++	+	+
Coumarins	+	+	_
Steroids	+	+	+
Alkaloids	_	+	+
Anthacyanin	_	_	_
B-cyanin	+	+	_

In this method, finely ground seed powder (1 gm) was extracted with 10 ml of chloroform, acetone and methanol in separate conical flasks in shaking condition with the help of a shaker. The process was repeated 3 times with the same material but using fresh solvent and each time the extract was decanted in to pre-weighed glass vials. The solvent in the extract was
removed by condensation. The extracted residues were weighed and re-dissolved in different solvents to yield 10mg/ml solutions for further analysis.

Preliminary bioactive compound Analysis

Various bioactive compounds present in the plant extracts were analysed with reference to [(Harborne, 1976) and (Evans et al., 1989)]

Test for Tannins

To 1 ml of the leaf extract, 1 ml of 5% ferric chloride was added. Formation of greenish black colour indicates the presence of tannins.

Test for Saponins

To 1 ml of leaf extract, 2ml of distilled water was added in a test tube. The solution was shaken for 15 minutes and was observed for stable persistent foam of about 0.5 to 1cm layer for the presence of saponins.

Test for Flavonoids

To 1ml of 2N NaOH, 1ml of leaf extract was added. Appearance of yellow colour indicates the presence of flavanoids.

Test for Quinones

To 1ml of leaf extract, 1.5 ml of conc. sulphuric acid was added. The solution was observed for the formation of red colour for the presence of quinones.

Test for Glycosides

To 1ml of leaf extract, 2ml of chloroform was added. To that 1ml of ammonium solution was added and formation of pink colour indicates the presence of glycosides.

Test for Cardioglycosides

To 1ml of leaf extract, 2ml of glacial acetic acid and 0.5 ml of 5% ferric chloride was added. To that 1.5 ml of conc. sulphuric acid is added and observed for the formation of brown colour for the presence of cardioglycosides.

Test for Terpenoids

1 ml of chloroform was added to 1ml of leaf extract and 1.5 ml of conc. sulphuric acid is added to it. Formation of reddish brown colour indicates the presence of terpenoids.

Test for Phenols

To 1ml of leaf extract 1ml of sodium carbonate was added .To that 1ml of folin's reagent was added. Formation of blue or green colour indicates the presence of phenols.

Test for Coumarins

Add 1ml of 10% sodium hydroxide to 1ml of leaf extract. Observe the solution for the appearance of yellow colour for the presence of coumarins.

Test for Steroids

Table 3 : Phytochemical Analysis Of *Ocimum Sanctum* (Hosur Super Market)

Solvent/ phytochemicals	Ethanol	Acetone	Aqueous
Tannin	+	+	+
Saponin	+	–	–
Flavanoids	++	+	–
Quinones	++	+	+
Glycosides	–	–	+
Cardioglycosides	–	+	–
Terpenoids	+	+	+
Phenols	+	+	+
Coumarins	+	+	–
Steroids	+	+	+
Alkaloids	–	+	–
Anthacyanin	–	–	–
B-cyanin	+	–	–

Table 4: Qualitative Analysis Of Antioxidant Activity

Plant name	Solvents	Result
	Aqeuous	Semipositive
Mentha spicata	Ethanol	Semipositive
	Acetone	Positive
Plectranthus	Aqueous	Positive
Amboinicus	Ethanol	Positive
	Acetone	Positive
Ocimum sanctum	Aqueous	Semipositive
	Ethanol	Semipositive
	Acetone	Positive

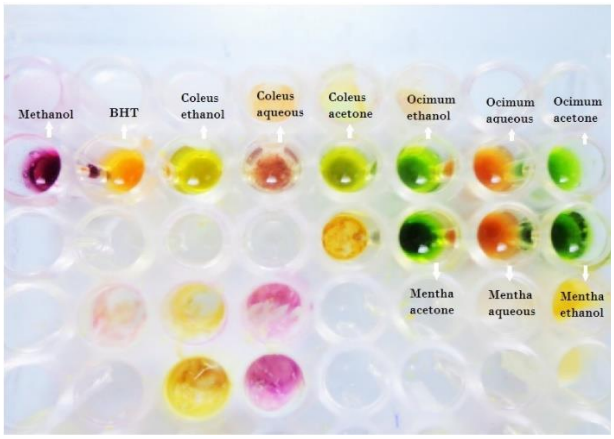


Figure 1: Qualitative analysis for DPPH Free Radical Scavenging Activity

To 1ml of leaf extract add 1ml of chloroform and 1.5ml of conc. sulphuric acid. The appearance, at the interphase, a reddish brown colour showed a positive reaction.

