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In vitro antioxidant potential of *Pterocarpus marsupium* bark

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ABSTRACT

Antioxidant activity of aqueous, ethyl acetate and methanol extracts of the bark of Indian Kino tree *Pterocarpus marsupium* have been tested using various antioxidant model systems, *viz* DPPH, ABTS, NO, OH, SO and inhibition of *in vitro* lipid peroxidation. The total antioxidant potential has been assessed by FRAP assay. Methanol extract of *Pterocarpus marsupium* is found to possess highest DPPH radical scavenging activity followed by aqueous and ethyl acetate extracts. The aqueous extract of *Pterocarpus marsupium* exhibits potent ABTS scavenging activity. The nitric oxide scavenging activity of *Pterocarpus marsupium* ethyl acetate extract records the highest. With respect to hydroxyl radical scavenging, all the three solvent extracts of *Pterocarpus marsupium* present high activities on par with each other. Methanol extract of *Pterocarpus marsupium* is found to possess higher superoxide scavenging activity at lower concentrations as compared to the other extracts. All the three extracts of *Pterocarpus marsupium* are also found to be good inhibitors of *in vitro* lipid peroxidation. Saturation in the scavenging activity has been attained in a concentration dependent manner. This study indicates significant free radical scavenging potential of *Pterocarpus marsupium* bark which can be exploited for the treatment of various free radical mediated ailments.

Key words: *Pterocarpus marsupium,* Antioxidant Activity, Aqueous, Methanol, Ethylacetate and Bark.

1. INTRODUCTION

Pterocarpus marsupium Roxb. (Fabaceae) is a medium to large, deciduous tree that can grow upto 30 metres tall. It is native to India, Nepal and Sri Lanka. In India, it occurs in parts of the Western ghats in the Karnataka-Kerala region. It is also known by the names Malabar Kino, Benga, Bijiayasal (in western Nepal), Piasal (Oriya) [1], Venkai (Tamil), and many others [2]. The heart wood, leaves, flowers and bark have useful medicinal properties. The heartwood of *P.marsupium* is astringent, bitter, acrid, anti inflammatory, anthelmintic and anodyne. It is used for the treatment of elephantiasis, leucoderma, diarrhoea, dysentery, rectalgia, cough and greyness of hair. It is also reported that an aqueous infusion of the wood is of use in diabetes and water stored in vessels made of the wood is reputed to have antidiabetic qualities. The bark is used as an astringent and also for relieving toothache. The bruised leaves are considered useful as an external application for boils, sores and skin diseases. *P.marsupium* is widely used in 'Ayurveda' as 'Rasayana' for management of various metabolic disorders including hyperlipidemia [3-4].

Oxidative stress caused by reactive oxygen species has been implicated in the etiology of various diseases like cancer, aging, diabetes, auto immune diseases, cardiovascular diseases and neurological degenerative disorders. The primary oxygen derived free radicals are superoxide anion, hydroxyl, hydroperoxyl, peroxyl and alkoxyl radicals and non free radicals are hydrogen peroxide, hypochlorous acid, ozone and singlet oxygen. These reactive intermediates are collectively termed reactive oxygen species (ROS). These Reactive Oxygen Species (ROS) such as superoxide anions, hydroxyl radical and nitric oxide inactivate enzymes and damage important cellular components causing injury through covalent binding and lipid peroxidation. Antioxidants are agents which may offer resistance against the oxidative stress by scavenging the free radicals, inhibiting the lipid peroxidation and by other mechanisms, reduce the damage due to oxidants by neutralizing the free radicals and thus prevent disease [5]. Antioxidants act as a cooperative network, employing a series of redox reactions. Phytoantioxidants, commonly available, less toxic, serving food and medicinal components have been

suggested to reduce the threat of wide range of ROS [6].

