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SCREENING OF THREE INDIAN MEDICINAL PLANT EXTRACTS FOR ANTIOXIDANT ACTIVITY

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Antioxidant activity, *Pongamia pinnata*, *Maytenus emarginata*, *Tribulus terrestris*

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ABSTRACT

Free radicals are implicated for many diseases including diabetes mellitus, arthritis, cancer, aging, *etc*. In the treatment of these diseases, antioxidant therapy has gained utmost importance. Currently there has been an increased interest globally to identify antioxidant compound that are pharmacologically potent and have low or no side effects. As plants are source of natural antioxidants, much attention has been gain to plants. The quest for natural antioxidants for dietary, cosmetic and pharmaceutical uses has become a major industrial and scientific research challenges over the last two decades. A variety of free radical scavenging antioxidants exists within the body in which many of them are derived from dietary sources like fruits, vegetables and teas. In this study, Antioxidant activity of the methanol extract of *Tribulus terrestris, Pongamia pinnata and Maytenus emarginata* was determined by DPPH free radical, Nitric oxide scavenging assays, Superoxide ion scavenging assays, ABTS and iron chelating methods. The results were analyzed statistically by the regression method. Their antioxidant activity was estimated by IC_{50} values. In all the testing, a significant correlation existed between concentrations of the extract and percentage inhibition of free radicals. The antioxidant property may be related to the phenols and flavonoids present in the extracts. These results clearly indicate that *Tribulus terrestris, Pongamia pinnata and Maytenus emarginata* are effective against free radical mediated diseases.

INTRODUCTION

In the past few years natural antioxidants have generated considerable interest in preventive medicine. There is increasing evidence that oxidative stress, defined as an imbalance between oxidants and antioxidant in favour of the former, leads to many biochemical changes and is an important causative factor in several human chronic diseases, such as atherosclerosis and cardiovascular diseases, mutagenesis and cancer, several neurodegenerative disorders, and the ageing process. The food industry also uses natural antioxidants as a replacement of conventional synthetic antioxidants in food by natural products that are considered to be promising and a safe source. As a result of which, much attention has been directed towards the characterisation of antioxidant properties of plant extracts/their fractions and identification of the constituents responsible for those activities. Antioxidants derived from fruits, vegetables, spices and cereals are very effective and have reduced interference with the body's ability to use free radicals constructively. [1-7]

Pongamia is a genus having one species only *Pongamia pinnata* which belongs to family Leguminosae and sub-family Papilionaceae. It is a medium sized glabrous, perennial tree grows in the littoral regions of South Eastern Asia and Australia. *Pongamia pinnata* is a preferred species for controlling soil erosion and binding sand dunes because of its dense network of lateral roots. Root, bark, leaves, flower and seeds of this plant also have medicinal properties and traditionally used as medicinal plants. All parts of the plant have been used as crude drug for the treatment of tumors, piles, skin diseases, wounds and ulcers.[8-11]

Maytenus is a genus of flowering plants in the staff vine family, Celastraceae. Members of the genus are distributed throughout Central and South America, Southeast Asia, Micronesia and Australasia, the Indian Ocean and Africa. They grow in a very wide variety of climates, from tropical to subpolar. Two new macrolide sesquiterpene pyridine alkaloids, emarginatine F and emarginatine G were isolated from *Maytenus emarginata*.[12]

Tribulus terrestris is a flowering plant in the family Zygophyllaceae, native to warm temperate and tropical regions of the Old World in southern Europe, southern Asia, throughout Africa, and Australia. It can thrive even in desert climates and poor soil. Like many weedy species, this plant has many common names, including puncturevine, caltrop, cathead, yellow vine, goathead, burra gokharu and bindii. The Latin name *tribulus* originally meant the caltrop (a spiky weapon), but in Classical times already the word meant this plant as well. *T. terrestris* is now being promoted as a booster for the purpose of increasing sex drive. Its use for this purpose originated from a Bulgarian

study conducted in the 1970s, which found effects on free testosterone and lutenizing hormone in men belonging to infertile couples.[13-16]

EXPERIMENTAL WORKDONE

Materials and methods

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2_-azinobis-3- ethylbenzothiazoline-6-sulfonic acid (ABTS), O-Phenanthroline, Ferric chloride, sulphanilamide, phosphoric acid, naphthylethylene diamine dihydrochloride, potassium ferricyanide, caffeic acid, quercetin and Ascorbic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA), Folin- Ciocalteus's phenol reagent and sodium carbonate were from Merck Chemical Supplies (Damstadt, Germany). All the chemicals used including the solvents, were of analytical grade.

