

RESEARCH ARTICLE

**QUANTIFICATION OF TOTAL PHENOL, FLAVONOID CONTENTS AND ANTIOXIDANT
ACTIVITY OF SOME SELECTED INDIAN MEDICINAL PLANTS**

C.RATHANAVEL¹ AND P.THILLAI ARASU²

¹Department of Chemistry, Manonmanium Sundaranor University, Abishekapatti,
Tirunelveli- 627 012 Tamil Nadu, India.

²Department of Chemistry, Kalasalingam University, Anand Nager, Krishnankovil,
Virudunagar-626 190 Tamil Nadu, India.

*Corresponding author e-mail: rathanavel.slm@gmail.com

Abstract

The study was conducted to investigate the phenol, flavonoid content and antioxidant activity in some selected, traditionally used Indian medicinal plants in ethyl acetate, and screened for their antioxidant and free radical scavenging properties using Ascorbic acid and α -tocopherol as standard antioxidants. Antioxidant was determined using spectrophotometric methods. Antioxidant activities of extracts were expressed as percentage of DPPH radicals inhibition and IC_{50} values. The total phenolic content ranged from 35.25 to 224.49 mg/100g of dry weight of extract, expressed as Gallic acid equivalents. The total Flavonoids concentration varied from 6.86 to 126.24 mg /100g of dry weight expressed as rutin equivalents. Results suggested a good potential of phenolic and flavonoid compounds and antioxidant activity in *Strychnos nux-vomica* (IC_{50} value 42.60), *Salacia Oblonga* (IC_{50} value 42.94) *Indigofera inctoria* (IC_{50} value 40.64) *Melothria maderaspatana* (IC_{50} value 38.21) and *Enicostemma littorale* (IC_{50} value 43.76) showed better activity than remaining medicinal plants.

Keywords: Antioxidant, radical scavenger, flavonoids, phenols, 1,1-diphenyl-2-picryl hydrazyl.

Introduction

Natural antioxidants in the plants scavenge harmful free radicals from our body. Free radical is any species capable of independent existence that contains one or more unpaired electrons which reacts with other molecules by taking or giving electrons and involved in many pathological conditions¹. Plants generally produce several secondary metabolic such as phenols, flavonoids, tannins, alkaloids, saponins quinines and sterols which are important source of biocides and many other pharmaceutical drugs²⁻³. The most important secondary metabolites are phenols and Flavonoids⁴⁻⁵. Antioxidants are used to preserve food quality mainly because they arrest oxidative deterioration of lipids⁶. It is possible to reduce the risk of chronic

diseases and prevent disease progression by either enhancing the body's natural antioxidant defenses or by supplementing with proven dietary antioxidants⁷.

The aim of this study is to determine total phenolic and flavonoid contents, and evaluate the antioxidant activity of medicinal plants and various part extracts using ethyl acetate solvent. The most commonly used antioxidant methods are DPPH is characterized by excellent reproducibility under certain assay conditions. The DPPH free radical (DPPH \cdot) does not require any special preparation. The ferric reducing antioxidant power (FRAP) method is based on the reduction of ferrioxal analogue, the Fe³⁺ complex of triphenyl triazine Fe

(TPTZ)³⁺ to the intensely blue-coloured Fe²⁺ complex Fe (TPTZ)²⁺ by antioxidants in acidic medium [13]. Oxygen consumption inherent in cell growth leads to the generation of a series of reactive oxygen species. They are continuously produced by the body's normal use of oxygen such as respiration. Oxidative stress due to increase free radical generation or impaired endogenous antioxidant mechanism is an important factor that has been implicated in various diseases.

The reactive oxygen species include such as superoxide anion radicals, hydroxyl radicals and non free radicals species such as hydrogen peroxide⁸. Reactive oxygen species are continuously produced during normal physiologic events and can easily initiate the peroxidation of membrane lipids, crucial bimolecular such as nucleic acids, proteins and carbohydrate⁹. Antioxidant can protect the human body from free radical and reactive oxygen species effect.