Plant based traditional system of medicine is still followed by a large Indian population. Kino juice obtained from the trunk of *P.marsupium* is primarily composed of kino tannic acid, a peculiar tannin. Phenolic compounds are associated with antioxidant properties. Hence it was considered to evaluate the antioxidant activity of *P. marsupium* bark for its potential use in prevention and treatment of degenerative diseases using variuos *in vitro* assays.

2. MATERIALS AND METHODS

2.1 Plant Collection

The bark of *Pterocarpus marsupium* was collected from Velliyangiri hills, a part of the Western ghats, Coimbatore and identified by the botanist from Botanical Survey of India, Tamil Nadu Agricultural University campus, Coimbatore, Tamil Nadu, India. The voucher specimen was preserved in Department of Biochemistry, Centre for Plant Molecular Biology, Tamil Nadu Agricultural University, Coimbatore for future reference.

2.2 Chemicals

DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS (2,2-azinobis(3-ethyl benzothiazoline-6-sulfonic acid) were purchased from Sigma Aldrich company Ltd. The standards curcumin and ascorbic acid were procured from High media. All other chemicals and solvents used in this study are of analytical grade.

2.3 Technological processes

2.3.1. Preparation of plant extracts

The bark of *Pterocarpus marsupium* was shade dried, ground to a fine powder and stored at room temperature. The dried bark was crushed into a coarse powder using a mechanical grinder. The bark powder was extracted in various solvents, *viz* aqueous, ethyl acetate and methanol.

2.3.1.1. Aqueous extract

Five gram of the powdered sample was taken and heated with 25ml of water in a water bath at 100°C for half an hour. The extract was centrifuged at 10,000 rpm for ten minutes. The supernatant, aqueous extract of *Pterocarpus marsupium* (PMA) was used for analysis.

2.3.1.2. Ethyl acetate extract

One part of the bark was macerated in five parts of ethyl acetate, kept in shaker for 24 hours at 40° C. Filtered and collected the solvent. The solvent was evaporated to obtain the ethyl acetate extract of *Pterocarpus marsupium* (PME).

2.3.1.3. Methanol extract

One part of the bark was macerated in five parts of methanol, kept in shaker for 24 hours at 40°C. Filtered and collected the solvent. The solvent was evaporated to obtain the methanol extract of *Pterocarpus marsupium* (PMM).

2.4. Analytical methods

2.4.1. Total antioxidant activity

The total antioxidant activity of the extracts of *Pterocarpus marsupium* viz PMA, PME and PMM was assayed by FRAP method (Ferric Reduction Antioxidant Power) [7]. The reaction mixture containing different volumes of extracts, made up to a final volume of 1.5 ml to which 1.5 ml of FRAP reagent was added and the absorbance measured at 593nm in 1cm light path at 37ºC. A standard solution of ascorbic acid was tested in parallel.

2.4.2. Total phenol content

The total phenol content was determined with Folin-Ciocalteu reagent using pyrocatechol as the standard. To 0.1 ml of ethanolic extract of *Pterocarpus marsupium* added 0.5 ml of diluted Folin Ciocalteau reagent followed by 2.0 ml of 20% Na_2CO_3 solution after 30 min and mixed thoroughly. The tubes were placed in a boiling water bath for exactly 1 minute. Cooled and measured the absorbance at 650 nm. From the standard graph calculated the amount of polyphenols and expressed as mg of phenols per g of the sample [8].

2.4.3. Free radical scavenging activity

A stock solution of 1mg/ml of *Pterocarpus marsupium* ethyl acetate extract (PME), methanol extract (PMM) was prepared. This was diluted to get various concentrations (20-100µg/ml) in the final volume of reaction mixture. Varying volumes (20-100 µl) of aqueous extracts were used for the assay. PME was dissolved in ethyl acetate, while PMM was dissolved in distilled water. The free radical scavenging activity of the extracts were analyzed by following the various standard *in vitro* radical generating model systems *viz.*,1,1-diphenyl-2-picryl hydrazyl (DPPH), 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), superoxide anion (SO), hydroxyl (OH) and nitric oxide (NO). Inhibition of *in vitro* lipid peroxidation was studied using goat liver as a model system. In all the experiments deionized water served as blank and reaction mixtures without extracts served as control samples. The percentage scavenging (or) inhibition was calculated according to the formula

$$\text{Percentage scavenging (or) Inhibition} = \frac{C-T}{C} \times 100$$

Where C is the absorbance of control and T is the absorbance of test. All the experiments were performed in triplicates and the mean values were taken for results.

