IN-VITRO ANTIOXIDANT ACTIVITY OF AERIAL PARTS OF *LIPPIA NODIFLORA* RICH.

Sangita Shukla*[1], Saluja A K[2], Pandya S S[3]

[1] Department of Pharmacognosy, Indukaka Ipcowala College of Pharmacy, New Vallabh Vidyanagar,P.O. Box 53, Po. Vitthal Udyognagar, Dist. Anand, Gujarat-388121.
Phone: +91 9898047135
[2] Department of Pharmacognosy, A.R.College of Pharmacy, Vallabh Vidyanagar, Gujarat-388120
[3] Department of Pharm.Chemistry, Babaria Institute of Pharmacy, Varnama, Gujarat-391240

Summary

As per Ayurveda, *Lippia nodiflora* Rich. [*Phyla nodiflora* (Linn.) Greene.] is used in various diseases, some of which occur due to generation of free radicals. In the present study, antioxidant and free radical scavenging activity of defatted and fractionated methanolic extract of aerial parts of *Lippia nodiflora* (MELN) was evaluated using *in vitro* methods like 1, 1-diphenyl, 2-picryl hydrazine (DPPH) radical scavenging activity, H_2O_2 scavenging activity, Nitric Oxide (NO) radical scavenging activity, Nitro Blue Tetrazolium (NBT) reduction assay, β-carotene-lineolate bleaching assay and Total reduction ability by Fe^{3+}-Fe^{2+} transformation (Reducing power assay). Ascorbic acid was used as a reference antioxidant compound. In DPPH radical scavenging activity, H_2O_2 scavenging activity, NO scavenging activity and NBT reduction assay the IC_{50} values obtained for MELN were found to be 799.74 µg/ml, 53.15 µg /ml, 61.51 µg/ml and 45.60 µg/ml respectively and for Ascorbic acid the IC_{50} values were found to be 511.36 µg/ml, 33.06 µg/ml, 42.40 µg/ml and 94.82 µg/ml respectively. In the reduction power assay increase in absorbance was observed in a dose dependant manner. Total phenolic content was measured using Folin-Ciocalteu phenol reagent which showed that 1 mg of the extract contained 114.89 µg/ml total phenolics equivalent to gallic acid. The results obtained in the present study indicate that the antioxidant activity of MELN may be due to the presence of flavonoids.

Key Words: *Lippia nodiflora*,antioxidant, flavonoids, total phenolic content.

Introduction

Free radicals are natural by-products produced during metabolic process in human/animal body. These are electrically charged molecules that attack our cells, tearing through cellular membranes to react and create havoc with nucleic acids, proteins, and enzymes present in the body. These attacks by free radicals, collectively known as oxidative stress, are capable of causing cells to lose their structure, function and can eventually destroy them. They are continuously produced by our body's own use of oxygen such as in respiration and some cell-mediated immune function **(1).** Normally there is a balance between the amount of free radicals generated in the body and the antioxidant defense systems that scavenge/ quench these free radicals preventing them from causing deleterious effects in the body **(2).** The antioxidant defense systems in the body can only protect the body when the amount of free radicals is within the normal physiological level. But when this balance is shifted towards more of free radicals, increasing their burden in the body either due to environmental conditions or produced within the body, it leads to oxidative stress, which may result in tissue injury and subsequent diseases **(3).** Since free radicals play such an important role in the disease scenario of an individual, a thorough understanding of the various physiologically significant free radicals is of paramount importance before the search of the radical scavengers or the antioxidant principles to treat the physiological disorders caused by them **(4).** Reactive oxygen species (ROS) is a collective term, which includes not only the oxygen radicals ($O_2^{\bullet -}$ and $OH^{\bullet}$) but also some non-radical derivatives of oxygen. These include hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl) and ozone (O_3) **(5).** The role played by ROS in stress induced gastric ulcer and inflammatory bowel diseases have been well established, as well as their involvement in the process of ageing **(6, 7).** Synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) commonly used in food have side effects and are carcinogenic **(8, 9).** This is one of the reasons why discovery and synthesis of novel and antioxidants is a major active area.

