Phytochemical screening and in vitro antioxidant activity of ethyl acetate leaf extracts of *Pterocarpus marsupium* Roxb (Fabaceae)

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Abstract

Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as, free radicals. The plants are susceptible to damage caused by active oxygen and thus develop numerous antioxidant defense systems resulting in formation. The potentially reactive derivatives of oxygen, continuously generated inside the human body are detoxified by the antioxidants present within the body of numerous potent antioxidants. The present study deals with the in vitro antioxidant activity of Ethyl Acetate leaf extract of *Pterocarpus marsupium* by using diphenyl-picryl-hydrazyl (DPPH) assay, ferric reducing antioxidant potential FRAP assay, ABTS assay, nitric oxide radical scavenging activity, hydroxyl radical scavenging activity, total peroxy radical trapping potential (TRAP) assay, hydrogen peroxide radical scavenging activity and reducing power assay. The percentage of scavenging activity at different concentration was determined. The leaf extract showed higher scavenging activity (i.e.) 71% in ferric reducing antioxidant potential (FRAP) assay at a concentration of 100 μg/ml. This study indicated that the Ethyl Acetate leaf extract of *Pterocarpus marsupium* have the ability for free radical scavenging activity.

Keywords: antioxidants, radical scavenging, phytochemicals, *Pterocarpus marsupium*

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Introduction

Living cells may generate free radicals and other reactive oxygen species byproducts as a results of physiological and biochemical processes. These Free radicals contributes more than one hundred disorders in humans including cancer, diabetes, atherosclerosis, arthritis, nerve injuries, AIDS etc. (Cook et al., 1996; Kumpulainen et al., 1997). Plants produces a wide variety of secondary metabolities such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are rich in antioxidant and antimicrobial activity (Wong et al., 2006; Baker, 2010). Due to depletion of immune system natural antioxidants in different maladies, consumption of antioxidants as free radical scavengers may be necessary (Younes, 1981; Halliwell et al., 1989). Studies have shown that many of these compounds possess anti-inflammatory, anti atherosclerotic, antitumor, anti mutagenic, and anti carcinogenic activities (Sala et al., 2002). The ingestion of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes, and other diseases associated with ageing. Hence, it should be include in our diet (Yang, 2001). In recent years, there has
been a worldwide trend towards the use of the natural phytochemicals present in berry crops, teas, herbs, oilseeds, beans, fruits and vegetables (Wang et al., 2000).

Kino is locally called as, Bija is nased native to India, Nepal and Sri Lanka where it occurs in parts of the We Pterocarpus marsupium Roxb. (Fabaceae) popularly known as, Indian Kino Tree, Malabar Kino Tree, Stern Ghats (Gamble, 1935; Mathew, 1983). Traditionally, the plant material has been used as a cooling external application for inflammations and headache as antipyretic, anti-helminthic, aphrodisiac, and in biliousness, mental aberrations and ulcers (Sambath et al., 2006). The wood and bark of P. marsupium are known for their anti-diabetic activity (Ivorra et al., 1989; Kameswara et al., 2001). Phytochemical studies on P. marsupium have shown that the plant contains iso-flavonoids, terpenoids and related phenolic compounds, β-sitosterol, lupenol, epicatechin, and aurone glycoside (Kumar et al., 1976; Mitra et al., 1983). The powdered bark is mixed with Schleichera oleosa and taken with cold water to treat dysentery (Mohanta et al., 2006). The flowers are used in fever and the gum is locally applied in leucorrhoea and passive hemorrhage. The bruised leaves are considered useful as an external application for boils, sores and skin diseases. The wood of the tree is useful in making water glasses of the diabetic patients (Reddy et al., 2008). The aim of the present study is to evaluate the in vitro antioxidant activity of ethyl acetate extract of Pterocarpus marsupium.

Materials and Methods

Collection of Plant material and extraction

Leaves of P. Marsupium were collected from Coimbatore region, Tamil Nadu, India, during August 2012. The flora of presidency of Madras (Gamble, 1935) and the flora of Tamil Nadu were used for identification and authentication of the plants. Collected material was washed thoroughly using running tap water, rinsed in distilled water and shade dried in open air and ground in to powder and was extracted with petroleum ether, chloroform, ethyl acetate, ethanol and water, by using soxhlet apparatus. The plant extracts were concentrated using rotary flash vaporator and stored in desicator.