2.4.3.1. DPPH scavenging activity

The effect of PMA, PME and PMM on DPPH scavenging activity was estimated as per the following method [9]. Aliquots containing varying volumes (20-100 µl) of aqueous extracts (PMA) and different concentrations (20-100 µg) of ethyl acetate extract (PME); methanol extracts (PMM) were made up to 1 ml. To this 2 ml of DPPH (0.1 mM) was added. In the control, 2 ml of DPPH and 1 ml of distilled water was added. All the tubes were incubated at 37ºC for 20 min. Absorbance of reaction mixtures was recorded at 517 nm.

2.4.3.2. ABTS radical decolourization assay

The ABTS stock reagent mixture was prepared by mixing 88µl of 140 mM potassium persulphate with 5 ml of 7 mM ABTS stock solution (pH 7.4). The working ABTS reagent was prepared by diluting the stock solution with ethanol to give an absorbance of 0.7 ± 0.5 at 734nm. Aliquots containing varying volumes (20-100 µl) of aqueous extracts (PMA) and different concentrations (20-100 µg) of ethyl acetate extract (PME), methanol extracts (PMM) were taken and the final volume was made up to 1ml with distilled water. One ml of ABTS cation working solution was added to the tubes and ABTS solution with equal amount of distilled water serves as control. The reaction mixtures were incubated at 28ºC for 30 min. Absorbance was measured at 734 nm. The effect of PMA, PME and PMM on ABTS scavenging activity was determined [10].

2.4.3.3. Nitric oxide scavenging activity

Aliquots containing varying volumes (20-100 µl) of PMA and different concentrations (20-100 µg) of PME and PMM were taken in different tubes. One ml of sodium nitroprusside (10 mM) was added to various tubes containing different extracts were made up to 500 µl using distilled water. Tubes were incubated at room temperature for 2.5 hrs. To the reaction mixture, 1 ml of greiss reagent (prepared by mixing an equal volume of 1% sulphanilamide in 2% orthophosphoric acid with 0.1% N-(napthyl) ethylene diamine hydrochloride in water) was added. One ml sodium nitroprusside and 500 µl of distilled water without the extracts served as control. Absorbance was recorded at 546 nm. Curcumin was used as a reference compound. The nitric oxide scavenging potential of the PMA, PME and PMM extracts was assayed [11].

2.4.3.4. Super oxide anion scavenging activity

Aliquots containing varying volumes (20-100 µl) of PMA and different concentrations (20-100 µg) of PME and PMM were taken in different tubes. To the extracts added 1 ml of nitroblue tetrazolium solution (156 µM in 100 mM phosphate buffer pH 7.4) and 1 ml of NADH solution (468 µM in 100 mM phosphate buffer pH 7.4). The volume was made up with distilled water and reaction started by adding 100µl of phenazine methosulphate solution (60 µM in 100 mM phosphate buffer pH 7.4). The reaction mixture was incubated at 25ºC for 5 min. Curcumin was used as a reference compound. Absorbance was measured at 560 nm and the superoxide anion scavenging activity of PMA, PME and PMM extracts was calculated [12].

2.4.3.5. Hydroxyl radical scavenging activity

Aliquots containing varying volumes (20-100 µl) of PMA and different concentrations (20-100 µg) of PME and PMM were taken in different tubes. The reaction mixture finally contains 1 ml of phosphate buffer, 100 µl of 1mM EDTA, 100 µl of 20 mM hydrogen peroxide, 100 µl of 2-deoxyribose (30 mM), 100 µl of 1mM ferric chloride and 100 µl ascorbic acid (1 mM). The tubes were incubated at 37ºC for 30 min. Added 1 ml of 2.8% trichloroacetic acid followed by 1ml of 1% thiobarbaturic acid to the tubes. Tubes were heated in a water bath maintained at 75ºC for 30 min and cooled. Absorbance was measured at 534nm and the PMA, PME and PMM extracts were assessed for hydroxyl radical scavenging activity [13].