Test for Alkaloids

To 1ml of leaf extract 1ml of conc. HCl was added. To that 1ml of Mayer's reagent is added. The formation of green or white precipitate was regarded as positive for the presence of alkaloids.

Test for β cyanin

To 1ml of leaf extract 1ml of 2N NaOH is added. The solution mixture is heated for 1minute in 100°C and the appearance of blue colour indicates the presence of anthocyanin. The formation of yellow colour indicates the presence of β cyanin.

Antioxidant Activity

Antioxidant assay on leaves extract of plants *Mentha spicata, Plectranthus amboinicus, Ocimum sanctum* were estimated for their free radical scavenging activity by using DPPH (1,1-Diphenyl-2picryl-hydrazyl) free radicals.

DPPH (1, 1-Diphenyl-2picryl-hydrazyl) is a stable free radical with purple colour (absorbed @ 517 nm).If free radicals have been scavenged, DPPH will degenerate to yellow colour. This assay is used to identify the free radical scavenging *activity.*

Qualitative analysis of antioxidant activity

100 µl of leaf extract of medicinal plants *Mentha spicata, Plectranthus amboinicus* and *Ocimum sanctum* were taken in the microtitre plate.100 µl of 0.1% methanolic DPPH was added over the samples. It was incubated for 30 minutes in dark condition. The samples were then observed for discoloration.

The colour changes from purple to yellow and pale pink were considered as strong and weak positive respectively. The antioxidant positive samples were subjected for further quantitative analysis. ((Nenadis and Tsimidou, 2003).

Quantitative assay of antioxidant activity

Leaf extract sample of 100 µl from qualitative assay were mixed with 2.7ml of methanol. Then 200 µl of 0.1% methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Subsequently, at every 5 minutes interval the absorption maxima of the solution were measured using a UV double beam spectra at 517nm. The antioxidant activity of the sample was compared with known synthetic standard of 0.16% of BHT (Butylated hydroxy toluene). (Nenadis and Tsimidou, 2003)

Table 5: Quantitative analysis of Antioxidant activity of *Mentha* spicata

S.No	Solvents	Time in minutes/ Inhibition percentage of Free Radical Scavenging						
		0	5	10	15	20	25	30
1.	Aqueous	45.08	67.21	76.22	80.32	81.96	82.80	85.24
2.	Ethanol	84.42	89.34	90.16	90.16	90.16	90.16	90.16
3.	Acetone	83.60	90.16	90.98	90.98	90.16	90.16	90.98

Table 6: Quantitative Analysis Of Antioxidant Activity Of *Plectranthus Amboinicus*

S.No	Solvents	Time in minutes/ Inhibition percentage of Free Radical Scavenging						
		0	5	10	15	20	25	30
1.	Aqueous	65.57	85.24	87.70	87.70	87.70	87.70	87.70
2.	Ethanol	53.27	59.83	65.57	67.21	72.13	74.59	77.04
3.	Acetone	52.45	70.49	61.47	66.39	68.85	70.49	72.13

The radical of sample is calculated by the following formula,

I nhibition =

$$\frac{\text{(Absorbance of control (Ac 517) – Absorbance of sample (As517)}}{\text{[(Absorbance control (Ac517)]}} \times 100$$

Estimation of Total Flavanoid Content

Aluminium calorimetric method

The aluminium chloride calorimetric method was modified from the procedure reported by Woisky and Salatino. Quercetin was used as standard. 10 mg of Quercetin was dissolved in 80% ethanol and then diluted to 25, 50 and 100µg/ml.