As per Ayurveda, *Lippia nodiflora* Rich. is acrid, cooling, aphrodisiac, astringent to the bowels, stomachic, vulnerary, anthelmintic, alexiteric; useful in diseases of the heart, the blood, the eye; improves taste; good for ulcers, wounds, burning sensation, asthma, bronchitis, thirst, loss of consciousness **(10).** It is found in wet places along bunds of irrigation channels, canal edges and river banks almost throughout India ascending upto 900m in the hills **(11).** *Lippia nodiflora* Rich. growing in India has revealed the presence of nodifloretin **(12)**; two 6-hydroxyluteolin glycosides; the 7-arabinose and the 4'-rhamnoside **(13)**; halleridone and hallerone **(14)** and monoterpenes like 2-phenethyl alcohol, 1-octen-3-ol, linalool, 2,6,- dimethyloctane, methyl salicylate, p-cymen-8-ol; sesqueterpenes like calamenene, β-caryophyllene, α-copaene, α-copaene, α-bergamotene, δ-cadinene, β-bisabolene **(15).**

Materials and Methods

Plant Material And Preparation Of Extract.

The aerial parts were collected from river bank of Mahi River, Anand District of Gujarat, India and authenticated by Pharmacognosist of Pharmacognosy department, Indukaka Ipcowala College of Pharmacy, New Vallabh Vidyanagar. A voucher specimen (IICP/07/02) has been preserved in Pharmacognosy laboratory of Indukaka Ipcowala College of Pharmacy. The collected plant material was dried under shade, powdered (# 60) with mechanical grinder and stored in air tight container. The dry

powdered material was defatted using petroleum ether (60-80^{o}) using Soxhlet's extractor. Defatted material was then extracted with chloroform followed by methanol. The solvents were completely recovered under reduced pressure. The methanolic extract (MELN) obtained as a semisolid mass and was selected for further studies after preliminary phytochemical analysis of all extracts **(16).**

DPPH Radical Scavenging Activity.

Free radical scavenging potentials of MELN was tested against a methanolic solution of DPPH **(17, 18).** 0.1 mM solution of DPPH in methanol was prepared and 1.0 ml of this solution was added to 3.0 ml of different concentrations (200-1000 µg/ml) of MEGA prepared in water. It was incubated at room temperature for 30 minutes and the absorbance was measured at 517 nm against the corresponding blank solution. Ascorbic acid was taken as reference. Percentage inhibition of DPPH free radical was calculated based on the control reading, which contain DPPH and distilled water without any extract using the following equation:

% Scavenging Activity = [(Ac- As) / Ac] × 100

Where, Ac is the absorbance of the control reaction and As is the absorbance in the presence of the sample of the extracts. The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration (in µg/mL) of extracts that inhibits the formation of DPPH radicals by 50%. The IC_{50} values were calculated from graph by linear regression analysis.

Scavenging Of Hydrogen Peroxide **(19)**

A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). The concentration of hydrogen peroxide was determined by recording the absorbance at 230 nm. Different concentrations of MELN (20-100 µg/ml) in distilled water added to a hydrogen peroxide solution (0.6 ml, 40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after ten minutes against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenged by the MELN and Ascorbic acid (standard) was calculated using following equation:

% Scavenging Activity = [(Ac- As) / Ac] × 100

Nitric Oxide Radical (NO) Scavenging Activity **(20, 21)**

Nitric oxide (NO) radicals were generated from sodium nitroprusside solution at physiological pH. Sodium nitroprusside (1 ml of 10 mM) was mixed with 1 ml of MELN in different concentrations (20-100 µg/mL) in phosphate buffer (pH 7.4). The mixture was incubated at 25^{o} C for 150 min. To 1 ml of incubated solution, 1 ml of Griess' reagent (1% sulphanilamide, 2% o-phosphoric acid and 0.1% naphthylethylenediamine dihydrochloride) was added. Absorbance was read at 546 nm and percentage inhibition was calculated using formula:

% Scavenging Activity = [(Ac- As) / Ac] × 100

Nitro blue Tetrazolium (NBT) Reduction Assay (**22)**

Antiradical activity is measured by the decrease in absorbance at 590 nm after illumination for 5 mins. Decrease in the absorbance in the standard and extracted was noted.0.2 ml riboflavin (1mg/ml) solution is mixed with 0.4ml EDTA (12mM) +

5.2 ml extract or ascorbic acid prepared in phosphate buffer (pH=7.4) (20 µg/ml-100 µg /ml) + 0.2 ml Nitro blue Tetrazolium. The reaction mixture was illuminated under light for 5 mins and absorbance measured at 590 nm. Percentage inhibition was calculated using formula:

% Scavenging Activity = [(Ac- As) / Ac] × 100

β -Carotene Linoleate Bleaching Assay

The antioxidant activity of the extract was assayed, based on the β-carotene bleaching method **(23).**Ascorbic acid was used as the standard. β-carotene (0.2mg in 1ml chloroform), linoleic acid (0.02ml) and tween-20(0.2ml) were transferred into a round bottom flask. The mixture was then added to 0.2 ml of different concentrations (200-1000 mcg/ml) of extract or standard or methanol (as control). Chloroform was removed at room temperature under vacuum at reduced pressure using a rotary evaporator. Following evaporation, 50ml of distilled water was added to the mixture, and then shaken vigorously to form an emulsion. 2 ml aliquots of the emulsion were pipetted into test tubes and immediately placed in a water bath at 50^{o} C. The absorbance was read at 30 min intervals for 2h at 470 nm. The antioxidant activity was based upon different parameters, namely antioxidant activity (AA), degradation rates (RD) and oxidation rate ratio (ROR).

Antioxidant activity (AA) was expressed as percent inhibition relative to control, using formula: **(24)**

$AA= [R_{control} - R_{sample\ of\ standard}/ R_{control}] X 100,$

Where $R_{control}$ and R_{sample} represent the bleaching rates of β -carotene without and with the addition of antioxidant, respectively.

Degradation rates (RD) were calculated according to first order kinetics: $RD=\ln (A_t/A_x) X 1/t_x$

Where ln is natural log, At is the absorbance at 470 nm at t=0 and Ax is the absorbance at 470 nm at t= 30, 60, 90, 120 min.

Reducing Power Assay **(25, 26)**

The different concentration of MELN (20-100 µg/ml) in 1 ml of deionized water were mixed with phosphate buffer (2.5 ml, 0.2M, pH 6.6) and 1% potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 ml). The mixture was incubated at 50 ^{o}C for 20 minutes. The reaction was stopped by adding trichloroacetic acid (2.5 ml, 10%) to the mixture,2.5ml of this mixture was mixed with distilled water (2.5ml) and ferric chloride (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Ascorbic acid was taken as reference.

Total phenolic content

The total phenolic contents of the extract were determined using the Folin-Ciocalteu reagent **(27).** The reaction mixture contained 1 ml of MELN, 0.5 ml of the Folin-Ciocalteu reagent, 3 ml of 20% sodium carbonate and 10ml of distilled water. After 2 h of reaction at the ambient temperature the absorbance at 765 nm was measured and used to calculate the phenolic contents using Gallic acid as a standard. The total phenolic content was then expressed as Gallic Acid Equivalent (GAE), in mg/g dry sample.