Materials and Methods

Chemicals and Reagents

1,1-diphenyl-2-picrylhydrazyl (DPPH) purchased from Sigma Aldrich, Bangalore. Ascorbic acid, Folin Ciocalteu's phenol reagent, sodium carbonate, gallic acid were purchased as analytical grade chemicals. Sodium nitrite, AlCl₃·H₂O, sodium hydroxide and H₂O₂ are analytical grade from Merck, Flavonoids(rutin) was purchased from sigma Aldrich. ethyl acetate, sodium phosphate buffer (P^H - 6.6), potassium ferric cyanide, trichloro acetic acid, ferric chloride and -tocopherol were obtained from Merck.

Preparation of crude extract

A known quantity of powder was filled in extraction thimble and soxhlet extracted successively for 48 hrs in ethyl acetate with constant temperature not exceeding the boiling point of the solvent. The extract was filtered using whatman filter paper (No.1) and concentrated in vacuum and dried at 45°C for solvent removal and extracts were kept in sterile bottles under refrigerated conditions until use. The dry weight of the plant extracts was obtained by solvent evaporation were weighed and dissolved in dimethyl sulfoxide (DMSO) to obtain the final concentrations (mg/ml). The extract thus obtained was directly used in the assay of Anti oxidant activity.

Estimation of Total phenolic content

Total phenolic content of the plant extracts obtained from Ethyl acetate were estimated using FolinCiocalteu assay by spectrophotometric method. Plant extracts with various concentrations (50µl, 100 µl,150 µl,200 µl and 250 µl) were mixed with 1ml of Folin Ciocalteu phenol reagent (1ml) and incubated room temperature for 3 minutes, followed by 1ml of 20% sodium carbonate solution was added to mixture and diluted to 10ml with distilled water. The reaction mixtures were incubated in dark for one hour and the absorbance of the resulting blue color was measured at 765nm with Shimadzu UV-VIS Spectrophotometer. Quantifications were done with respect to the standard curve of Gallic acid (20-100µg/ml). Results were expressed as mg of Gallic acid equivalent per 100g of the dry weight of the plant material extracts(GAEs) Table 3.1. All determinations were performed in triplicates (n=3).

Determination of Total Flavonoids

The Flavonoids rutin (quercetin-3-rutinoside) is a flavonol glycoside comprised of the flavonol quercetin and the disaccharide rutinose. Rutin was found in many medicinal plant and well known antioxidant and as natural compound with wide range of medicinal properties¹⁰ some studies suggest that flavonoids may be useful in the treatment of many impaired conditions¹¹. In fact, many of the medicinal action of herbs of pollens and propolis are now known to be directly related to their flavonoid content. Rutin as a flavonoid and its aglucone quercetin (P Vitamin) have a ability to increase intracellur ascorbic acid levels decrease capillary permeability and fragility scavenge oxidants and free radicals and unique ability to bind to collagen structure directly (prolin hydroxylation to hydroxyprolin) as well as inhibit destruction of bone⁸.

Total Flavonoids contents of the plant extracts were determined by Aluminum chloride colorimetric assay ethyl acetate extracts with different concentrations (50µl, 100 µl, 150 µl, 200 µl and 250 µl) were prepared and mixed with 1.25ml of distilled water and 75 µl of 5% sodium nitrite solution was added. After 5 minutes 150 µl of 10% AlCl₃·H₂O solution was added. After 6 minutes 500 µl of 1M sodium hydroxide and 275 µl of distilled water were added to the mixture. The solution was mixed well and the absorbance was measured against a