2.4.3.6. Extent of inhibition of *in vitro* lipid peroxidation

Aliquots containing varying volumes (20-100 µl) of PMA and different concentrations (20-100 µg) of PME and PMM were taken in different tubes. To 50 µl of 5% liver homogenate, extracts were added. Added 50 µl of ferrous sulphate to induce oxidation and the final volume was made up to 500 µl with cold TBS (10 mM Tris, 0.15 M sodium chloride pH 7.4). Control was prepared for each sample, containing respective extract (150 µl), and liver homogenate (50 µl) and made up to a final volume of 500 µl with cold TBS. A blank was set containing no plant extract, no liver homogenate but only ferrous sulphate and TBS. The final volume was made up to 500 µl with distilled water. A medium corresponding to 100% oxidation was prepared by adding all constituents except the plant extracts and volume was made up to 500 µl. The experimental medium corresponding to auto oxidation contained only liver homogenate and TBS made up to final volume. All tubes were incubated at 37ºC for 1

hour. After that 500 µl of 70% alcohol was added to stop the reaction. One ml of 1% TBA was added to all the tubes, followed by boiling in a hot water bath for 20 min. After cooling the tubes were centrifuged. To the clear supernatants collected *in toto* added 500 µl of acetone. Thio barbituric acid reactive substance (TBARS) was measured at 535 nm and the inhibition of *in vitro* lipid peroxidation was calculated [14].

3. RESULTS AND DISCUSSION

Free radicals are chemical entities that can exist separately with one or more unpaired electrons. The generation of free radicals can bring about thousands of reactions and thus cause extensive tissue damage. Lipids, proteins and DNA are all susceptible to attack by free radicals [15]. Antioxidants may offer resistance against oxidative stress by scavenging the free radicals or protecting the antioxidant defense mechanisms. Antioxidant effectiveness is measured by monitoring the inhibition of oxidation of a suitable substrate. Various methods are available for determining free radical scavenging effects.

The bark of *P.marsupium* extracted in various solvents *viz* aqueous, ethyl acetate and methanol was tested for its antioxidant potential using FRAP assay. Free radical scavenging activities of *P.marsupium* was tested using various models *viz* DPPH, ABTS, NO, OH, SO and inhibition of *in vitro* lipid peroxidation.

The transformation of Fe^{3+} into Fe^{2+} in the presence of various fractions was measured to determine the reducing power ability. The reducing ability of a compound generally depends on the presence of reductones, which exert the antioxidant activity by breaking the free radical chain by donating a hydrogen atom. The antioxidant potential of the bark of *P.marsupium* is expressed as µg equivalence of ascorbic acid. 1g of *P.marsupium* extracts was found to be equivalent to 530 µg of ascorbic acid in terms of antioxidant activity (Table 1). The antioxidant principles present in PMA, PME and PMM extracts of *P.marsupium* caused the reduction of Fe^{3+} / ferricyanide complex to the ferrous form, and thus proved the reducing power ability.

Table -1: Total antioxidant potential and total phenol content of *Pterocarpus marsupium*

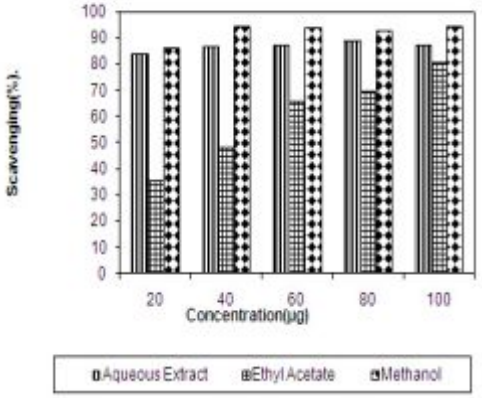
Total antioxidant potential[a] Ascorbic acid equivalents (µg)	Total phenol content [b] mg/g
530 ±4.3	17.4 ± 0.6

Values are mean ± SD of triplicates

Sample extract – [a]Aqueous extracts (5 g in 25ml of distilled water), [b]Ethanol extract, Table shows conversion of 1g of sample extracts.

