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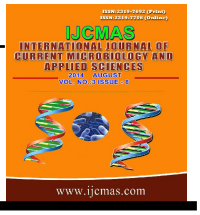

Original Research Article

Antioxidant activity, phenol and flavonoid contents of some selected Indian medicinal plants

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Keywords

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

A B S T R A C T

In present study, we have extracted twenty traditionally used Indian medicinal plants in chloroform, ethyl acetate and methanol, were screened for their antioxidant and free radical scavenging properties using α-tocopherol as standard antioxidant. 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 7.86 to 254.10 mg/100g of dry weight of extract, expressed as gallic acid equivalents. The total Flavonoids concentration varied from 2.65 to 133.90 mg /100g of dry weight expressed as rutin equivalents. Methanolic extract of *Plumbago zeylancia* showed the highest phenolic content and Flavonoids concentration and showed strong antioxidant activities.

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 conditions[1]. 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 drugs[2-3]. The most important secondary metabolites are phenols and Flavonoids [4-5]. Antioxidants are used to preserve food quality mainly because they arrest oxidative deterioration of lipids [6]. 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 antioxidants [7].

The aim of this study is to determine total phenolic and flavonoid contents, and evaluate the antioxidant activity of

medicinal plants extracts using methanol as 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 ferroin analogue, the Fe^{3+} complex of triphyridyl triazine Fe (TPTZ) $^{3+}$ to the intensely blue-coloured Fe^{2+} complex Fe $(TPTZ)^{2+}$ by antioxidants in acidic medium. 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 peroxide[8]. 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 carbohydrate [9]. 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 ($DPPH^{\cdot}$) purchased from Sigma Aldrich, Bangalore. ascorbic acid, Folin Ciocalteu's phenol reagent, sodium carbonate, gallic acid were purchased as analytical grade chemicals.Sodium nitrite, $AlCl_3.H_2O$, sodium hydroxide and H_2O_2 are analytical grade from Merck, Flavonoids(rutin) was purchased from sigma Aldrich. Methanol, sodium phosphate buffer (P^H - 6.6), potassium ferric cyanide, trichloro acetic acid, ferric chloride and α-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 methanol 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 45ºC 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 in methanol were estimated using FolinCiocalteu assay by colorimetric 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 properties [10] some studies suggest that flavonoids may be useful in the treatment of many impaired conditions[11.] 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 bone[8].

Total Flavonoids contents of the plant extracts were determined by Aluminum chloride colorimetric assay. Methanol 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% $AlCl_3H_2O$ 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 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 per100g 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 (methanol) were studied by the evaluation of the free radical-scavenging effect on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) by the modified method[12]. Various concentrations (0.02-0.08mg/ml) of plant extracts (0.3ml) were mixed with 2.7 ml of appropriate solvent (methanol) 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. % RSA = $[(A_{DPPH} - As)/ A_{DPPH}] \times 100$

Where,

As = 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 methanol and 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 50ºC 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 α– tocopherol equivalent as milligrams per gram of dry material (TE/g dw) using α – tocopherol standard curve.

Hydrogen peroxide scavenging assay

The ability of extracts to scavenge hydrogen peroxide was determined according to the method[13]. 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 α-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

The amounts of total phenolic contents were measured by FolinCiocalteu method varied widely in plant material in methanol solvent extracts. In methanol extract TPC was observed ranged from 25.10 -254.10 mg GAE/100g dw. High concentration level of TPC was noted in root sample of *Plumbago zeylancia* 254.10 mg GAE/100g and low concentration level was observed in plant sample of *Oldenlandia umbellate* 25.10 mg GAE/100g dw. TPC high concentrations > 200 mg GAE/100g dw was observed in leaf sample of *Melothria maderaspatana* 250.10 mg GAE/100g dw seeds of *Citrullus colocynthis* 248.60 mg GAE/100g dw, tubers of *Mirabilis jalapa* 218.29 mg GAE/100g dw and roots of *Plumbago zeylancia* 254.10 mg GAE/100g dw. TPC ranged from 100-200 mg GAE/100g dw was observed in plant samples of *Acalypha indica* 143.18 mg GAE/100g dw, leaf samples of *Aristolochia bracteolate* 160.18 mg GAE/100g dw. and *Achyranthes aspera* 136.40 mg GAE/100g dw, seeds of *Mucuna Prurita* 102.30 mg GAE/100g dw, *Strychnosnux-vomica* 123.36 mg GAE/100g dw and *Strychnos potatorum* 162.00 mg GAE/100g dw, tubers of *Corallocarpus Epigaeus* 162.24 mg GAE/100g dw and *Asparagus racemosus* 138.19 mg GAE/100g dw, roots of *Glycyrrhiza glabra* 141.80 mg GAE/100g dw and low concentrations level of TPC <

100 mg GAE/100g dw was observed in plants of *Indigofera tinctoria* 89.24 mg GAE/100g dw, *Oldenlandia umbellate* 25.10 mg GAE/100g dw ,*Indigofera aspalathoides* 34.16 mg GAE/100g dw, *Eclipta alba* 64.16 mg GAE/100g dw *Enicostemma littorale* 43.74 mg GAE/100g dw, roots of *Salacia oblonga* 79.13 mg GAE/100g and bark sample of *Lannea coromandelica* 40.8 mg GAE/100g dw. The results obtained in the methanol extract showed that medicinal plants and various parts were relatively high content of phenolic contents.