Results

The results obtained in this study indicated dose dependant inhibition of DPPH, H_2O_2, NO and Reduction of NBT by MELN (Fig 1-a, b, c, d). The IC_{50} values for MELN in DPPH, H_2O_2, NO radical scavenging activity and NBT reduction assay were found to be 799.4 µg/ml, 53.15µg/ml, 61.15 µg/ml and 45.60 µg/ml respectively and for Ascorbic acid the IC_{50} values were 511.36 µg/ml, 33.06 µg/ml, 42.40 µg/ml and 94.82 µg/ml respectively. In the reducing power assay there was an increase in the absorbance with increase in concentration of the extract, which indicates that the extract possesses reducing ability. The results of MELN showed 50% inhibition at high concentration in DPPH, H_2O_2, NO radical scavenging activity and at low concentration in NBT reduction assay as compared to ascorbic acid.

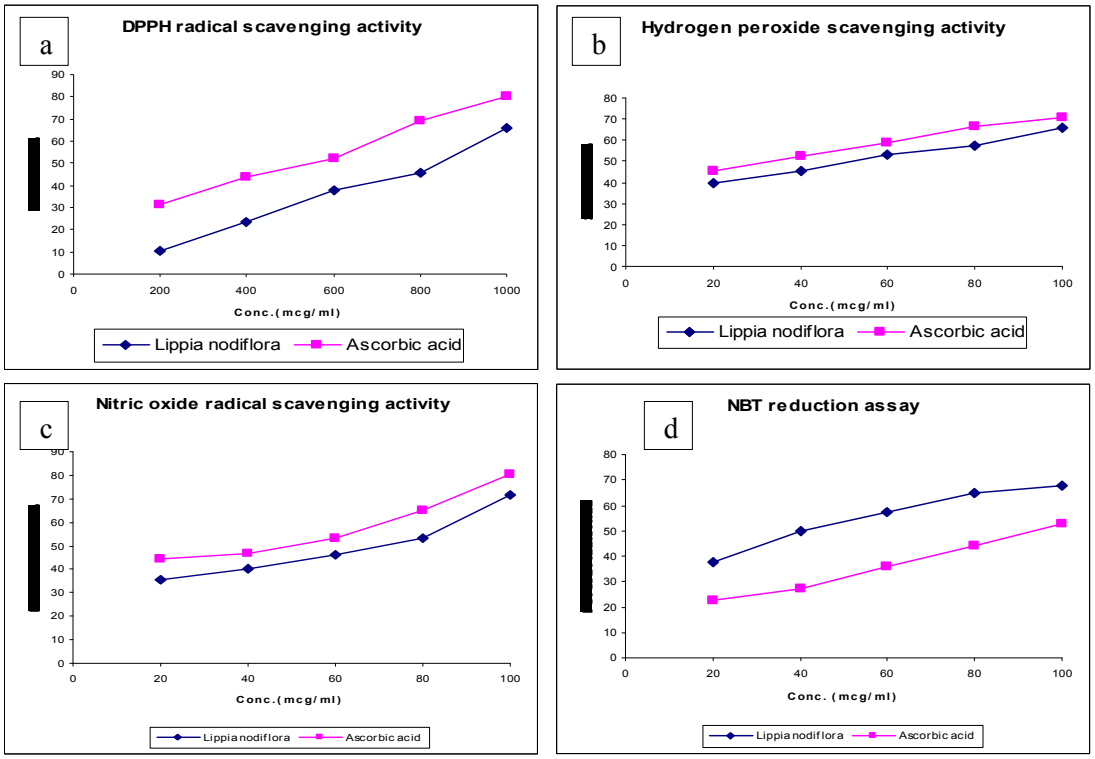

Fig.1Effect of MELN and Ascorbic acid on **a)** DDPH radical scavenging activity **b)** H_2O_2 scavenging activity **c)** Nitric oxide scavenging activity **d)** NBT reduction assay.

The reducing power of MELN was observed to be dose dependant as shown in Table 1.

Concentration in µg/ml	Absorbance MELN	Absorbance Ascorbic acid
20	0.107	0.232
40	0.194	0.304
60	0.254	0.328
80	0.35	0.433
100	0.407	0.484

Table 1. Absorbance of MELN and Ascorbic acid at different concentration showing Total reductive ability.