Table 3.1 Total Phenolic contents in Medicinal Plants

Botanical Name of Medicinal plants & Parts Investigated	Total phenol content Ethyl acetate Extract(mg GAE/100g dw)
<i>Indigofera tinctoria</i> (Whole plant)	109.42 ± 0.26
<i>Oldenlandia umbellata</i> (Whole plant)	202.23 ± 0.00
<i>Acalypha indica</i> (Whole plant)	138.69 ± 0.51
<i>Indigofera aspalathoides</i> (Whole plant)	141.84 ± 1.18
<i>Eclipta alba</i> (Whole plant)	224.49 ± 0.00
<i>Enicostemma littorale</i> (Whole plant)	131.21 ± 0.37
<i>Aristolochia bracteolata</i> (Leaf)	142.51 ± 0.60
<i>Melothria maderaspatana</i> (Leaf)	217.24 ± 12.5
<i>Achyranthes aspera</i> (Leaf)	66.08 ± 0.04
<i>Mucuna Prurita</i> (Seeds)	114.67 ± 1.30
<i>Strychnos nux-vomica</i> (Seeds)	75.09 ± 1.10
<i>Citrullus colocynthis</i> (Seeds)	224.19 ± 0.00
<i>Strychnos potatorum</i> (Seeds)	115.96 ± 3.98
<i>Corallocarpus Epigaeus</i> (Tuber)	35.25 ± 0.03
<i>Mirabilis jalapa</i> (Tuber)	54.37 ± 0.22
<i>Asparagus racemosus</i> (Tuber)	36.07 ± 0.03
<i>Glycyrrhiza glabra</i> (Root)	148.77 ± 0.76
<i>Plumbago zeylancia</i> (Root)	214.19 ± 0.00
<i>Salacia Oblonga</i> (Root)	132.22 ± 0.00
<i>Lannea coromandelica</i> (Bark)	93.80 ± 1.90

Table 3.2 Flavonoids contents in Medicinal Plants

Botanical Name of Medicinal plants & Parts Investigated	Flavonoids Ethyl acetate Extract(mg Rutin/100g dw)
<i>Indigofera tinctoria</i> (Whole plant)	104.90 ± 4.74
<i>Oldenlandia umbellata</i> (Whole plant)	12.56 ± 0.9
<i>Acalypha indica</i> (Whole plant)	61.36 ± 1.59
<i>Indigofera aspalathoides</i> (Whole plant)	126.24 ± 4.57
<i>Eclipta alba</i> (Whole plant)	23.42 ± 0.69
<i>Enicostemma littorale</i> (Whole plant)	79.78 ± 2.5
<i>Aristolochia bracteolata</i> (Leaf)	50.50 ± 0.63
<i>Melothria maderaspatana</i> (Leaf)	126.83 ± 6.8
<i>Achyranthes aspera</i> (Leaf)	20.09 ± 0.69
<i>Mucuna Prurita</i> (Seeds)	46.09 ± 0.64
<i>Strychnos nux-vomica</i> (Seeds)	12.94 ± 0.57
<i>Citrullus colocynthis</i> (Seeds)	6.86 ± 0.14
<i>Strychnos potatorum</i> (Seeds)	47.72 ± 0.71
<i>Corallocarpus Epigaeus</i> (Tuber)	12.88 ± 0.84
<i>Mirabilis jalapa</i> (Tuber)	79.18 ± 1.21
<i>Asparagus racemosus</i> (Tuber)	44.01 ± 2.43
<i>Glycyrrhiza glabra</i> (Root)	108.89 ± 7.32
<i>Plumbago zeylancia</i> (Root)	119.74 ± 4.39
<i>Salacia Oblonga</i> (Root)	116.68 ± 9.8
<i>Lannea coromandelica</i> (Bark)	118.76 ± 10.4

freshly prepared reagent blank at 510nm. Results are shown in Table-3.2. Total Flavonoids content of the extracts was expressed as percentage of rutin equivalent per 100g dry weight of sample. Standard solution of rutin (10-100 µl) was used to calculate the standard curve with respective of solvent used for extracts.

Determination of antioxidant capacity

Free radical-scavenging ability by the use of a stable DPPH radical

The antioxidant capacities of the plants and different parts solvent extract (ethyl acetate) were studied by the evaluation of the free radical-scavenging effect on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) by the modified method¹². Various concentrations (0.02-0.08mg/ml) of plant extracts (0.3ml) were mixed with 2.7 ml of appropriate solvent (ethyl acetate) containing DPPH radical (0.1mM). The radical stock solution was prepared fresh daily. Ascorbic acid was used as standard in 20-100 µg/ml solution. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 60 minutes.

The decrease in absorbance of the resulting solutions was monitored at 517nm. The radical scavenging activity was calculated as a percentage of DPPH discoloration, using the equation.

$$\% \text{ RSA} = [(A_{\text{DPPH}} - A_s) / A_{\text{DPPH}}] \times 100$$

Where,

A_s = absorbance of the solution when the sample extracts were added at a particular level

A_{DPPH} = absorbance of DPPH radical solution,

All determinations were performed in triplicate. The IC_{50} was calculated as the amount of antioxidants present in the sample necessary to reduce the initial DPPH concentration by 50%.