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. (sharififer et al, 2008). In methanol extract Flavonoids contents are ranged from 2.6 - 17.0 mg Rutin/100g dw .The concentrations level of > 10 mg Rutin/100g dw was showed in plant sample of *Indigofera tinctoria* 10.9 mg Rutin/100g dw and *Acalypha indica*14.8 mg Rutin/100g dw leaf samples of *Melothria maderaspatana* 16.5 mg Rutin/100g dw and *Achyranthes aspera* 15.6 mg Rutin/100g dw, seeds of *Strychnosnux-vomica, 13.9* mg Rutin/100g dw, tubers of *Mirabilisj alapa 17.0* and *Asparagus racemosus 13.6* mg Rutin/100g dw, roots of *Plumbago zeylancia* 16.1mg Rutin/100g dw and bark sample of *Lannea coromandelica* 11.6 mg Rutin/100g dw were investigated. The concentrations level < 10 mg Rutin/100g dw medicinal plants namely *Oldenlandia umbellate* 9.8 mg Rutin/100g dw,*Indigofera aspalathoides* 4.5 mg Rutin/100g dw and *Eclipta alba* 7.9 mg Rutin/100g dw, leaf sample *Aristolochia bracteolate* 9.4 mg Rutin/100g dw, seeds of *Mucuna Prurita* 8.2 *Citrullus colocynthis* 8.2 *and Strychnos potatorum 9.2* mg Rutin/100g dw respectively. Tubers of *Corallocarpus Epigaeus* 8.1 mg Rutin/100g dw and roots of *Glycyrrhiza glabra 8.6* and *Salacia oblonga 8.3* 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 methanol 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 genotopic and environmental differences (Climate, Location, Temperature, Fertility diseases and pest exposes) 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 with methanol.

The antioxidant activity of twenty medicinal plants and various parts with methanol 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 different solvent extracts of medicinal plants is expressed interms of percentage of inhibition (%) and IC_{50} 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 α-tocopherol IC_{50} values were obtained and compared to the value of antioxidant activity (Fig.1.7).

The results indicate that antioxidant activity of methanol extract of *Achyranthes aspera* (IC_{50} value 29.49) and *Strychnosnux-vomica* (IC_{50} value 29.33) are higher than that of the ascorbic acid. The antioxidant activity of methanolic extract of *Eclipta alba* (IC_{50} value 32.45), *Salacia Oblonga* (IC_{50} value 32.99) were nearly same when compared to ascorbic acid. However other medicinal plants extracts were found to be less active than ascorbic acid and since the IC_{50} values are found to be higher (Fig).

The antioxidant activity indicates that methanolic extracts of *Melothria maderaspatana* (IC_{50} value 42.86), *Strychnos potatorum*(IC_{50} value 32.45), *Corallocarpus Epigaeus*(IC_{50} value 32.45) and *Mirabilis jalapa* (IC_{50} value 35.23) were higher than α-tocopherol (Fig). The free radical screening activity of methanol extract was confirmed in the medicinal plants investigation.

Table.3.1 Total Phenolic contents in Medicinal Plants

Botanical Name of Medicinal plants &Parts Investigated	Total phenol content (mg GAE/100g dw) in Methanol Extract
Indigofera tinctoria (Whole plant)	89.26 ± 0.18
Oldenlandia umbellate (Whole plant)	25.10 ± 0.00
Acalypha indica (Whole plant)	143.18 ± 0.34
Indigofera aspalathoides(Whole plant)	34.16 ± 0.42
Eclipta alba (Whole plant)	64.16 ± 0.32
Enicostemma littorale (Whole plant)	43.74 ± 0.01
Aristolochia bracteolate (Leaf)	160.18 ± 4.4
Melothria maderaspatana (Leaf)	250.10 ± 0.00
Achyranthes aspera (Leaf)	136.40 ± 0.26
Mucuna Prurita (Seeds)	102.30 ± 10.0
Strychnosnux-vomica (Seeds)	123.36 ± 0.29
Citrullus colocynothis (Seeds)	248.60 ± 0.00
Strychnos potatorum (Seeds)	162.00 ± 0.00
Corallocarpus Epigaeus (Tuber)	162.24 ± 06.42
Mirabilis jalapa (Tuber)	218.29 ± 0.00
Asparagus racemosus (Tuber)	138.19 ± 0.29
Glycyrrhiza glabra (Root)	141.80 ± 0.58
Plumbago zeylancia (Root)	254.10 ± 0.00
Salacia Oblonga (Root)	79.13 ± 0.06
Lannea coromandelica (Bark)	40.8 ± 0.33