In β-carotene bleaching assay there was a co-relation between degradation rate and the bleaching of β-carotene; the extract with the lowest degradation rate exhibited highest antioxidant activity. (Fig.2-a and b)

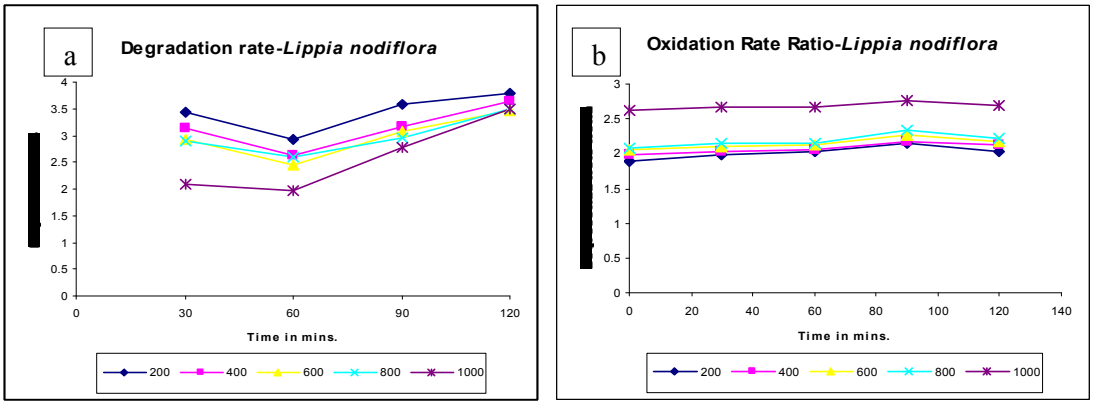

Fig. 2 a and b: Degradation rate and oxidation rate ratio of methanolic extract of *Lippia nodiflora* respectively, by β-carotene-linoleate bleaching assay.

Determination of Total Phenolic Content: The absorbance of MELN (1mg/ml) measured at 765 nm was found to be 0.562.From the standard curve of gallic acid (Fig.3) the amount of total phenolics in MELN was calculated by linear regression analysis using the equation y=0.0064x-0.0073(R^2=0.9922). 1 mg of MELN contained 114.89 µg/ml of total phenolics equivalent to Gallic acid.

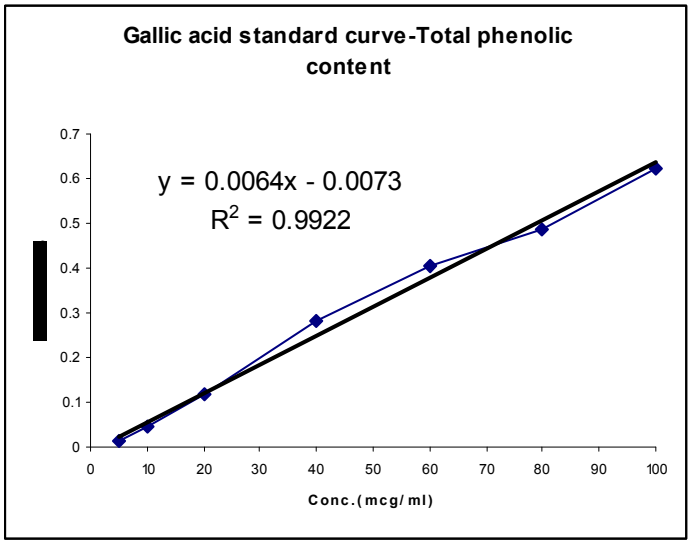

Fig.3 Gallic acid standard curve for determination of total phenolics.

Discussion

DPPH scavenging activity has been used by various researchers as a quick and reliable parameter to assess the *in vitro* antioxidant activity of the crude plant extracts **(28, 29).** In the DPPH test the ability of a compound to act as donor for hydrogen atoms or electrons was measured spectrophotmetrically. In the present investigation MELN demonstrated significant DPPH radical scavenging activity in a dose dependant manner indicating its ability to act as radical scavengers.