Plant collection

Leaves of *Pongamia pinnata, Maytenus emarginata* and *Tribulus terrestris,* were collected from Jaipur, Rajasthan, India during the month of September, 2010. The plants were identified by Dr.Vinod kumar sharma, Department of Botany, University of Rajasthan, Jaipur, India. The voucher specimen of the plants (RUBL20907, 20908 and 20909) respectively has been kept in the Department of Botany, University of Rajasthan, Jaipur, for the future references.

Extraction

Leaves were shade-dried, powdered. They were extracted successively with each of petroleum ether, chloroform, ethylacetate, and methanol in a soxhlet extractor for 18-20hrs. The extracts were concentrated to dryness under reduced pressure and controlled temperature (40-50ºC) in a rotator evaporator.

Phytochemical screening

Phytochemical screenings were performed using standard procedures.[17-18]

Test for phenols

To 0.5 g each of the extract, 2 ml of ferric chloride was added. A reddish brown colouration at the interface indicates the presence of phenols.

Test for terpenoids (Salkowski test)

To 0.5 g each of the extract, 2 ml of chloroform was added. Concentrated H_2S0_4 (3 ml) was carefully added to form a layer. A reddish brown colouration at the interface indicates the presence of terpenoids.

Test for flavonoids

Three methods were used to test for flavonoids. First, diluted ammonia (5 ml) was added to a portion of an aqueous filtrate of the extract followed by addition of concentrated sulphuric acid (1 ml). A yellow coloration that disappears on standing indicates the presence of flavonoids. Second, a few drops of 1% aluminium solution were added to a portion of the filtrate. A yellow colouration indicates the presence of flavonoids. Third, a portion of the extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of diluted ammonia solution. A yellow colouration indicates the presence of flavonoids.

Test for saponins

To 0.5 g of extract 5 ml of distilled water was added in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for tannins

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

Test for alkaloids

0.5 g of extract was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate 2 ml of diluted ammonia was added followed by addition of 5 ml of chloroform and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Draggendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Draggendorff's reagent) was regarded as positive for the presence of alkaloids.

QUANTITATIVE ESTIMATION OF PHENOLS

Extraction Procedure

Each of the deproteinized test samples (200 mg) was homogenized in 10 ml of 80% ethanol for 2 hrs. and left overnight at the room temperature. Each of these was centrifuged and the supernatant was collected separately, the volume of which was raised 40 ml with 80% ethanol in each case.

Quantification

Total phenols were quantified in each test samples, following the protocol of Bray and Thorpe, 1954which included the preparation of a regression curve of standard phenol (Gallic acid).

A stock solution of the standard phenol (Gallic acid) was prepared in 80% ethanol (400 mg/ml) out of which 0.1 to 0.9 ml was taken into separate test tubes and the volume of each was

raised to 1 ml with 80% ethanol, To each tube, 1 ml Folin-Ciocalteau reagent (diluted with distilled Water in 1:2 ratio, just before use) was added followed by 2 ml of 20% Na_2CO_3 solution and this mixture was shaken vigorously. Such samples were placed in a boiling water bath for exactly 1 min and later, cooled under 3 times running tap water. Each of the reaction mixture was diluted to 25 ml with distilled water and ODs were taken at 750 nm against a blank using a spectrophotometer. Five replicates were taken for each concentration and the average OD was plotted against the respective concentration to compute a regression curve which followed the Beer's Law. From the mean values, total levels of phenols were calculated (with reference to gallic acid) by referring the ODs experimental samples with the standard regression curve. Values in milligram gallic acid equivalents (mg gallic acid/g extract) was reported.

QUANTITATIVE ESTIMATION OF FLAVONOIDS[19]

The amount of total flavonoids content for each extract was determined by the method of (Kim et al., 2003) .To 1ml sample: water (50:50, v/v) or standard solutions quercetin (0-500 mg L^{-1}) was added to 4ml water in a 10ml volumetric flask. After 5 min., 0.3 ml $AlCl_3$ (100 g L^{-1}) was added. At 6min, 2ml 1mol L^{-1} NaOH were added to the mixture and immediately diluted with 204ml of water. Absorbance of the mixture was read at 510nm vs. water blank. Values in milligram quercetin equivalents (mg quercetin /g extract) was reported.

PREPARATION OF PLANT EXTRACT.

About 50 g of each plant powder was taken and extracted in a soxhlet extractor with methanol (0.2 Lit.). The crude extract was concentrated to dryness in a rotary flash evaporator under reduced pressure and controlled temperature (40–50 ^oC). The extracts were preserved in vacuum desiccators for subsequent use in study.

DETERMINATION OF ANTIOXIDANT ACTIVITY

DPPH radical scavenging assay.