Determination of ferric reducing antioxidant power (FRAP)

The ferric reducing property of medicinal plants extracted with ethyl acetate solvents were determined, by various concentrations of extracts were mixed with 2.5ml of 200mM sodium phosphate buffer (P^H - 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes, then 2.5 ml of 10% trichloroacetic acid

was added and the mixture was centrifuged at 1000 rpm for 8 minutes. The upper layer (5ml) was mixed with 5 ml of distilled water and 1ml of 0.1% ferric chloride and the absorbance was measured Spectrophotometrically at 700 nm. The absorbance obtained was converted to -tocopherol equivalent as milligrams per gram of dry material (TE/g dw) using -tocopherol standard curve.

Hydrogen peroxide scavenging assay

The ability of extracts to scavenge hydrogen peroxide was determined according to the method¹³. A solution of hydrogen peroxide (40 mM) was prepared in 40 mM phosphate buffer saline of pH 7.4. Various concentrations of solvents extracts (50-250 µg/ml) were added to hydrogen peroxide solution (0.6 ml 40mM). Absorbance of hydrogen peroxide at 230 nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide.

For each concentration, a separate blank sample was used for background subtraction. For control sample take Hydrogen Peroxide solution and take absorbance at 230 nm. Results are shown in table the percentage inhibition activity was calculated from the formula

$$\% \text{ Scavenged } [H_2O_2] = [(A_0 - A_1) / A_0] \times 100,$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of extract/standard taken as -tocopherol (10–100 µg/ml).

Statistical analysis

The direction and magnitude of correlation between variables was done using analysis of variance (ANOVA) and quantified by the correlation factor "r"

Results and Discussion

Total phenolic contents showed in ethyl acetate extracts of investigated medicinal plants ranged from 35.25 - 224.49 mg GAE/100g dw. High concentration levels of > 200 mg/100g was observed in plant sample of *Oldenlandia umbellata* 202.23 mg GAE/100g dw and *Eclipta alba* 224.49 mg GAE/100g dw, leaf sample of *Melothria maderaspatana* 217.24 mg GAE/100g dw seeds of *Citrullus colocynthis* 224.49 mg GAE/100g dw and roots of *Plumbago zeylancia* 214.19 mg GAE/100g dw and the phenolic content ranged from (100 - 200 mg/100g) was showed in plant *Indigofera tinctoria*

109.42 mg GAE/100g dw *Acalypha indica* 138.69 mg GAE/100g dw *Indigofera aspalathoides* 141.84 mg GAE/100g dw and *Enicostemma littorale* 131.21 mg GAE/100g dw, leaf sample of *Aristolochia bracteolata* 142.51 mg GAE/100g dw, seeds of *Mucuna Prurita* 114.67 mg GAE/100g dw and *Strychnos potatorum* 115.96 mg GAE/100g dw, roots of *Glycyrrhiza glabra* 148.77 mg GAE/100g dw and *Salacia oblonga* 132.22 mg GAE/100g dw and the concentrations below 100mg was observed in leaf sample of *Achyranthes aspera* 66.08 mg GAE/100g dw, seeds of *Strychnosnux-vomica* 75.09 mg GAE/100g dw and tubers of *Corallocarpus Epigaeus* 35.25 mg GAE/100g dw *Mirabilisj alapa* 54.37 mg GAE/100g dw and *Asparagus racemosus* 36.07 mg GAE/100g dw and bark sample of *Lannea coromandelica* 93.80 mg GAE/100g dw the total phenolic contents are low in tuber samples.

Flavonoids are class of secondary plant metabolites with significant antioxidant and chelating properties. Antioxidant activity of Flavonoids depends on the structure and substitution pattern of hydroxyl group.