The 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical is widely used as a model system to investigate the free radical scavenging activities of several plant extracts. DPPH is stable, nitrogen-centered free radical which produces violet colour in ethanol solution. It was reduced to a yellow coloured product, diphenyl picryl hydrazine, with the addition of the extracts. The reduction in the number of DPPH molecules can be calculated with the number of available hydroxyl groups. The scavenging activity of PMA, PME and PMM extracts of *Pterocarpus marsupium* was found to increase in a concentration dependent manner (Fig.1).

Fig. 1: DPPH free radical scavenging activity of *P.marsupium* extracts



PMA exhibited a maximum scavenging of 87.2% at 100 µl (1:5 w/v). At a concentration of 100 µg/ml PME exhibited a scavenging of 80.2%. Among all the three extracts, PMM recorded the highest scavenging activity of 94.4% at a concentration of 40 µg/ml. All the extracts attained saturation in the scavenging activity with further increase in the concentration. PMA, PME and PMM were found to be very good scavengers of the stable DPPH free radical in comparison with standard ascorbic acid as reported earlier [16]. The DPPH and ABTS free radical scavenging activity by ascorbic acid is shown in Figures 2a and 2b.

The ABTS assay is based on the inhibition of the absorbance of the radical cation $ABTS^{+}$, which has a characteristic long wavelength absorption spectrum. The ABTS radical scavenging activities of *P. marsupium extracts* is depicted in Fig.3. The scavenging activity of PMA, PME and PMM were found to increase in a concentration dependent manner. PMA showed a maximum scavenging of 96.1% at 60 µl (1:5 w/v). PME exhibited a scavenging of 95.1% at a

concentration of 100 µg/ml. Of all the three extracts tested, PMM recorded the highest scavenging activity of 97.7% at a concentration of 100 µg/ml. The scavenging activity attained saturation with further increase in the concentration of extracts. The results imply that PMA, PME and PMM were found to be very good inhibitors and scavengers of the ABTS radical.