To the Methanol solution of DPPH (1 mM) an equal volume of the extract dissolved in alcohol was added at various concentrations from 2 to 1000 µg/ml in a final volume of 1.0 ml. An equal amount of alcohol was added to the control. After 20 min, absorbance was recorded at 517 nm. Experiment was performed in triplicate.[20-21]

ABTS radical scavenging assay.

To the reaction mixture containing 0.3 ml of ABTS radical, 1.7 ml phosphate buffer and 0.5 ml extract was added at various concentrations from 2 to 500 µg/ml. Blank was carried out without drug. Absorbance was recorded at 734 nm. Experiment was performed in triplicate. [20-21]

Nitric oxide radical scavenging.

Sodium nitroprusside (5µM) in standard phosphate buffer solution was incubated with different concentration of the test extracts dissolved in standard phosphate buffer (0.025M, pH 7.4) and the tubes were incubated at 25 °C for 5 hr. After 5 h, 0.5 ml of incubation solution was removed and diluted with 0.5 ml Griess reagent (prepared by mixing equal volume of 1% sulphanilamide in 2% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in water). The absorbance of chromophore formed was read at 546 nm. The control experiment was also carried out in similar manner, using distilled water in the place of extracts. The experiment was performed (in triplicate) and % scavenging activity was calculated using the formula 100−[100/blank absorbance × sample absorbance]. The activity was compared with ascorbic acid, which was used as a standard antioxidant. [22]

Superoxide scavenging.

Alkaline DMSO was used as a super oxide generating system. To 0.5 ml of different concentrations of the test compound, 1 ml of alkaline DMSO and 0.2 ml of NBT 20 mM in phosphate buffer pH 7.4 was added. The experiment was performed in triplicate.[23]

Iron chelating activity assay.

The reaction mixture containing 1 ml O-Phenanthroline, 2 ml Ferric chloride, and 2 ml extract at various concentrations ranging from 2 to 1000 µg/ml in a final volume of 5 ml was incubated for 10 minutes at ambient temperature. The absorbance at 510 nm was recorded. Ascorbic acid was added instead of extract and absorbance obtained was taken as equivalent to 100% reduction of all ferric ions. Blank was carried out without extract.

Experiment was performed in triplicate.[20,21,24]

STATISTICAL ANALYSIS

The experimental results were expressed as mean ± standard deviation (SD) of three replicates. Where applicable, the data were subjected to one way analysis of variance (ANOVA) and two way analysis of variance (ANOVA). All these analysis was done by Minitab Software programme. *P-v*alues < 0.05 were regarded as significant.

OBSERVATIONS:

Table -1 Phytochemical analysis of *Pongamia pinnata* leaves

CONSTITUENTS	EXTRACTS					
	Petroleum ether	Diethyl ether	Chloroform	Acetone	Methanol	Aqueous
Total % by weight in 50 g/ dry weight	2.054	1.420	0.546	0.440	2.258	3.380
Tannins	-	-	+	-	++	-
Flavonoids	-	-	+	-	+	++
Alkaloid	-	+	-	-	-	-
Steroids	+	-	-	-	-	-
Saponin	--	-	-	-	-	-
Tri terpenoid	+	-	-	-	-	-

Where'++' (High amounts), '+' (Available) and '-` (Absent)

Table- 2. Phytochemical analysis of *Maytenus emarginata* leaves

CONSTITUENTS	**EXTRACTS**					
	Petroleum ether	**Diethyl ether**	**Chloroform**	**Acetone**	**Methanol**	**Aqueous**
Total % by weight in 50 g/ dry weight	1.469	0.860	0.676	0.537	6.790	6.547
Tannins	++	-	-	-	+	-
Flavonoids	+	-	-	-	-	-
Alkaloid	-	+	-	-	++	-
Steroids	-	-	-	-	++	+
Saponin	++	+	-	-	-	-
Tri terpenoid	-	++	-	-	-	-

Where'++' (High amounts), '+' (Available) and '-` (Absent)

Table- 3 Phytochemical analysis of *Tribulus terrestris* leaves

CONSTITUENTS	EXTRACTS					
	Petroleum ether	Diethyl ether	Chloroform	Acetone	Methanol	Aqueous
Total % by weight in 50 g/ dry weight	3.092	1.365	0.768	0.367	11.086	4.030
Tannins	++	-	+	-	+	-
Flavonoids	+	-	+	-	-	-
Alkaloid	-	-	-	-	++	-
Steroids	-	-	+	+	+	++
Saponin	++	+	+	-	-	-
Tri terpenoid	-	++	-	-	-	-

Where'++' (High amounts), '+' (Available) and '-` (Absent)

Table- 4 Quantitative Estimation of Total Phenol content in *P.pinnata, M.emarginata* and *T.terrestris*-