In ethyl acetate extract the Flavonoids levels are ranged from 6.8-126.8 mg Rutin/100g dw. The higher concentration level > 100 mg Rutin/100g dw was observed in plant samples of *Indigofera tinctoria* 104.9 mg Rutin/100g dw and *Indigofera aspalathoides* 126.20 mg Rutin/100g dw, leaf sample of *Melothria maderaspatana* 126.8 mg Rutin/100g dw, roots of *Glycyrrhiza glabra* 108.8 mg Rutin/100g dw, *Plumbago zeylancia* 119.7 mg Rutin/100g dw and *Salacia oblonga* 116.6 mg Rutin/100g dw and bark sample of *Lannea coromandelica* 118.7 mg Rutin/100g dw. The concentrations level of Flavonoids ranged between 50-100 mg Rutin/100g dw was observed in whole plant sample of *Acalypha indica* 61.3 mg Rutin/100g dw and *Enicostemma littorale* 79.7 mg Rutin/100g dw, leaf sample of *Aristolochia bracteolata* 50.5 mg Rutin/100g dw and tuber sample of *Mirabilisj alapa* 79.1 mg Rutin/100g dw and low concentration < 50 mg Rutin/100g dw was showed in plant sample of *Oldenlandia umbellate* 12.5 mg Rutin/100g dw and *Eclipta alba* 23.4 mg Rutin/100g dw, leaf samples of *Achyranthes aspera* 20.0 mg Rutin/100g dw seeds of *Mucuna Prurita* 46.0 mg Rutin/100g dw, *Strychnosnux-vomica* 12.9 mg Rutin/100g dw, *Citrullus colocynthis* 6.8 mg Rutin/100g dw and *Strychnos potatorum* 47.7 mg Rutin/100g dw, tubers of *Corallocarpus Epigaeus* 12.8 mg Rutin and *Asparagus racemosus* 44.0 mg Rutin/100g dw.

In the present studies showed that the results of Total phenolic content in medicinal plants and different parts were extracted with ethyl acetate solvent and investigated that, high values of total phenolic contents. The results are presented in Table 3.1 showed that significant difference between the results were likely due to genotypic and environmental differences within the medicinal plants, choice of part tested, time of taking samples and determination results.

Flavonoids is one of the most diverse and widespread groups of natural products which are probably the most important natural phenolics. Several Flavonoids have been reported to quench active oxygen species and inhibit *in vitro* oxidation of low-density lipoproteins. The results presented in Table 3.2 showed that total Flavonoids (rutin) contents were varied widely in selected medicinal plants.

The antioxidant activity of twenty medicinal plants and various parts with ethyl acetate solvent extracts were determined using DPPH reagent. DPPH is very stable free radical, unlike *in vitro* generated free radicals such as the ferric reducing and hydrogen peroxide radicals, DPPH has the advantage of being unaffected by certain side reaction, such as metal ion chelation and enzyme inhibition. A freshly prepared DPPH solution exhibits a deep purple colour with an absorption maximum at 517nm. The purple color generally fades when antioxidant molecules quench DPPH free radicals (ie, by providing hydrogen atom or by electron donation, conceivably via a free radical attack on the DPPH molecule) and convert them into a colourless product, resulting in a decrease in absorbance at 517nm.

The antioxidant activity of the ethyl acetate solvent extracts of medicinal plants is expressed in terms of percentage of inhibition (%) and IC₅₀ values (µg/ml) (Fig 1.1 to 1.6) parallel to examination of the antioxidant activity of plant extracts, the value for the standard compounds ascorbic acid and -tocopherol IC₅₀ values were obtained and compared to the value of antioxidant activity (Fig.1.7).

The antioxidant activity indicates that, ethyl acetate extracts of *Strychnosnux-vomica* (IC₅₀ value 42.60) *Salacia Oblonga* (IC₅₀ value 42.94) *Indigofera tinctoria*(IC₅₀ value 40.64) *Melothria maderaspatana*(IC₅₀ value 38.21) and *Enicostemma littorale*(IC₅₀ value 43.76) were higher