The diluted standard solutions (0.5ml) were separately mixed with 1.5ml of 95% ethanol, 0.1ml of 10% Aluminium chloride, 0.1ml of 1m potassium acetate and 2.8ml of distilled water. After incubation at room temperature for 30 minutes, the absorbance of the reaction mixture was measured at 415 nm with a spectrophotometer. (Chang et al., 2002)

RESULTS AND DISCUSSIONS

Phytochemical Analysis

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents (M. Devi *et al*.,2012). The pharmacological action of crude drugs and other therapeutic uses are due to their therapeutically active constituents. So the preliminary phytochemical analysis revealed the importance of secondary metabolites (M. Wink *et al*.,1999).

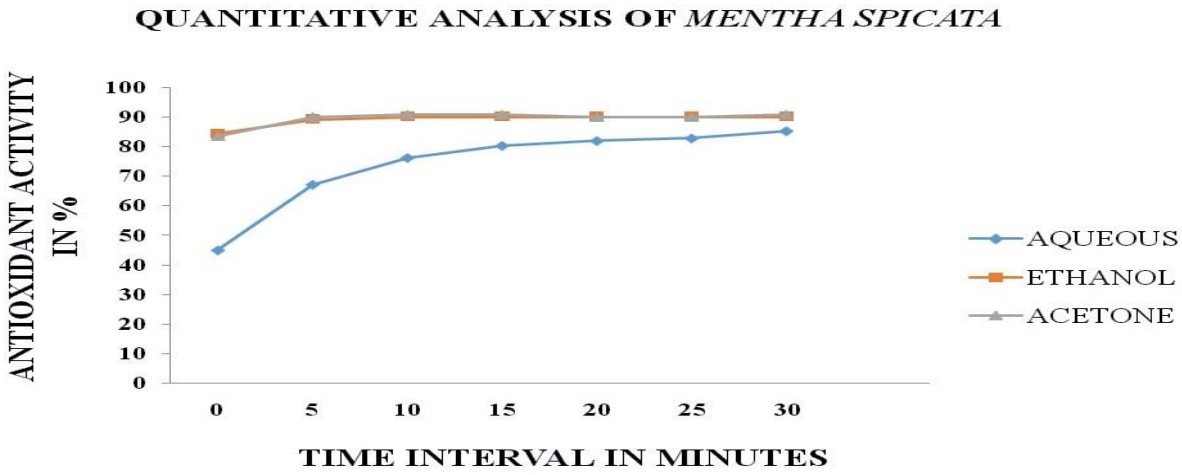
From this analysis ethanolic leaf powder extracts were found to have more chemical constituents compared to other extracts. Phytochemical analysis of *Mentha spicata* from the Hosur market accession (table 1) indicates the presence of Tannin, Flavonoids, Glycosides, Terpenoids, Quinones, Phenol, Steroids, Coumarin and β-cyanin. Analysis of *Plectranthus amboinicus* from the Chengalpet market accession (table 2) indicates the presence of Tannin, Flavonoids, Terpenoids, Quinones, Phenol, Steroids, Coumarin, Alkaloids and β-cyanin. Analysis of *Ocimum sanctum* from the Hosur market accession (table 3) indicates the presence of Tannin, Flavonoids, Cardioglycosides, Alkaloids, Terpenoids, Quinones, Phenol, Steroids, Coumarin and β-cyanin. Further Aqueous extract showed the presence of Flavonoids, Quinones, Terpenoids, Phenols, Coumarins, Steroids and β-cyanin commonly in all the three plants in high quality.