A Comparative study

Plant	**Phenol content(mg gallic acid /g extract)**
P.pinnata	8.64mg/g
M.emarginata	10.69 mg/g
T.terrestris	7.84 mg/g

(*M.emarginata*; 10.69 mg/g > *P.pinnata*; 8.64 mg/g > *T.terrestris*; 7.84 mg/g)

Table- 5 Quantitative Estimation of Total Flavonoid content in *P. pinnata, M. emarginata* and *T. terrestris*- A Comparative study

Plant	Flavonoid content(mg quercetin /g extract)
P.pinnata	2.37 mg/g
M.emarginata	1.56 mg/g
T.terrestris	1.28 mg/g

(*P.pinnata*; 2.37 mg/g > *M.emarginata;* 1.56 mg/g > *T.terrestris*; 1.28 mg/g)

Figure 1: **Study on DPPH scavenging activity in *P.pinnata, M.emarginata* and *T.terrestris*- A Comparative study**

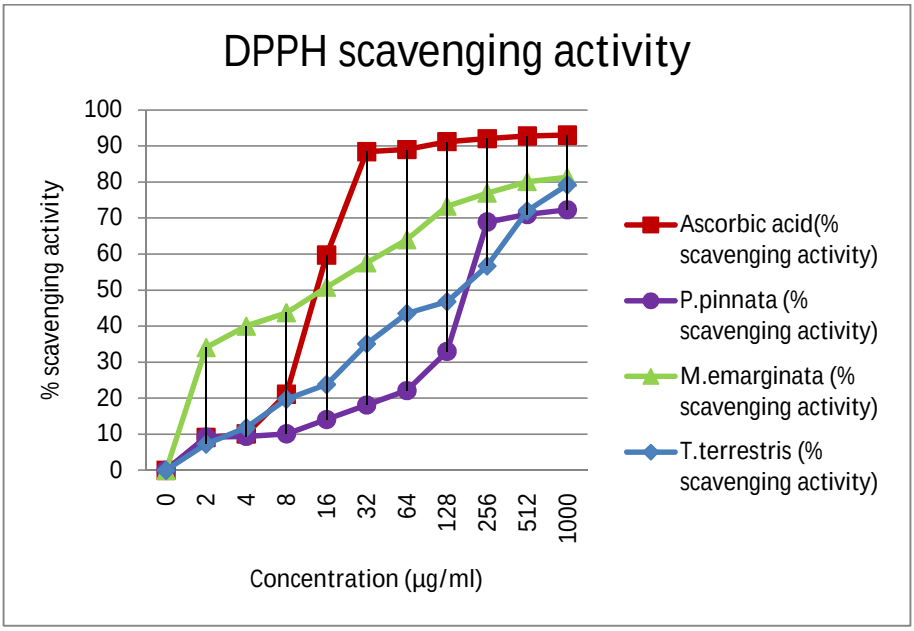

Table- 6 Study on DPPH scavenging activity in *P.pinnata, M.emarginata* and *T.terrestris*- A Comparative study

Concentration (µg/ml)	Ascorbic acid(% scavenging activity)	*P.pinnata* (% scavenging activity)	*M.emarginata* (% scavenging activity)	*T.terrestris* (% scavenging activity)
0	0	0	0	0
2	9.1±0.004	9.2±0.008	34.1±0.001	7.3±0.012
4	10.1±0.005	9.4±0.001	40±0.002	11.8±0.004
8	21.1±0.002	10.1±0.004	43.7±0.005	19.7±0.007
16	59.7±0.007	14.1±0.007	50.8±0.004	23.8±0.004
32	88.4±0.008	18.1±0.006	57.6±0.006	35.1±0.003
64	89±0.001	22.1±0.004	64±0.003	43.5±0.004
128	91.2±0.004	33±0.005	73.3±0.004	46.8±0.005
256	92±0.007	68.9±0.004	76.9±0.005	56.7±0.003
512	92.8±0.005	71±0.003	80.1±0.003	72±0.005
1000	93±0.007	72±0.007	81.3±0.004	79.2±0.004

Values are mean ± SD of six separate experiments

Statistical comparison has been done by student't' test

Figure 2: **Study on ABTS scavenging activity in *P.pinnata, M.emarginata* and *T.terrestris*- A Comparative study**