Chemicals

2,2-diphenyl-picrylhydrazyl (DPPH), ascorbic acid, 2,4,6-triprydyl-s-triazine (TPTZ), ferric chloride hexahydrate, 2,2-azinobis (3-ethylbenzothiozoline-6-sulfonic acid) diammonium salt (ABTS•+), 2,7-dichloroflurecain (DCF) diacetate, sodium phosphate, 2,2-azobis (2- amino prop dihydro chloride (AAPH), sodium nitroprusside, sulphanilamide, griess reagent, deoxy ribose, phenyl hydrazine, trichloro acetic acid (TCA), TBA, hydrogen peroxide, potassium ferrocyanide.

Physicochemical studies

Ash value

The residue remaining after incineration is the ash content of the drug, which simply represents inorganic salts, naturally occurring in drug or adhering to it or deliberately added to it as a form of adulteration. It is used to determine quality and purity of a drug.

Total ash value

A quantity of test sample equivalent to 2-3 gm of air dried plant materials were weighed separately in a charred platinum or silica disc and incinerated in muffle furnace at temperature not exceeding 450°C until free from carbon. It was cooled and weighed. The percentage of ash with reference to the air dried leaves was calculated.

Acid insoluble ash value

The ash obtained was boiled with 25 ml of 2 N HCl for 5 min. The insoluble matter was collected in a gouch
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crucible, washed with hot water, ignited and weighed. The percentages of acid insoluble ash with reference to air dried drugs were calculated.

**Water soluble ash value**

The ash obtained was boiled with 25 ml of distilled water for 5 min. The soluble matter was collected in a Gooch crucible, washed with hot H₂O, ignited and weighed. The percentage of water soluble ash with reference to air dried drugs was calculated.

**Determination of moisture (loss on drying)**

About 2 gm of air dried plant material were weighed in to a moisture dish which was previously dried in an oven and weighed. The dish was covered and was transferred to desiccators and weighed quickly as possible as the dish was cooled. The heating and weighing procedure was repeated until successive weight. Loss in weights was recorded.

**Extractive value**

5 g air dried coarsely powdered leaf material was macerated separately with 100 ml of each solvent (petroleum ether, chloroform, ethylacetate, ethanol and water) in a closed container for 24 hr, it was shaken frequently during the first 6 hr and allowed to stand for 18 hr, and then filtered, 25 ml of the filtrate was taken from each flask and evaporated to dryness in a tarred flat-bottomed shallow dish, dried at 105°C and weighted. The percentages of different soluble extractive values were calculated with reference to the air dried powder.

**Preliminary phytochemical screening**

The ethyl acetate extract of *P. marsupium* was subjected to, to evaluate the presence of phytoconstituents such as alkaloids, flavanoids, steroids, glycosides, triterpinoids, amino acids and tannins. This was carried by using standard procedure (Edeogal et al., 2005).

**Thin Layer Chromatography**

TLC was done by the standard procedure, by using ethyl acetate extract of leaf. The selection of solvent system was based on increasing the order of polarity. Based on the chemical tests and nature of phytoconstituent present, the solvent systems were selected.

**In vitro Antioxidant studies**

**Determination of 2,2′-diphenyl-1-picrylhydrazyl DPPH radical scavenging activity**

The free radical scavenging activity of the extracts was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH (2, 2′-diphenyl-1-picrylhydrazyl). 0.1 mM solution of DPPH in ethanol was prepared and 1.0 ml of this solution was added to 2.5 ml of extract solution in water at different concentrations (20-100 μg/ml). Thirty minutes later, the absorbance was measured at 518 nm. Ascorbic acid was used as a standard (Blois, 1958). Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

\[
\text{DPPH Scavenged (\%) = } \left( \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \right) \times 100
\]

Where, A control is the absorbance of the control reaction and A test/std is the absorbance in the presence of the extracts.

**Ferric reducing/antioxidant power (FRAP) assay**

FRAP reagent (900 μl), prepared freshly and incubated at 37°C, was mixed with 90 μl of distilled water and 30 μl of test sample or methanol. The test samples and reagent blank were incubated at 37°C for 30 min in a water bath (Pulido et al., 2000). The final dilution of the test sample in the reaction mixture was 1/34. The FRAP reagent contained 2.5 ml of 20 mmol/l TPTZ solution in 40...
mmol/l HCl plus 2.5 ml of 20 mmol/l FeCl₃, 6H₂O and 25 ml of 0.3 mol/l acetate buffer (pH 3.6) (Siddhuraju et al., 2003). At the end of incubation, the absorbance readings were taken immediately at 593 nm, using a spectrophotometer. Methanolic solutions of known Fe (II) concentration, (FeSO₄.7H₂O) were used for the preparation of the calibration curve.