Quantitative Analysis

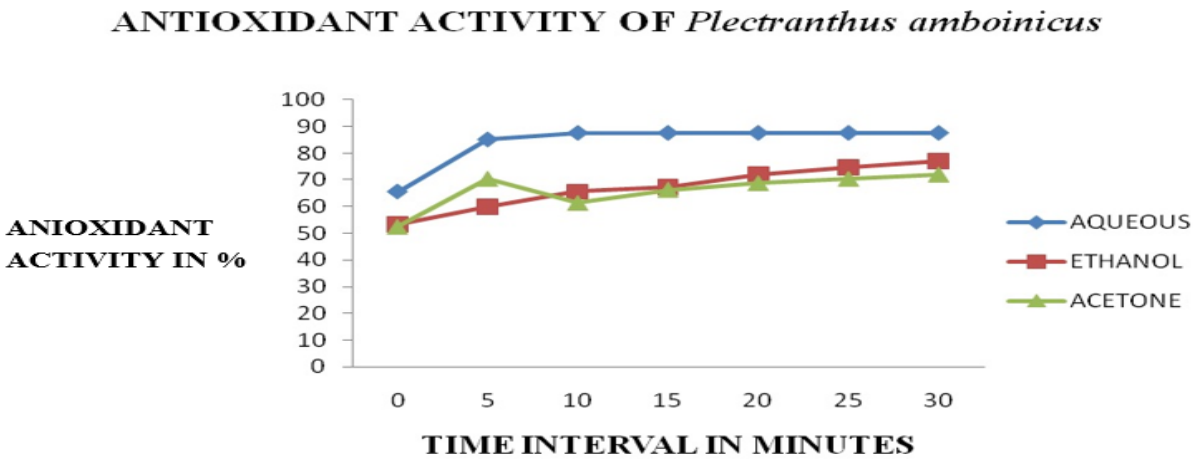
Free radicals are chemical entities that can exist individually with one or more unpaired electrons. The generation of free radicals can bring about thousands of reactions and thus cause extensive tissue damage. Lipids, proteins and DNA are all susceptible to attack by free radicals (Cotran *et al*.,1999). There is a evidence that phytochemical could be used as effective antioxidants for improving human and preventing or delaying degenerative diseases, including cardiovascular diseases (Cui, Dai, Li, Zhang, Fang 2000). The plant derived antioxidants are characterized by this ability to scavenge free radicals. Free radical scavenging action is an important attribute of antioxidants which is measured by the DPPH radical scavenging activity (Yamaguchi *et al*.,1998).The present study (table 4) shows that the ethanol and acetone extract of *Mentha spicata* exhibit strong antioxidant activities compared to that of standard compound BHT (Butylated Hydroxy Toluene). In *Plectranthus amboinicus* an aqueous extract(table 5) exhibit strong antioxidant activity compared to that of standard compound BHT (Butylated Hydroxy Toulene). In *Ocimum sanctum* an acetone extract (table 6) exhibit strong antioxidant activity compared to that of the standard compound BHT (Butylated Hydroxy Toulene). Hence this investigation suggested that the plant naturally having rich source of antioxidants could be used in the prevention of free radical diseases.

Table 7: Quantitative analysis of Antioxidant activity of *Ocimum sanctum*

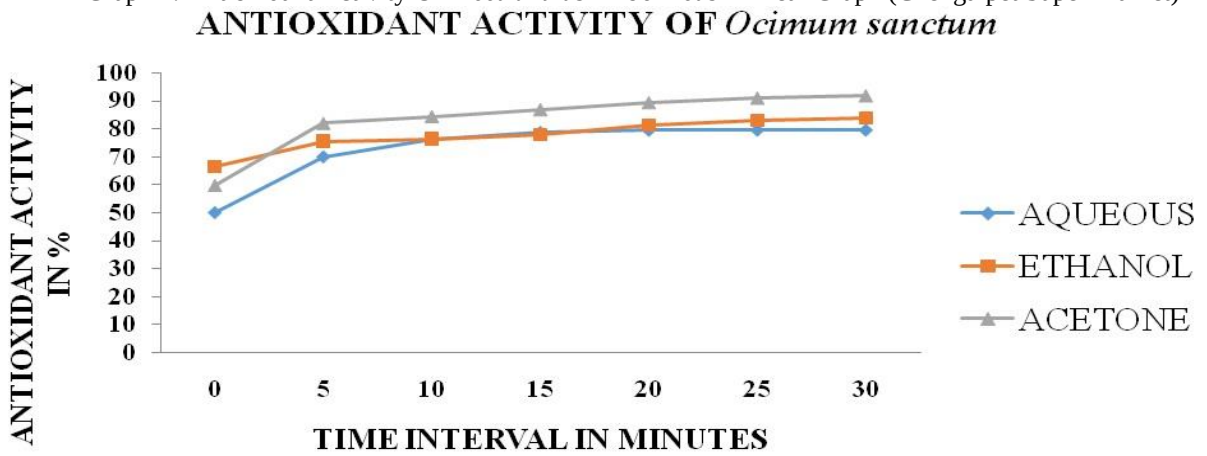
S.No	Solvents	Time in minutes/ Inhibition percentage of Free Radical Scavenging Activity						
		0	5	10	15	20	25	30
1.	Aqueous	50	69.67	76.22	78.68	79.50	79.50	79.50
2.	Ethanol	66.39	75.40	76.22	77.86	81.14	82.78	83.60
3.	Acetone	59.83	81.96	84.42	86.88	89.34	90.98	91.80