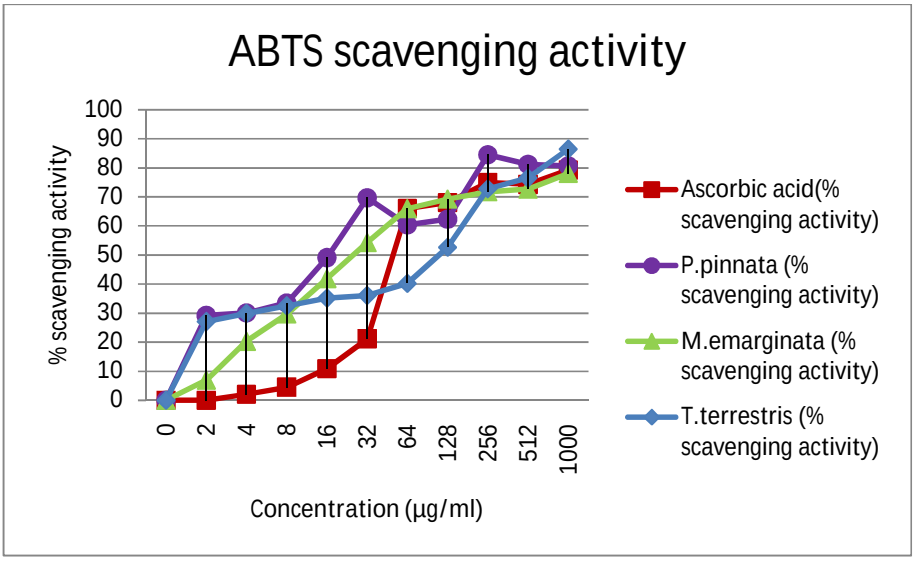

Table -7 Study on ABTS scavenging activity in *P.pinnata, M.emarginata* and *T.terrestris*- A Comparative study

Concentration (µg/ml)	Ascorbic acid(% scavenging activity)	*P.pinnata* (% scavenging activity)	*M.emarginata* (% scavenging activity)	*T.terrestris* (% scavenging activity)
0	0	0	0	0
2	0±0.011	29.2±0.012	6.9±0.015	27.1±0.011
4	2.1±0.014	30.1±0.011	20.3±0.011	29.9±0.012
8	4.5±0.017	33.5±0.014	29.7±0.016	32.5±0.011
16	10.9±0.011	49.1±0.017	41.9±0.012	35.2±0.014
32	21.2±0.015	69.7±0.012	54.3±0.017	36.1±0.015
64	66±0.013	60.4±0.011	66±0.018	40.2±0.011
128	68±0.014	62.3±0.013	69.3±0.014	52.65±0.012
256	75±0.015	84.5±0.014	71.8±0.011	72.8±0.013
512	74.3±0.016	81.2±0.015	72.7±0.018	76.5±0.014
1000	79.2±0.017	80.7±0.012	78.1±0.011	86.5±0.011

Values are mean ± SD of six separate experiments

Statistical comparison has been done by student 't' test

Figure 3: Study on Nitric oxide scavenging activity in *P.pinnata, M.emarginata* and *T.terrestris*- A Comparative study

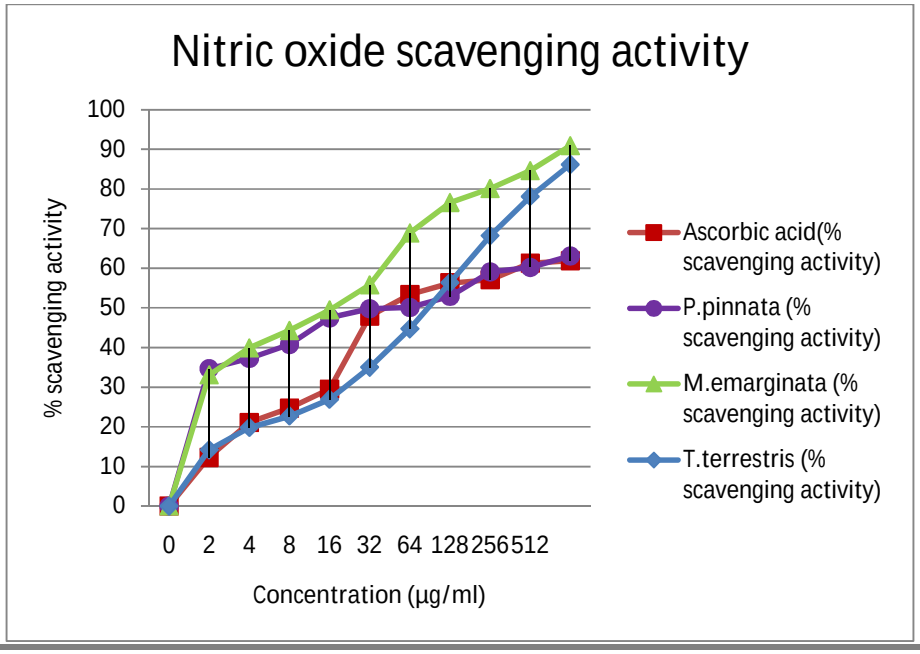

Table- 8 Study on Nitric oxide scavenging activity in *P.pinnata, M.emarginata* and *T.terrestris*- A Comparative study