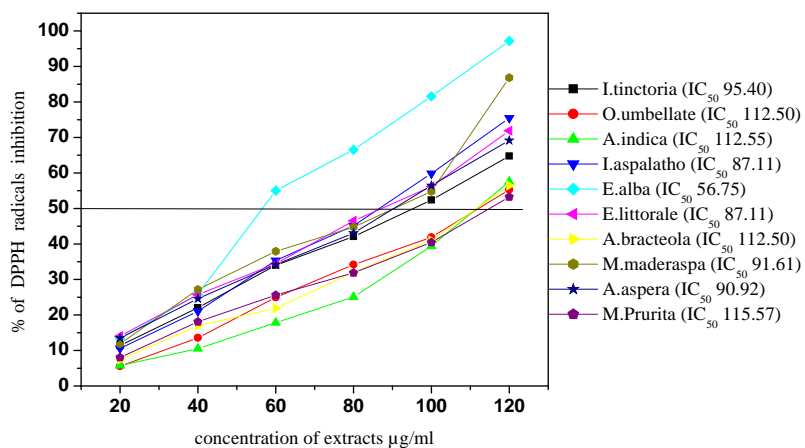


Fig 1.1

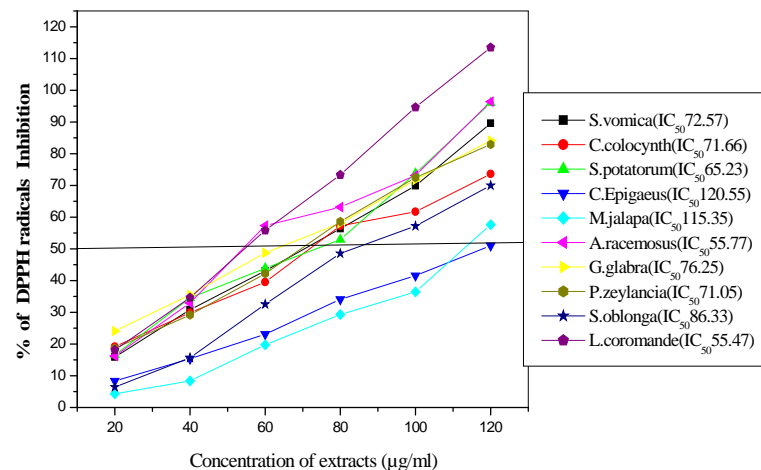


Fig 1.2

Figure 1.1 & 1.2 - IC₅₀ (µg/ml) values of medicinal plants of ethyl acetate extracts for free radical scavenging activity by DPPH radical