Graph 1: Antioxidant Activity Of *Mentha Spicata* –Linear Graph (Hosur Super Market)



Graph 2: Antioxidant Activity Of *Plectranthus Amboinicus* –Linear Graph (Chengalpet Super Market)



Graph 3: Antioxidant Activity of Ocimum sanctum –Linear Graph (HOSUR SUPER MARKET)

Estimation Of Total Flavonoid Content

sanctum

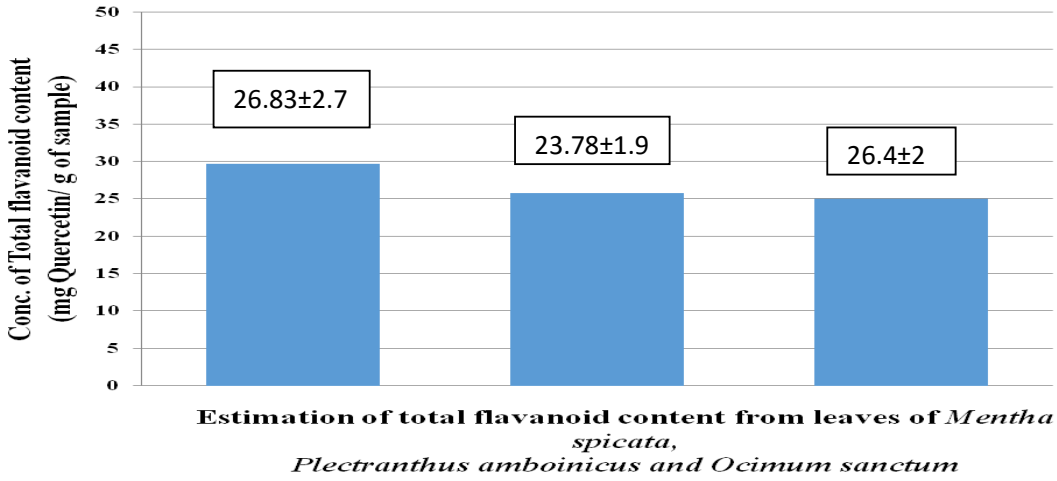
The amount of flavonoid content varied slightly and ranged from 29.70 to 25 mg of QE/g sample for all the three plant samples of acetone extract namely *Mentha spicata, Plectranthus amboinicus* and *Ocimum sanctum* (table 6, 7, 8). The highest flavonoid content was found in *Plectranthus amboinicus* when compared to the other two plant samples.

CONCLUSION

The phytochemical tests revealed the presence of Saponin, Flavanoids, Tannin, Quinones, Phenol, Steroids,Terpenoids.The antioxidant activity of *Mentha spicata, Plectranthus amboinicus* and *Ocimum sanctum*

Table 8: Determination Of Flavonoid Content

S.NO	Plant Samples	Total flavanoid content conc. (mg QE/ g sample)
1.	*Mentha spicata*	26.83±2.7
2.	*Plectranthus amboinicus*	23.78±1.9
3.	*Ocimum sanctum*	26.4±2



Graph 4: Flavonoid Estimation of *Mentha spicata, Plectranthus amboinicus, Ocimum*

was screened by DPPH assay method. The maximum scavenging activity of sample was found in the acetone extract of *Ocimum sanctum* (Hosur super market) compared with the *Mentha spicata* and *Plectranthus amboinicus*.The acetone extract of *Plectranthus amboinicus* (Chengalpet super market) showed the maximum total flavonoid content.