The scavenging of hydrogen peroxide by MELN and ascorbic acid after incubation for 10 minutes increased with increase in concentration. The IC_{50} value of MELN and ascorbic acid were found to be 53.15 µg/ml and 33.06 µg/ml.While hydrogen peroxide is not very reactive **(30)**, it can generate highly reactive hydroxyl radical (OH) through Fenton reaction (Equation 1) **(31,32).**

Thus, the scavenging of hydrogen peroxide is an important antioxidant defense mechanism **(33).**

$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH + OH^-$ (Equation 1)

The decomposition of hydrogen peroxide to water involves the transfer of electrons as in Equation 2 **(34).**

$H_2O_2 + 2H^+ + 2e^- \longrightarrow 2H_2O$ (Equation 2)

The scavenging of hydrogen peroxide by phenolic compounds has been attributed to their electron-donating ability **(35).**

Nitric Oxide (NO) is a potent pleiotropic mediator of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical which plays many roles as an effector molecule in diverse biological systems including neuronal messenger, vasodilation, antimicrobial and antitumor activities **(36).** Excess of NO is associated with several diseases **(37, 38).** Oxygen reacts with excess nitric oxide to generate nitrite and peroxynitrite anions, which act as free radicals **(39, 40).**In the present study the extract MELN competed with oxygen and reacted with NO and thus inhibited the generation of anions.

In NBT reduction assay the antiradical activity was measured by the decrease in absorbance at 590 nm after illumination of the reaction mixture for 5 min. This was caused by the reduction of NBT by riboflavin. In the present study MELN required less dose (IC_{50}= 49.6 µg/ml) for 50% reduction as compared to ascorbic acid (IC_{50}= 94.82 µg/ml) **(22).**

In the β-carotene bleaching assay, linoleic acid produces hydroperoxides as free radicals during incubation at 50 ^oC. The presence of antioxidants in the extract minimizes the oxidation of β-carotene by hydroperoxides. Hydroperoxides formed in this system were neutralized by the antioxidants from the drug. In the present study, we evaluated antioxidant activity of MELN by β-carotene linoleate bleaching assay because β-carotene shows strong biological activity and is physiologically important compound **(41, 42).**

Phenolic constituents are very important in plants because of their scavenging ability due to their hydroxyl groups **(43).**Phenolic compound are widely distributed in plants**(44)**.These have gained much attention due to their antioxidant activity and free radical scavenging ability, which potentially have beneficial implications for human health **(45-47).** One mg of MELN extract contained 114.89µg/ml of GAE of phenols. Phenolic compounds are famous powerful chain breaking antioxidants **(48).**

Preliminary phytochemical analysis showed the presence of flavonoids in the methanolic extract of aerial parts of *Lippia nodiflora*. The flavonoids may, therefore act as a preventive agent in pathologies where reactive oxygen species have been implicated. Thus the antioxidant potential of MELN could be attributed to the presence of the flavonoids present in the plant.