Table.3.2 Flavonoids contents in Medicinal Plants

Botanical Name of Medicinal plants & Parts Investigated	Flavonoids (mg Rutin/100g dw) in Methanol Extract
Indigofera tinctoria (Whole plant)	10.90 ± 0.62
Oldenlandia umbellate (Whole plant)	9.86 ± 0.13
Acalypha *indica* (Whole plant)	14.87 ± 3.25
Indigofera aspalathoides (Whole plant)	4.51 ± 0.15
Eclipta alba (Whole plant)	7.99 ± 0.33
Enicostemma littorale (Whole plant)	2.65 ± 0.06
Aristolochia bracteolate (Leaf)	9.46 ± 0.57
Melothria maderaspatana (Leaf)	16.50 ± 4.5
Achyranthes aspera (Leaf)	15.65 ± 4.5
Mucuna Prurita (Seeds)	8.28 ± 0.11
Strychnosnux-vomica (Seeds)	13.98 ± 2.85
Citrullus colocynothis (Seeds)	8.25 ± 0.11
Strychnos potatorum (Seeds)	9.25 ± 0.33
Corallocarpus Epigaeus (Tuber)	8.18 ± 0.24
Mirabilis jalapa (Tuber)	17.02 ± 13.10
Asparagus racemosus (Tuber)	13.65 ± 3.2
Glycyrrhiza glabra (Root)	8.64 ± 0.23
Plumbago zeylancia (Root)	16.13 ± 6.05
Salacia Oblonga (Root)	8.38 ± 19.32
Lannea coromandelica (Bark)	11.76 ± 1.80

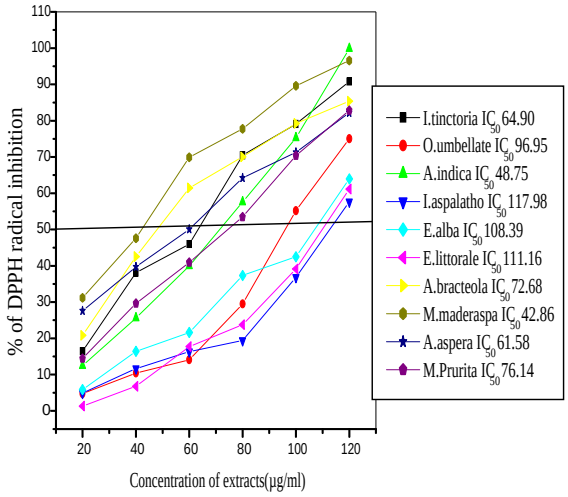

Fig 1.1

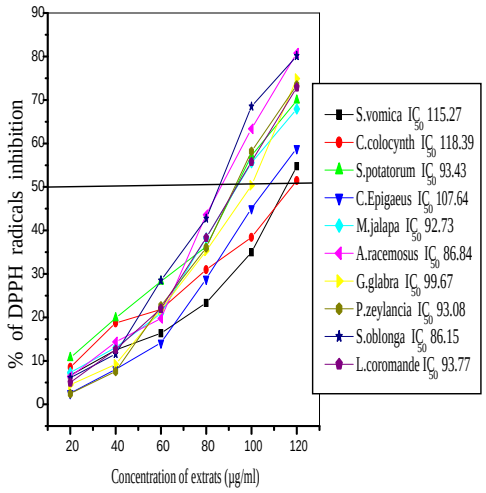

Fig 1.2

Figure 1.1 & 1.2 - IC_{50} (µg/5l) values of medicinal plants of methanol extracts for free radical scavenging activity by DPPH radical