Fig. 2a: DPPH free radical scavenging activity of ascorbic acid

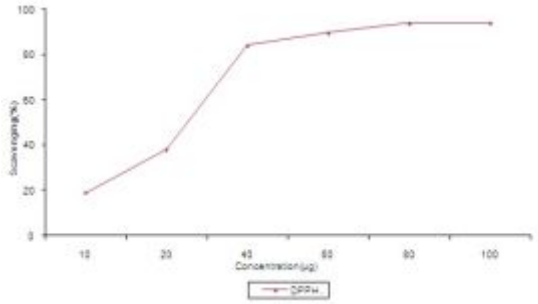


Fig 2 b: ABTS free radical scavenging activity of ascorbic acid

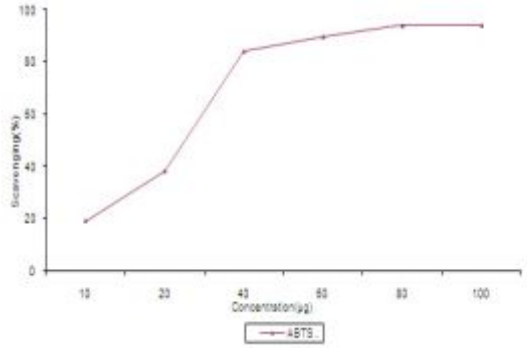
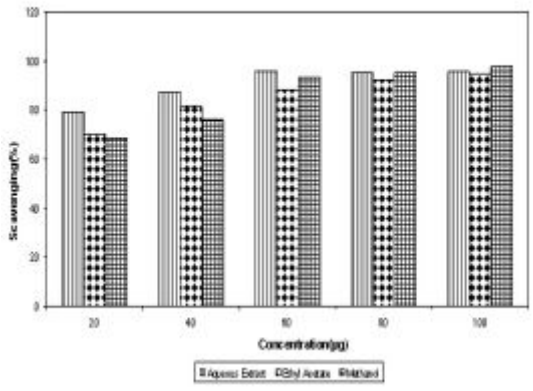
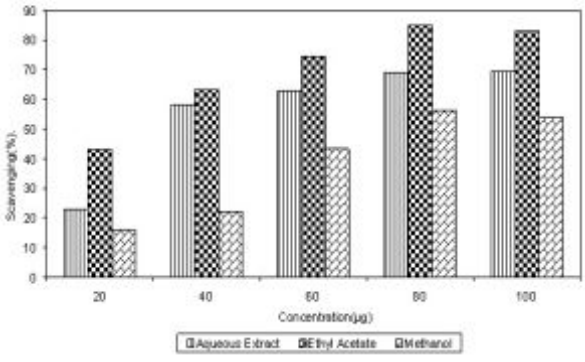


Fig.3: ABTS radical scavenging activity of *P.marsupium* extracts



Nitric oxide is a free radical generated by endothelial cells, macrophages, neurons etc and involved in the regulation of various physiological processes[17]. Excess concentration of NO is associated with several diseases. *In vitro* inhibition of nitric oxide radical is a measure of antioxidant activity of plant drugs. Scavenging of nitric oxide radical is based on the generation of nitric oxide from sodium nitroprusside in buffered saline, which reacts with oxygen to produce nitrite ions that can be measured by using Griess reagent [18]. The absorbance of the chromophore is measured at 546 nm in the presence of the extracts. The nitric oxide scavenging activities of the extracts of *P. marsupium* are presented in Fig.4. The scavenging activity of PMA, PME and PMM were found to increase in a concentration dependent manner. PMA showed a scavenging activity of 69.4% at 100 µl (1:5 w/v). While PME exhibited a scavenging of 85% at 80 µg/ml PMM recorded scavenging activity of 56.2% at 80 µg/ml concentration attaining saturation with further increase in the concentration of extracts. All the extracts of *P. marsupium* decreased the amount of nitrite generated from the decomposition of sodium nitroprusside *in vitro*. This may be due to the antioxidant principles in the extracts which compete with oxygen to react with NO, thereby inhibiting the generation of nitrite ions. Curcumin was used as a reference compound. The IC_{50} values of P. *marsupium* extracts PMA, PME, PMM were found to be 27, 33 and 23 µg respectively, whereas the IC_{50} values of curcumin was 20.4.

Fig.4: Nitric oxide scavenging activity of *P.marsupium* extracts



The hydroxyl radical scavenging activity is measured as the percentage of inhibition of hydroxyl radicals generated in the Fenton's reaction mixture by studying the competition between deoxyribose and the extract for hydrogen radicals generated from Fe^{3+}/ EDTA/ H_2O_2 systems. The hydroxyl radicals attack deoxy ribose which eventually results in TBARS

formation. The NO, SO, OH radical scavenging activity of standard curcumin is given in Fig 5a. The hydroxyl radical scavenging activity of PMA, PME and PMM of *Pterocarpus marsupium* was found to increase in a concentration dependent manner (Fig.5b). PMA recorded the highest scavenging activity of 96.3% at 100 µl (1:5 w/v), PME exhibited a scavenging of 94.7% at 100 µg/ml while PMM recorded the scavenging activity of 94.9% at 100 µg/ml. All the three extracts attained saturation in the scavenging activity with further increase in concentration. PMA, PME and PMM were found to be very good scavengers of hydroxyl radical in comparison with standard curcumin.