References

1. Li Y, Trush M A. Reactive oxygen dependant DNA damage resulting from the oxidation of phenolic compounds by a copper redox cycle. Cancer Research 1994; 54: 1895s-1898s.
2. Nose K. Role of reactive oxygen species in regulation of physiological functions. Biological and Pharmaceutical Bulletin 2000; 23: 897-903.
3. Finkle T, Holbrook N J. Oxidants, oxidative stress and biology of ageing. Nature 2000; 408: 239-247.
4. Govindarajan R, Vijayakumar M, Pushpagandhan P. Antioxidant approach to disease management and the role of 'Rasayana' herbs of Ayurveda. Journal of Ethnopharmacology 2005; 99:167.
5. Bandhopadhyay U, Das D, Banerjee R K. Reactive oxygen species: oxidative damage and pathogenesis. Current Science 1999; 77:658-666.
6. Harman D. Aging: phenomenon and theories. American NewYork Academy of Sciences 1998; 854:1-7.
7. Ferrari C K B. Oxidative stress physiology: searching for an effective antioxidant protection. International Medical Journal 2001; 8: 175-184.
8. Branen A L. Toxicology and biochemistry of butylated hydroxyl anisole and butylated hydroxyl toluene. Journal of the American Oil Chemists Society 1975; 52: 59-63.
9. Ito N, Fukushima S, Hassegawa A et al. Carcinogenicity of butylated hydroxyl anisole in F344 rats. Journal of National Cancer Institute 1983; 70: 343-347.
10. Kirtikar K R, Basu B D. Indian Medicinal Plants, 2nd ed Vol III. Dehradun: India: Bishen Singh and Mahendra Pal Singh, 1991:1916-1917.
11. Anonymous. The Wealth of India (A dictionary of Indian Raw Materials and Industrial Products). Raw Materials-Vol VI, New Delhi: CSIR,1969:142-143.
12. Barna A K, Chakrabarti P, Sanyl P K. Nodifloretin- a new flavone from *Lippia nodiflora.* J Indian Chem. Soc 1969; 46: 271.
13. Nair A G R, Ramesh P, Nagarajan S, Subramanian S S. New flavone glycosides from *Lippia nodiflora.* Indian J Chem 1973; 11: 1316.
14. Ravikanth V, Ramesh P, Diwan P V, Venkateswarlu Y. Halleridone and Hallerone from *Phyla nodiflora* as taxonomic markers. Biochemical Systematics and Ecology 2000; 28: 905-906.
15. Terblanche F C, Kornelius G.Essential oil constituents of the genus Lippia (Verbenaceae) - A literature review. Journal of Essential Oil Research 1996; 8: 471-485.
16. Kokate C K. Practical Pharmacognosy. 4thed. 1994. Vallabh Prakashan, Delhi:107-111.
17. Blois M S. Antioxidant determination by the use of a stable free radical. Nature 1958; 181: 1199-1200.
18. Lai L S, Chous S T, Chao W W. Studies on the antioxidant activities of hsian-tsao (*Mesona procumbens* Hemsl) leaf gum. J Agric Food Chem 2001; 49: 963-968.
19. Ruch R J, Cheng S J, Klaunig J E. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis 1989; 10: 1003-1008.
20. Sreejayan N, Rao M N A. Nitric oxide scavenging by curcuminoids. J Pharm Pharmacol 1997; 49:105.