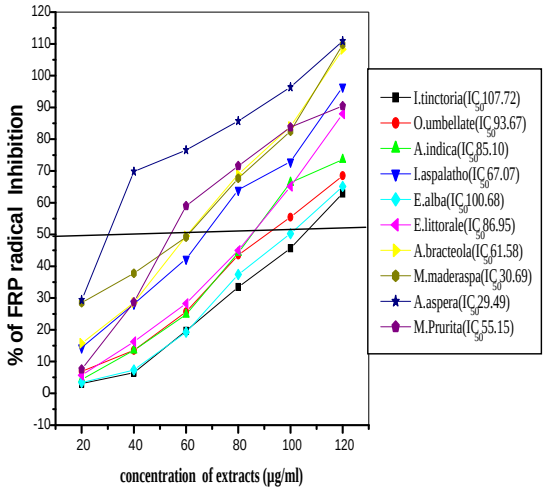

Fig 1.3

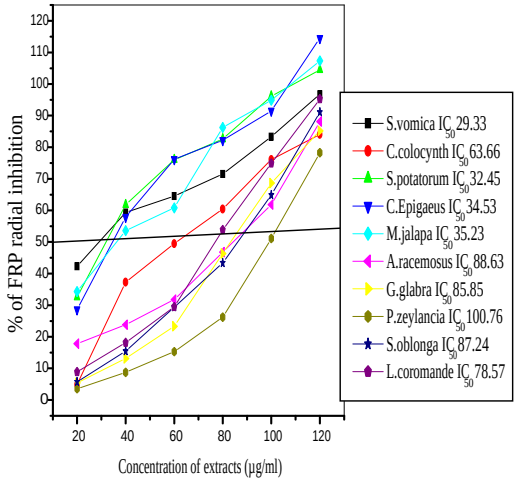

Fig 1.4

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

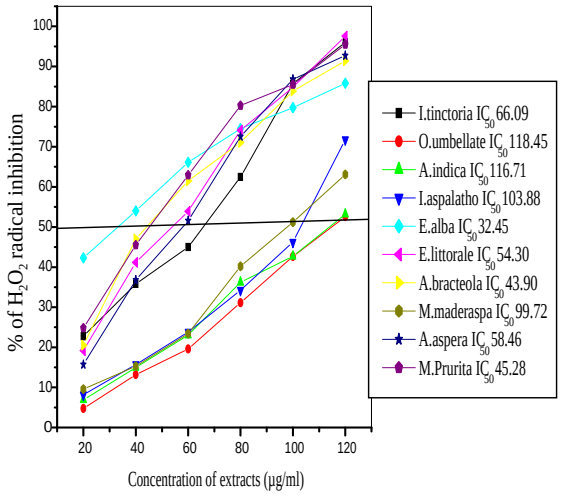

Fig 1.5

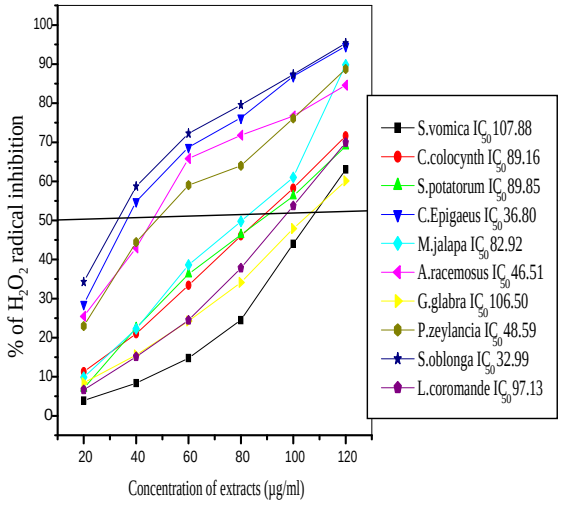

Fig 1.6

Figure 1.5 & 1.6- IC50 (µg/ml) values of medicinal plants of methanol extracts for free radical scavenging activity by H_2O_2 radical

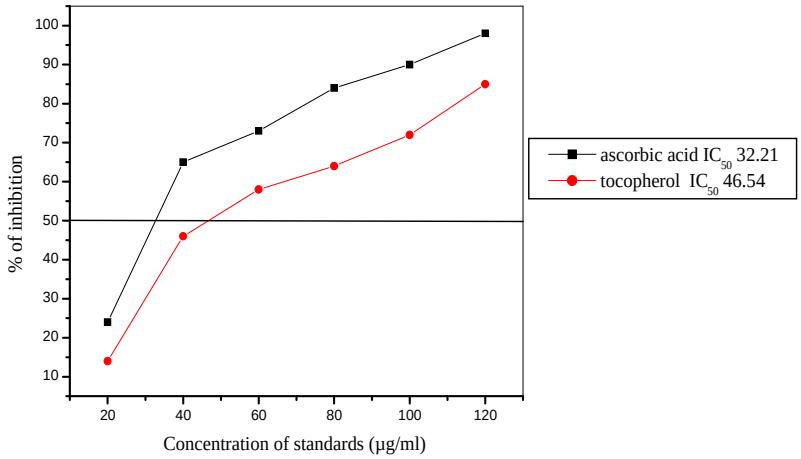

Figure 1.7 IC50 (µg/ml) values of standards ascorbic acid and α-tocopherol

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