**Antioxidant activity by the ABTS•⁺ assay**

The total antioxidant activity of the samples was measured by ABTS radical cation decolorization ABTS•⁺ was produced by reacting 7 mM ABTS aqueous solution with 4.9 mM ammonium persulfate in the dark for 12–16 h at room temperature (Re et al., 1999). Prior to assay, this solution was diluted in ethanol and equilibrated at 30°C to give an absorbance at 734 nm of 0.700 ± 0.02. After the addition of 950 μl of diluted ABTS solution to 50 μl of sample (final concentration 100-500 μg) in ethanol, absorbance was measured at 30°C exactly 30 min after the initial mixing. Appropriate solvent blanks were also run in each assay and percentage inhibition was calculated of the blank absorbance at 734 nm.

**Determination of nitric oxide radical scavenging activity**

Nitric oxide was generated from sodium nitroprusside and measured by the Griess reaction (Sreejayan et al., 1997). Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitric ions that can be estimated by use of Griess reagent. Scavenger of nitric oxide competes with oxygen leading to reduced production of nitric oxide. Sodium nitroprusside (10 mM) in phosphate-buffered saline buffer (5 pH 7.4). The mixture was then incubated at 37°C for 1 h. To 1.5 ml of the incubated sample, 1.5 ml Griess reagent (1% sulphanilamide, 0.1% naphthylethylene diamine hydrochloride in 2% H₃PO₄) was added. The absorbance of chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with naphthylethylene diamine was measured at 546 nm.

**Hydroxyl radical scavenging activity**

Various concentrations (20, 40, 60, 80 and 100 μg) of extracts were taken. The reaction volume is made up to 2 ml with 0.6ml of ribose (1 mM), 0.4 ml phenyl hydrazine (0.2 mM), 0.4 ml of sample, 0.6 ml phosphate buffer (10 mM, pH 7.4) and incubate for 2 hrs at room temperature (Klein et al., 1991). After incubation add 1 ml of TCA (2-8% in distilled water) and 1ml of TBA. Kept all the mixture incubated at 80-90°C for 15 min in a water bath. The reaction mixture without sample was used as control. The intensity of the color formed was measured spectroscopically at 532 nm against reagent blank. The % hydroxyl radical scavenging activity is calculated by the following formula,

\[
\% \text{ HRSA} = \frac{(A_0 - A_1)}{A_0} \times 100
\]

where A₀ is the absorbance of the control and A₁, is the absorbance of the extract/standard

**Total peroxy radical trapping potential (TRAP)**

A water soluble azo initiator 2, 2’ azo bis (2-amidino propane) dihydrochloride (AAPH) produced the peroxyl radicals while a spectrophotometric analysis of 2,7 dichlorofluresecin-diactate (DCF) monitored the scavenging activity of the plant extracts (Valkonen et al., 1997). A 350 μl of 1 mM stock of DCF in ethanol was mixed with 1.75 ml of 0.01 N sodium hydroxide and allowed to stand for 20 min before the addition of 17.9 ml of 25 mM sodium phosphate buffer (pH 7.2). The reaction mixture contained 0.5 ml of various concentration of plant extract in methanol, 150 μl of activated DCF solution and 25 μl of AAPH (56 mM). The reaction was initiated with
the addition of the AAPH. Absorbance was read at 490 nm. Ascorbic acid was used as standard.

Hydrogen Peroxide scavenging activity

A solution of hydrogen peroxide (2 mmol/l) was prepared in phosphate buffer (pH 7.4). Extracts (20-100 μg/ml) were added to hydrogen peroxide solution (0.6 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide (Ruch et al., 1984). The percentage scavenging of hydrogen peroxide of *P. marsupium* and standard compounds was calculated using the following formula:

\[
\text{Percentage scavenging} = \left( \frac{A_{blank} - A_{sample}}{A_{blank}} \right) \times 100
\]

Determination of reducing power assay

Different concentrations of ethyl acetate extract of *P. marsupium* (20, 40-100 μg/ml) in 1ml of methanol were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferrocyanide (2.5 ml, 1%). The mixture was incubated at 30°C for 20 min (Oyaizu, 1986). A portion (2.5 ml) of trichloro acetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl₃ (0.1%) and the absorbance was measured at 700 nm and compared with standard. Increased absorbance of the reaction mixture indicated increased reducing power.