Concentration (µg/ml)	Ascorbic acid(% scavenging activity)	*P.pinnata* (% scavenging activity)	*M.emarginata* (% scavenging activity)	*T.terrestris* (% scavenging activity)
0	0	0	0	0
2	12.3±0.04	34.7±0.05	33.1±0.01	14.2±0.05
4	21.1±0.05	37.3±0.06	39.9±0.05	19.8±0.06
8	24.7±0.03	40.8±0.07	44.3±0.06	22.7±0.04
16	29.5±0.05	47.5±0.04	49.5±0.04	26.9±0.06
32	47.9±0.06	49.8±0.06	55.8±0.07	35.01±0.05
64	53.4±0.01	50.1±0.01	68.9±0.04	44.7±0.01
128	56.3±0.04	52.9±0.02	76.6±0.02	56.4±0.02
256	57.1±0.02	59.1±0.01	80.1±0.08	68.2±0.03
512	61.3±0.04	60.2±0.06	84.6±0.01	78.1±0.01
1000	61.9±0.01	63.1±0.05	90.9±0.06	86.2±0.07

Values are mean ± SD of six separate experiments

Statistical comparison has been done by student 't' test

Figure 4: **Study on Superoxide ion scavenging activity in *P.pinnata, M.emarginata* and *T.terrestris*- A Comparative study**

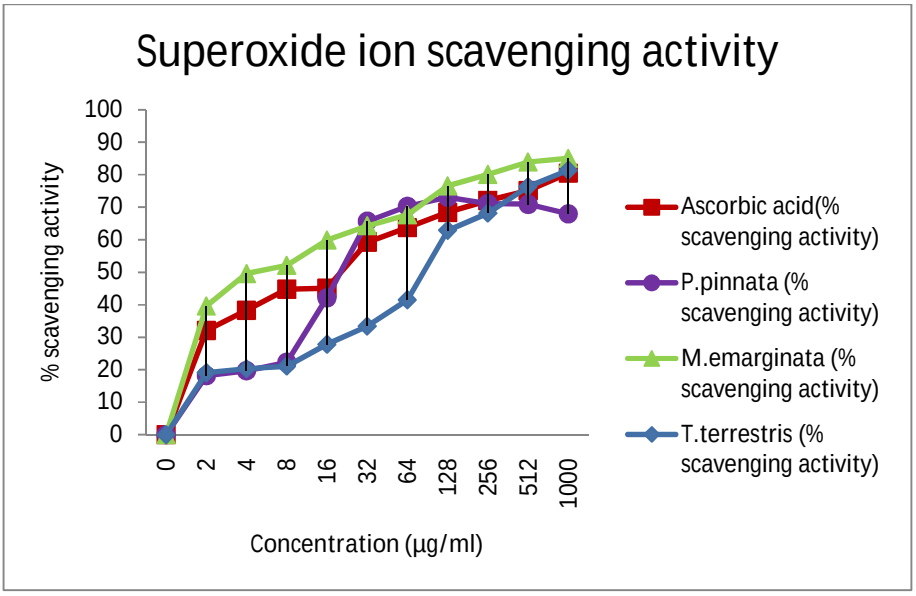

Table- 9 Study on Superoxide ion scavenging activity in *P.pinnata, M.emarginata* and *T.terrestris*- A Comparative study

Concentration (µg/ml)	Ascorbic acid(% scavenging activity)	*P.pinnata* (% scavenging activity)	*M.emarginata* (% scavenging activity)	*T.terrestris* (% scavenging activity)
0	0	0	0	0
2	32.1±0.020	18.2±0.021	39.6±0.023	19.1±0.021
4	38.3±0.022	19.7±0.022	49.6±0.021	20.3±0.022
8	44.8±0.027	22.3±0.024	52.1±0.023	21.1±0.026
16	45.1±0.021	42.1±0.021	59.9±0.021	27.8±0.023
32	59.3±0.023	65.7±0.023	64.3±0.025	33.4±0.025
64	63.7±0.026	70.3±0.025	67.7±0.027	41.5±0.026
128	68.4±0.024	73.1±0.026	76.6±0.025	62.9±0.026
256	72.1±0.022	71.1±0.027	80.1±0.026	68.2±0.027
512	75.1±0.021	70.9±0.028	83.9±0.021	76.3±0.028
1000	80.5±0.027	68±0.029	85±0.020	81.4±0.029