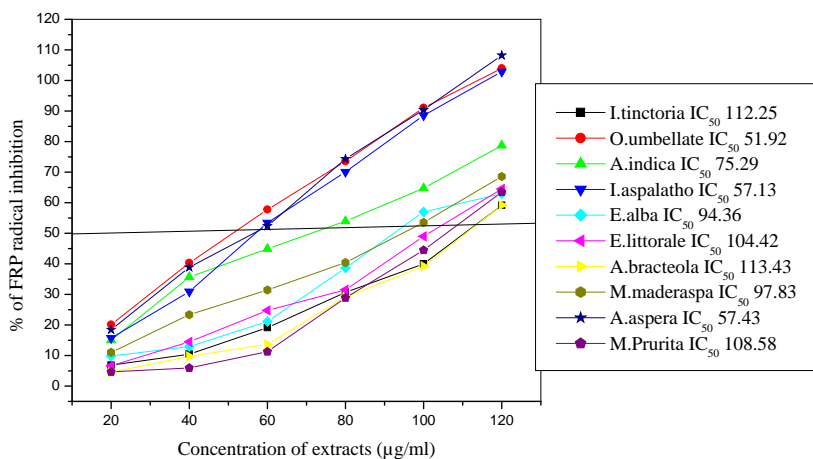


Fig 1.3

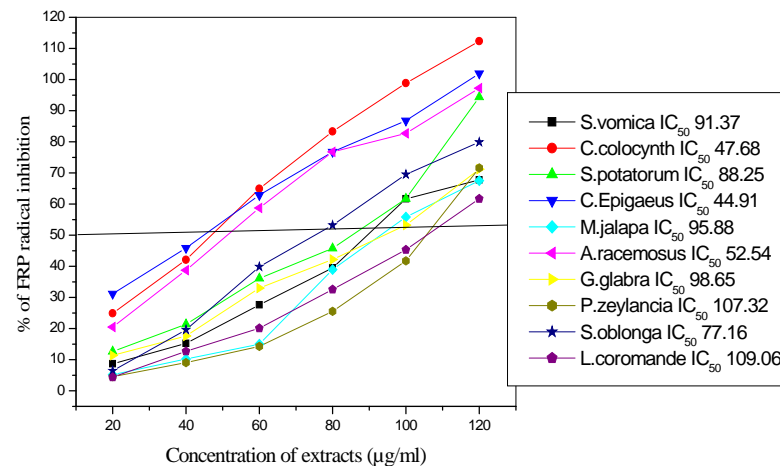


Fig 1.4

Figure 1.3 & 1.4 - IC₅₀ (µg/ml) values of medicinal plants of ethyl acetate extracts for free radical scavenging activity by FRAP radical

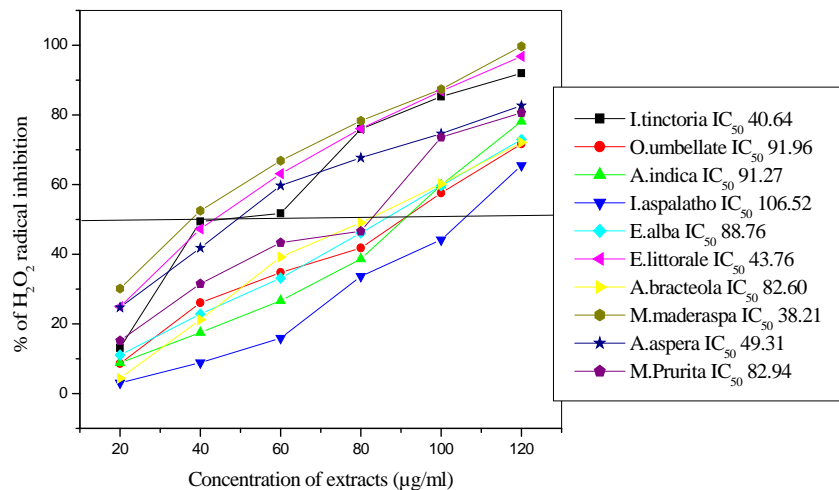


Fig 1.5

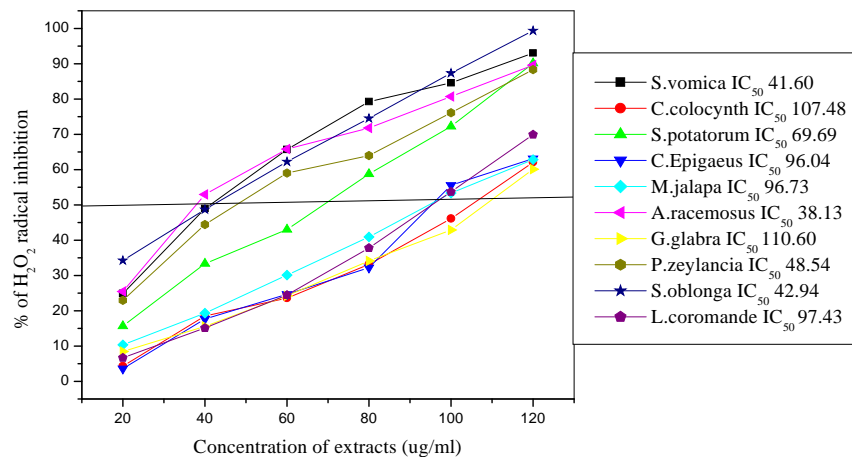


Fig 1.6

Figure 1.5 & 1.6 IC₅₀ (µg/ml) values of medicinal plants of ethyl acetate extracts for free radical scavenging activity by H₂O₂ radical