21. Marcocci L, Maguire J, DroyLefaix M T et al. The nitric oxide scavenging properties of Ginkgo biloba extract EGB761. Biochem Biophy Res Commun 1994; 201:748.
22. Yashisaknono. Arch Biochem and Biophysics 1978; 136(1): 189-195.
23. Othman A, Ismail A, Ghani N A et al. Antioxidant capacity and phenolic content of cocoa beans.Food Chemistry 2007; 100:1523-1530.
24. Suja K P, Jayalakshmi A, Arumughan C. Antioxidant activity of sesame cake extract. Food Chemistry 2005; 91:213-219.
25. Oyaizu M. studies on product of browning reaction prepared from glucose amine. Jpn J Nutr 1986; 44: 307-315.
26. Jayaprakash G K, Singh R P, Sakariah K K. Antioxidant activity of grape seed extracts on peroxidation models in vitro. J agric food Chem 2001; 55: 1018-1022.
27. Zhou K, Yu L. Total phenolic contents and antioxidant properties of commonly consumed vegetables grown in Colorado. Lebensmittel-Wissenschaft und-technologie 2006; 39: 1155-1162.
28. Navarro M C, Montilla M P, Martin A, Jimennez J, Utrilla M P. Free radicals and anithepatotoxic activity of *Rosmarinus tomentosus*. Planta Med 1992; 59: 312-314.
29. Thabrew M I, Hughes R D, McFarlane I G. Antioxidant activity of *Osbeckia aspera*. Phytotheapy Res 1998; 12: 288-290.
30. Namiki M. Antioxidants / antimutagens in foods. Crit Rev Food Sci Nutr 1990; 29:273-300.
31. Cohen G, Heikkila R E. The generation of hydrogen peroxide, Superoxide radical and hydroxyl radical by 6- hydroxyl radical by 6-hydroxydopamine, dialuric acid, and related cytotoxic agents. J Biol Chem 1974; 249:2447-2452.
32. Halliwel B. The biological toxicity of free radicals and other reactive oxygen species. In Aruoma O I, Halliwell B, eds.Free Radicals and Food Additives. London: Taylor and Francis, 1991; 37-49.
33. Duh P D, Tu Y Y, Ten G C. Antioxidant activity of water extract of Harng Jyur (*Chrysanthemum moifolium* Ramat). Lebensm-Wiss Technol 1999;32: 269-277.
34. Wettasinghe M, Shahidi F. Antioxidant and free radical-scavenging properties of ethanolic extracts of defatted borage (*Borago officinalis* L.) seeds. Food Chem 1999; 67: 399-414.
35. Wettasinghe M, Shahidi F. Scavenging of reactive-oxygen species and DPPH free radicals by extracts of borage and evening primrose meals. Food Chem 2000; 70: 17-26.
36. Hagerman A E, Riedl K M, Jones G A, Sovik K N, Ritchard N T, Hartzfeld P W et al. High molecular weight plant polyphenolics (tannins) as biological antioxidants. J Agric and Food Chem 1998; 46: 1887-1892.
37. Ialenti S, Moncada M, Di Rosa. Modulation of adjuvant arthritis by endogenous nitric oxide. Br J Pharmacol 1993; 110:701-705.
38. Ross R. The pathogenesis of artherosclerosis: a perspective for the 1990's. Nature 1993; 362:801.
39. Cotran R S, Kumar V, Collins T. Robbin's Pathological basis of diseases, 6th ed . Noida, Delhi:Thomson Press, 1999: 1-43.
40. Sainani G S, Manika J S, Sainani R G. Oxidative stress: a key factor in pathogenesis of chronic diseases. Med Update 1997; 1:1.

41. Kumazawa S, Taniguchi M, Suzuki Y et al. Antioxidant activity of polyphenols of carob pods. Journal of Agricultural and Food Chemistry 2002; 50: 373-377.
42. Sarkar A, Bishayee A, Chatterjee M. Beta carotene prevents lipid peroxidation and red blood cell membrane protein damage in experimental hepato carcinogenesis. Cancer Biochemistry and Biophysics 1995; 15: 111-125.
43. Hatano T, Edamatsu R, Mori A et al .Effects of tannins and related polyphenols on Superoxide anion radical and on DPPH radical. Chem Pharm Bull 1989; 37: 2016-2021.
44. Li BB, Smith B, Hossain Md. M. Extraction of phenolics from citrus peels I. Solvent extraction method. Separation and Purification Technology 2006; 48: 182-188.
45. Govindarajan R, Singh D P, Rawat A K S. High Performance Liquid Chromatographic Method for quantification of phenolics in 'Chyavanprash' a potent Ayurvedic drug. Journal of Pharmaceutical and Biomedical Analysis 2007; 43: 527-532.
46. Imeh U, Khokhar S. Distribution of conjugated and free phenols in fruits; Antioxidant and cultivar variations. Journal of Agricultural and Food Chemistry 2002; 50: 6301.
47. Ross J A, Kasum C A. Dietary flavonoids: Bioavailability, metabolic effects and safety. Annual review of Nutrition 2002; 22: 19.
48. Shahidi F, Wanasudara PKJPD. Phenolic antioxidants. Crit Rev Food Sci Nutr 1992; 32: 67-103.