Values are mean ± SD of six separate experiments

Statistical comparison has been done by student 't' test

Figure 5: **Study on Iron chelating activity in *P.pinnata, M.emarginata* and *T.terrestris*- A Comparative study**

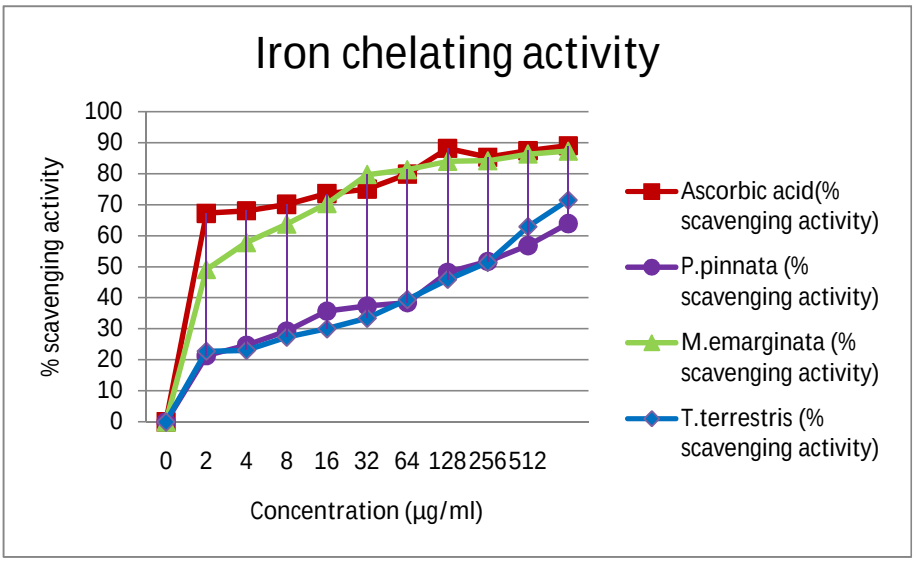

Table- 10 Study on Iron chelating activity in *P.pinnata, M.emarginata* and *T.terrestris*- A Comparative study

Concentration (µg/ml)	Ascorbic acid(% scavenging activity)	*P.pinnata* (% scavenging activity)	*M.emarginata* (% scavenging activity)	*T.terrestris* (% scavenging activity)
0	0	0	0	0
2	67.2±0.013	21.3±0.018	49.1±0.021	22.8±0.021
4	68±0.018	24.7±0.014	57.8±0.017	23.1±0.012
8	70.1±0.011	29.3±0.02`	63.7±0.013	27.3±0.014
16	73.6±0.024	35.7±0.027	70.3±0.021	30±0.017
32	75±0.027	37.3±0.014	79.7±0.026	33.4±0.011
64	79.8±0.016	38.4±0.023	81.3±0.025	39.4±0.016
128	88.1±0.018	48.2±0.025	83.9±0.024	45.9±0.010
256	85.3±0.012	51.7±0.027	84.1±0.022	51.4±0.021
512	87.4±0.019	56.8±0.024	86.3±0.020	62.9±0.023
1000	89±0.021	63.9±0.023	87.2±0.025	71.4±0.026

Values are mean ± SD of six separate experiments
Statistical comparison has been done by student 't' test

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical screening of the extracts revealed the presence of alkaloids, flavonoids, saponins, steroids and tannins (Table 1, 2, 3).

Total phenolics

Results obtained in the present study revealed that the level of these phenolic compounds in the methanol extract of the leaves of *Maytenus emarginata* is highest (Table 4). Polyphenolic compounds are known to have antioxidant activity and it is likely that the activity of the extracts is due to these compounds. This activity is believed to be mainly due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. In fact, many medicinal plants contain large amounts of antioxidants such as polyphenols. Many of these phytochemicals possess significant antioxidant

capacities that are associated with lower occurrence and lower mortality rates of several human diseases. The result strongly suggests that phenolics are important components of this plant, and some of its pharmacological effects could be attributed to the presence of these valuable constituents.[25-29]

The phenolic content determined by the Folin-Ciocalteu method for the *Tribulus terrestris,Pongamia pinnata and Maytenus emarginata leaves* is shown in Table 4.