Fig. 5a: Nitric oxide, hydroxyl radical and super oxide scavenging activity of curcumin

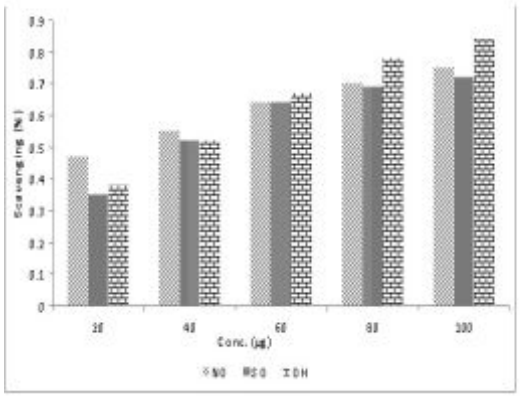
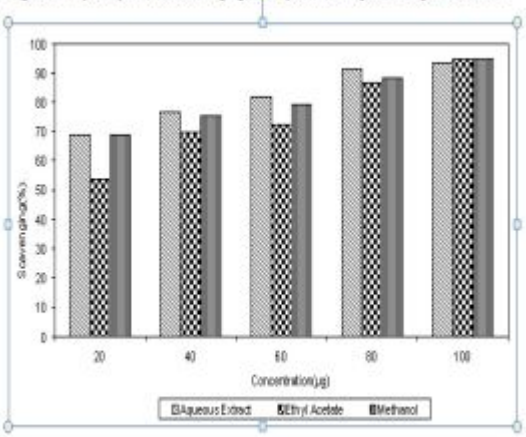
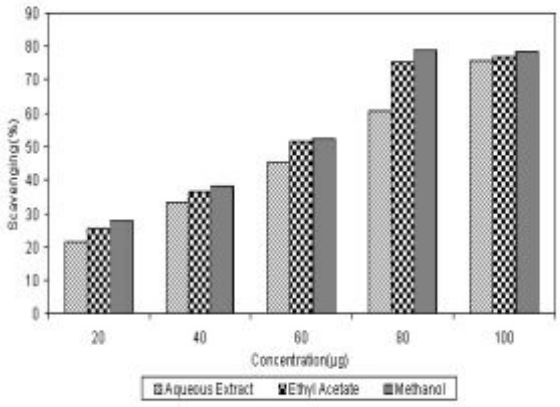


Fig. 5b: Hydroxyl radical scavenging activity of *Pterocarpus marsupium* extracts



Superoxides are produced from molecular oxygen due to oxidative enzymes [19] of body as well as via non-enzymatic reaction such as antioxidation by catecholamines [20]. The decrease in absorbance at 560nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture. The superoxide scavenging activity of PMA, PME and PMM was found to increase in a concentration dependent manner (Fig.6). PMA showed maximum scavenging of 75.6% at 100 µl (1:5 w/v). PME exhibited 76.8% scavenging activity at a concentration of 100 µg /ml and PMM reported 78.7% scavenging activity at a concentration of 80 µg/ml.

Fig. 6: Super oxide radical scavenging activity of *P.marsupium* extracts



PMA, PME and PMM were found to be very good scavengers of superoxide radical in comparison with standard curcumin. The IC_{50} values of *P. marsupium* extracts PMA, PME and PMM on superoxide radical scavenging activity was found to be 30, 34 and 36 respectively , and that of curcumin was 5.95. The results indicate that the extracts of *P. marsupium* have a potent scavenging activity with increasing percentage inhibition. The probable mechanism of scavenging the superoxide anions may be due to the inhibitory effect of the extract towards generation of superoxides in the *in vitro* reaction mixture.