REFERENCES

1. Adam, S., Yahya, A., Salih, W., Abdelgadir, W., On Antimoicrobial Activity of the Masticatory *Cola acuminata* Nut (Gooro). Current Research Journal of Biological Sciences 3(4): 357-362, (2011).
2. Allen, J.C and W.L. Wreiden. 1982a. Influence of milk proteins on lipid oxidation in aqueous emulsion I. Casein, Whey protein and R- Lactalbumin. J. Dairy Res. *49*:239-248
3. Alok, S., Kumar Jain, S., Verma, A., Kumar, M., Herbal antioxidant in clinical practice. Asian Pac J Trop Biomed. (2014); 4(1): 78-84, 169 1(14) 60213-6.
4. Bansod sS, Rai M. Antifungal activity of essential oils fromIndiam Medicinal Plants against human pathogenic *Asperillus fumigates and A.niger. World J Med Sci, 2008*, 3(2): 81-88.
5. Barlow SM (1990).Toxicological aspects of antioxidants used as Food Additives. In Food Antioxidants, Hudoson BJF (ed.) Elsevier, London, pp 253-307.
6. Borris RP (1996).Natural productions research: Perspectives from a major pharmaceutical company. J. Ethnopharmacol *51*: 29-38.
7. Branen AL (1975). Toxicological and biochemistry of butylated hydroxy anisole & butylated hydroxytoulene. J.American Oil Chemists Society *5*: 59-63.
8. Espin, J.C., Soler- Rivas, C., Wichers, H.J., (2000), Characterization of the total free radical scavenger capacity of vegetable oils and oil fractions using 2,2-diphenyl1-1-picryl hydrazyl radicals, *J. Agric- Food Chemicals, 48*, (pp.648-656).
9. Frankel E (1995) Nutritional Benefits Of Flavonoids. International Conference on Food Factor: Chemistry and Cancer Prevention, Hammamatsu, Japan. Abstracts, C6-2.
10. Gulluce M, Sokman M, Daferera D, Agar G, Ozkan H, Kartal N, Polissiou M, Sokmen A, Shin F(2003). Invitro antibacterial, antifungal and antioxidants activities of the essential oil and the ethanol extracts of herbal parts and callus cultures of *Satureja hortensis* L. J. Agr, Food Chem. 51: 3958-3965.
11. Gryglewski RJ, Korbut R, Robak J (1987). On the mechanism of antithrombotic action of flavonoids. Biochemicals Pharmacol 36:317-321.
12. Halliwell B, Gutteridge JMC (1999). Free radicals in biology and medicine. Oxford University Press, Oxford.
13. Halliwell B (1995). How to characterize an antioxidant: an update. Biochem. Soc. Symp. *61*: 73-101.
14. Hu C., Kitts D.D. (2000): Studies on the antioxidant activity of Echinacea root extracts. J. Agric. Food. Chem., *48:*1466-1472.
15. Kanner J, Frankel E, Granit R, German B, Kinsella JE (1994). Natural Antioxidants in Grapes and Wines. J. Agric. Food Chem. *42*: 64-69.
16. Katsuzaki, H.; Kawakishi, S.; Osawa, T. (1993). Strucrure of novel antioxidative lignan triglucoside isolated from sesame seed. *Heterocycles, 36* 933-936.
17. Koleva ll, Van Beek TA, Linseen JPH, de Groot A, Evstatieva LN (2002). Screening of plant extracts for antioxidant activity: a comparative study on three testing methods phytochemical . Analysis.*13*:8-17.
18. Leighton,F., Cuevas,A., Guasch,V., Perez,D., Strobel,P., San Martin,A.,