Total flavonoids

The flavonoid content of *Tribulus terrestris, Pongamia pinnata and Maytenus emarginata leaves* is shown in Table 5. Results obtained in the present study revealed that the Flavonoid Content is maximum in the methanol extract of the leaves of *Pongamia pinnata.*

Antioxidant assays

DPPH radical scavenging activity

The proton radical scavenging action is known to be one of the various mechanisms for measuring antioxidant activity. The DPPH test provides information on their activity of the test compounds with a stable free radical. DPPH is one of the compounds that possess a proton free radical and shows absorption at 517 nm. When DPPH encounters proton radical scavengers, the absorption reduces and the DPPH solution is decolourised as the colour changes from deep violet to light yellow. This assay determines the scavenging of stable radical species of DPPH by antioxidants. Figure 1 shows the dose-response curve of DPPH radical scavenging activity of the methanol extracts of the *Tribulus terrestris, Pongamia pinnata and Maytenus emarginata leaves,* compared with ascorbic acid. The degree of reduction in absorbance measurement is indicative of the radical scavenging (antioxidant) power of the extract. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability.Though the DPPH radical scavenging abilities of the extracts were less than ascorbic acid but *Pongamia pinnata*, showed same results at concentrations between 512-1000µg/ml (Table 6).The study showed that the extracts have the proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. This study suggests that these plants possess antioxidant activity.[30]

ABTS radical scavenging activity

ABTS, a protonated radical, has characteristic absorbance maxima at 734 nm which decreases with the scavenging of the proton radicals (Mathew and Abraham 2006). The scavenging of the ABTS+ radical by the extracts was found to be much higher than that of DPPH radical. Factors like stereoselectivity of the radicals or the solubility of the extract in different testing systems have been reported to affect the capacity of extracts to react and quench different radicals. It was found that some compounds which have ABTS+ scavenging activity did not show DPPH

scavenging activity. This is the case in this study. This further showed the capability of the extracts to scavenge different free radicals in different systems, indicating that they may be useful therapeutic agents for treating radical-related pathological damage.[31-32]

The methanol extracts of the leaves of *Tribulus terrestris, Pongamia pinnata and Maytenus emarginata* were fast and effective scavengers of the ABTS radical (Figure 2).It was found that their activity was more than that of Ascorbic acid at 2, 4, 8, 16 and 32 µg/ml (Table 7). The Ascorbic acid exhibited higher activity than the extracts at128 µg/ml. Also all extracts exhibited same scavenging at 1000µg/ml indicating that Proton radical scavenging is an important attribute of their antioxidant action.

Nitric oxide scavenging

Sodium nitroprusside serves as a chief source of free radicals. The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with napthylethylene diamine is used as the marker for NO scavenging activity.[33]

Figure 3 shows that the *Maytenus emarginata* leaf extract possess significant free radical scavenging action against nitric oxide (NO) induced release of free radicals at the concentrations between 2-1000 µg / ml, showing 33.1% to 90.9% of NO inhibition, respectively. Ascorbic acid was used as reference.

Superoxide scavenging activity

Superoxide radical scavenging activity exhibited by the Methanol extracts of *Tribulus terrestris, Pongamia pinnata and Maytenus emarginata* at different concentration in presented in Figure 4. This shows that the superoxide scavenging activity of *Tribulus terrestris and Pongamia pinnata* was less as compared to the standard .The probable mechanism may be due to the non-inhibitory effect of Methanol extract of the leaf towards generation of superoxides in the reaction mixture. But the superoxide scavenging activity of *Maytenus emarginata* was more than the standard between 2-16 µg / ml and at 512 µg / ml concentrations. The results suggested that the *M. emarginata* plant extract is a good superoxide radical scavenger and efficiency of *T. terrestris* is low compared to ascorbic acid.

Iron chelating activity assay.

O-phenanthroline quantitatively forms complexes with Fe^{+2} which get disrupted in the presence of chelating agents. The methanol of *Maytenus emarginata* extract interfered with the formation of a ferrous-o-phenanthroline complex, thereby suggesting that the extracts had metal chelating activity (Figure 5).The results (Table 10)showed that *Tribulus terrestris and Pongamia pinnata* exhibited

less Iron chelating activity as compared to the standard, where as activity of *Maytenus emarginata* is best as comparative to other plants studied.[34]

CONCLUSIONS

The current study clearly indicates that *Pongamia pinnata, Maytenus emarginata* and *Tribulus terrestris,* are rich source of phytonutrients having immense antioxidant potential. In particular, spectrophotometrical analysis identified phenolics as abundant phytonutrients in *Maytenus emarginata* whereas total flavonoids were abundant in *Pongamia pinnata.* The results obtained thus indicate that these extracts have potent antioxidant activity, achieved by scavenging abilities observed against DPPH, ABTS, Nitric-oxide, Superoxide and Iron chelating. The plants have been reported to contain flavonoid and isoflavonoid glycosides which are known antioxidants, hence the antioxidant activity of the Methanol extract of the plants also showed good antioxidant potential. Further, the isolation of the compounds responsible for the activity has to be taken up which may result in a modern drug from these plants. These plants can be used to discover bioactive natural products that may serve as leads for the development of new pharmaceuticals that address hither to unmet therapeutic needs. It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin.

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