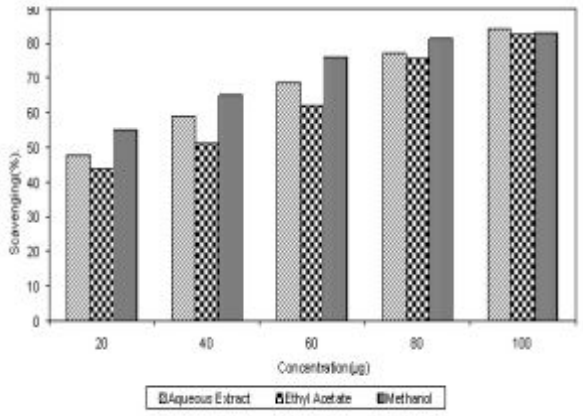
Lipid peroxidation is the oxidative degeneration of polyunsaturated fatty acids and involves formation of lipid radicals leading to membrane damage. Free radicals induce lipid peroxidation in poly unsaturated lipid rich areas like brain and liver [21]. Initiation of lipid peroxidation by ferrous sulphate takes place through hydroxyl radical by Fenton's reaction. The inhibition could be caused by absence of ferryl-perferryl complex or by scavenging the hydroxyl radical on the superoxide radicals or by changing the Fe^{3+}/ Fe^{2+} or by reducing the rate of conversion of ferrous to ferric or by chelating the iron itself. The Inhibition of *in vitro* lipid peroxidation of PMA, PME and PMM was found to increase in a concentration dependent manner (Fig.7). *In vitro* lipid peroxidation was inhibited at a level of 84.2% at 100µl (1.5w/v) by PMA, 82.7% at 100 µg/ml by PME and 83% at 100 µg/ml by

PMM. PMA, PME and PMM were found to be good inhibitors of *in vitro* lipid peroxidation. The IC_{50} values for DPPH, ABTS, NO, SO, OH radical scavenging and inhibition of *in vitro* lipid peroxidation are presented in Table 2.

Table -2: IC_{50} values (µg) for antioxidant scavenging activities of extracts of *P. marsupium*

Extract	DPPH	ABTS	NO	SO	OH	LPO
PMA	32	44	27	30	37	20
PME	28	40	33	34	32	17
PMM	40	38	23	36	34	22
Standard ascorbic acid	23	21	-	-	-	-
Standard curcumin	-	-	20.4	5.95	70	-

Fig. 7: Inhibition of *in vitro* lipid peroxidation by *P.marsupium* extracts



Phenolics are diverse secondary metabolites abundant in plant tissues [22]. Polyphenols possess ideal structural chemistry for free radical scavenging activity, and they have been shown to be more effective antioxidants *in vitro* than tocopherols and ascorbate. The total phenol content of bark of *P.marsupium* was estimated as 17.4 mg/g (Table 1). Antioxidative properties of polyphenols arise from their high reactivity as hydrogen or electron donors, from the ability of the polyphenol derived radical to stabilize and delocalize the unpaired electron (chain breaking function) and from their ability to chelate transition metal ions (termination of the Fenton reaction)[23]. Another mechanism underlying the antioxidative properties of phenolics is the ability of flavanoids to alter peroxidation kinetics by modification of the lipid packing order and to decrease fluidity of membranes [24]. These changes could sterically hinder diffusion of free radicals and restrict peroxidative reactions. Moreover, it has been shown recently that phenolic compounds can be involved in the hydrogen peroxide scavenging cascade in plant cells [25].

Medicinal plants are known to contain a variety of antioxidants. Numerous substances have been suggested to appear as antioxidants. It has been revealed that various phenolic antioxidants such as flavanoids, tannins, coumarins, xanthones and more recently procyanidins scavenge radicals dose dependently, thus they are viewed as promising therapeutic drugs for free radical pathologies [26]. The results of the present study which demonstrates the radical scavenging of PMA, PME and PMM, indicate that the use of *Pterocarpus marsupium* for the treatment of free radical mediated ailments (various inflammatory diseases) seems reasonable and useful.

4. CONCLUSION

In summary, the bark of *Pterocarpus marsupium* may be considered as good source of natural antioxidants for free radical mediated ailments. Further investigation on the isolation, purification and identification of antioxidative and antidiabetic constituents is being performed in our laboratory. This will certainly help to identify the possible mechanism of action and the potency of the compound.

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