19. Urzua,U., Diez ,M.S., Foncea,R., Castillo,O., Mizon,C., Espinoza,M.A., Urquiaga,I., Rozowski,J., Maiz,A.and Germain,A.(1999) 'Plasma Polyphenols and antioxidants, oxidative DNA damage and endothelialfunction in a diet and wine intervention study in humans', Drugs Exp. Clin, Res., pp. 133-141.
20. Lin, C.C. and P.C. Huang,2002. Antioxidant and hepatoprotective effects of Acathopanax senticosus Phytother. Res. *14*:489-494.
21. Nosrati, S., EsmailzadehHosseini, S., Srapeleh, A., SoflaeiShhrbabak, M., Soflaeishshrbabak, Y., On the Antifungal Activity of Spearmint (*Mentha spicata* L.) Essential oil on *Fusarium oxysporum f.sp. radicis-Cucumerinum* the casual Agent of stem and Crown Rot of Greenhouse Cucumber in Yazd, Iran., (2011)International Conference On Environmental and Agricultural Enginnering IPCBEE vol. 15(2011)
22. Okamura, H.; Mimura, A.; Yakou, Y.; Niwano, M.; Takahara, Y. (1993). Antioxidant activity of tannins and flavonoids in *Eucalyptus rostrata*. *Phytochemistry*, *33*, 557-561.
23. Parasakthy, K.; Shanthi, S.; Deepalokshmi, P.; and Niranjali, S.D (1996): The antioxidant effect of eugenol and carbon tetrachloride induced erythrocyte damage in rats: *J. Nutr. Biochem*. *7*: 23-28.
24. Paton A. A synopsis of *Ocimum* L. (Labiatae) in Africa. Kew Bull. 1992; 47: 403-36. http://dx.doi.org/10.2307/4110571
25. Perry, E.K., A.T. Pickering, W.W. Wang, P.J. Houghton, and N.S. Perru 1999. Medicinal plants and Alzhemier's disease: from ethnobotany to phytotherapy.J.Pharm.Pharmacol. *51*:527-534.
26. Repetto, M.G. and S.F. Lleusy. 2002. Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. Braz. J. Med. Biol. Res. *35*:523-534.
27. Romani A, Pinelli P, Galardi C, Mulinacci N, Tattini M (2002). Identification and quantification of Galloyl derivatives, Flavanoids, Glycosides and anthocyanins in leaves of *Pistacia lentiscus L.* Phytochem. Anal. 13: 79.86.
28. Rice- Evans CA, Miller NJ, Papanga G (1997). Antioxidant properties of Phenolic compounds. Trend.Plant sci. *4*:152:159.
29. S.y. Ramesh, K. Sandeep, D,Anupam, Antifungal properties of essential oil *Mentha spicata 1.* Var. MSS-5. Indian J. Crop science. 2006.1:197-200.
30. Sethi., Prakash, O., Chandra., Punetha, H., Pant, A.K., On Antifugal activity of essential oils of some *Ocimum* species collected from different locations of Uttarkhand (2013)., Indian Journal of Natural Products and Resources vol, 4(4), December 2013, pp.392-397.
31. Suh HJ, Chung MS, Cho YH, Kim JW, Kim DH, Han KW, Kim CJ (2005). Estimated daily intakes of Butylated Hydroxy Anisole (BHA), Butylated Hydroxy Toulene (BHT), tert- Butyl hydroquinones antioxidants in Korea, Food. Addit. Contam. 22 (12):1176-1188.
32. Tattini M, Guidi l, Morassi- Bonsi, Pinelli P, Remorini D, Innocenti E, Giordano C, Mossai R, Agati G (2005). On the role of flavanoids mechanisms of response of *Ligustrum vulgare* and *Phillyrea latifolia* to high solar radiation, N. Phytol., 167;457-470.
33. Yen, G. C., Duh, P. D., and Chuang. D. Y. (2000). Antioxidant activity of anthraquinone and anthrone. *Food Chemistry*, *70*, 307-315.