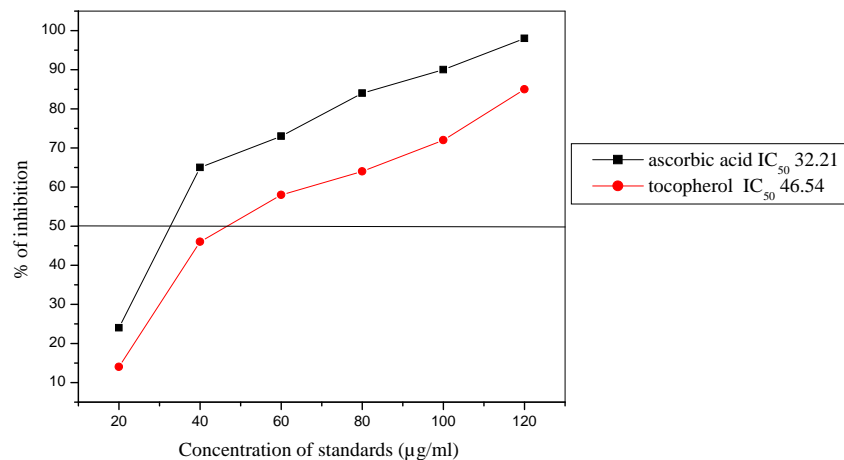


Fig 1.7

Figure 1.7 IC₅₀ (µg/ml) values of standards ascorbic acid and -tocopherol

than -tocopherol (Fig). The free radical screening activity of ethyl acetate extract was confirmed in the medicinal plants investigation.

Conclusion

The highest concentration of phenolic compounds in extracts were obtained using ethyl acetate solvent extracts manifested greater power of extraction for phenolic compounds. The high concentration of phenolic compounds indicated that these compounds contribute to the strong antioxidants activity. The phytochemical tests indicated the presence of phenolic compounds and Flavonoids in the crude ethyl acetate extract. Several of such compounds are known to possess potent antioxidant activity¹⁴. Hence, the observed antioxidant activity may be due to the presence of phenolic compounds and Flavonoids. The above medicinal plants exhibited strong anticancer, hepato-protective, antiviral and several other activities. These properties may be due to its antioxidant activity.

References

1. Stanner S A, Hughes J, Kelly C.N, Buttriss. S.A review of the epidemiological evidence for the 'antioxidant hypothesis' Public Health Nutrition. 2004 May;7(3):407-22.
2. William C. S. Cho, MicroRNAs in Cancer Translational Research.
3. Ho Won Jung, Timothy J, Tschaplinski, Linwang, Jane Glazebrook. Priming in Systemic Plant Immunity, *Science* 3 April 2009: Vol. 324 no. 5923 pp. 89-91
4. Justine B. Wells , Morten H. Christiansen , David S. Race , Daniel J. Acheson Maryellen MacDonald, Experience and sentence processing: Statistical learning and relative clause comprehension Department of Psychology, University of Wisconsin Cognitive Psychology 58 (2009) 250–271
5. Ruch R.T., Cheng S.J., Klaunig J.E (1984). methods in enzymology , 105: 198-209.
6. Murim Choi , Ute I. Scholl , Weizhen Ji , Tiewen Liu , Irina R. Tikhonova , Paul Zumbo , Ahmet Nayir , Anita Farhi , Shrikant Mane and Richard P. Lifton Genetic diagnosis by whole exome capture and massively parallel DNA sequencing

19096–19101 PNAS November 10, 2009 vol. 106 no. 45

7. Middleton E. Jr (1984) The flavonoids. Trends Biol. Sci. 5, 335-338.
8. Cody V., Middleton E. Jr and Harborne J. B. (1986) Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological and Structure Activity Relationships.
9. Valko M, Izakovic M, Mazur M, Rhodes CJ, Telser J Role of oxygen radicals in DNA damage and cancer incidence. Molecular Cell Biochem. 2004 Nov;266(1-2):37-56.
10. Choi H.R., Choi J.S., Han Y.N., Bae S.J. and Chung H.Y., (2002). Peroxynitrite scavenging activity of herb extracts. Phytother. Res. Volume 16, Issue 4, Pages 364-367.
11. Havsteen B. Flavonoids, a class of natural products of high pharmacological potency. Biochem Pharmacol 1983; 32(7):1141-1148.
12. Braca A. Sortino C, Politi M. Anti-oxidant activity of Flavonoids from *Licania licaniaeflora* J Ethnopharmacol 79;379-381,2002.
13. Ruch R.J, Cheng S.J, and Klaunig J.E, (1989) Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis, 10.pp 1003-1008
14. Lee J, Koo N, Min DB. Reactive oxygen species, aging and antioxidative nutraceuticals. Comp Rev Food Sci Food Safety 3;21-33,2004.