Results

In the present study, the ethyl acetate extract prepared from the dried leaves of *Pterocarpus marsupium* was subjected to, physiochemical studies, preliminary phytochemical screening, thin layer cromatography and *In vitro* antioxidant studies. Phychochemical studies of ethyl acetate extract of *Pterocarpus marsupium* reveals the presence of physical constant values like extractive value, total ash value, acid insoluble ash value, water insoluble ash value and moisture content (Table 1). Preliminary phytochemical screening of ethyl acetate extract of *Pterocarpus marsupium* revealed the presence of active phytoconstituents (Table 1). Thin Layer Chromatography (TLC) using ethyl acetate extract of *Pterocarpus marsupium* reveals the separation and identification of active phytoconstituents by using the principle of adsorption. *In vitro* antioxidant assay reveals the free radical scavenging activity of antioxidants present in Ethyl acetate extract of leaf of *Pterocarpus marsupium*. The analysis is done by the different assays and compared with standard (ascorbic acid) by graphical representation (Tables 1, 2 and Figs. 1 to 3).

Table 1. Qualitative analysis of Ethyl Acetate leaf extract of *Pterocarpus marsupium*

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Ethyl acetate extract of <em>Pterocarpus marsupium</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>++</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+++</td>
</tr>
<tr>
<td>Sterols and Triterpinoids</td>
<td>+++</td>
</tr>
<tr>
<td>Tannins</td>
<td>++</td>
</tr>
<tr>
<td>Resins</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>Proteins and amino acids</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>++</td>
</tr>
<tr>
<td>Gum &amp; mucilage</td>
<td>-</td>
</tr>
<tr>
<td>Flavanoids</td>
<td>+++</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>-</td>
</tr>
</tbody>
</table>

+++ represents higher concentration, ++ - medium concentration, + represents mild concentration, - represents absence

Discussion

Plants are known to have beneficial therapeutic effects documented in Traditional Indian System of Medicine. Much work has been done on ethnomedicinal
plants in India. Interest in a large number of traditional natural products has increased. It has been suggested that phytochemical extracts from plants holds promise to be used in allopathic medicine as they are potential sources of antiviral, antitumoral and antimicrobial agents (Nair et al., 2005). Phytochemical analysis showed the presence of alkaloids, flavonoids, glycosides terpenoids steroids and tannins. These constituents have diverse pharmacological properties including antioxidant and antimicrobial activity (Bhandary et al., 1995). Antioxidant and antimicrobial activity of *Pterocarpus marsupium* may be due to the synergistic effect of two or more chemical constituents of the plant extract.

Antioxidants exert their mode of action by suppressing the formation of reactive oxygen species either by inhibition of enzymes or by chelating trace elements. DPPH is widely used to evaluate the free radical scavenging effect of natural antioxidant. DPPH is a stable free radical at room temperature. As the electron became paired in the presence of free radical scavenging the absorption vanishes and the resulting discoloration stochiometrically coincides with the number of electrons taken up. The bleaching of DPPH absorption is representative of the capacity of the test drugs to scavenge free radicals independently. The bleaching of DPPH molecules can be correlated with the number of available hydroxyl groups. We can infer that, the activity of the extract may be probably due to the presence of substance with an available hydroxyl group. The extracts are able to reduce the stable radical DPPH to the yellow coloured diphenyl picrylhydrazine (Ghaisas et al., 2008). Radical scavenging activity increased as the concentration of extract of *P. marsupium* increased the leaf extract demonstrated H-donor activity. It is postulated that *P. marsupium* extract reduces the radicals to the corresponding hydrazine when it reacts with the hydrogen donor in the antioxidant activity.

FRAP assay directly measures antioxidants or reductants in a sample that react with ferric tripyridyltriazine (Fe3+ TPTZ) complex and produce a coloured ferrous tripyridyltriazine (Fe2+ TPTZ). The phenolic compounds exhibited redox properties by acting as reducing agents, hydrogen donators and singlet oxygen quenchers (Rice-Evans et al., 1997). There is a highly positive relationship between total phenolics and antioxidant activity in many plant species (Oktay et al., 2003). Therefore, the sample extracts can act as electron donors and react with free radicals and convert them to stable products, thus terminating the radical chain reaction. Similarly it is observed that radical scavenging activity increased as the concentration of extract of *P. marsupium* increased. ABTS assay is an excellent tool to determine the antioxidant activity of hydrogen donating and chain breaking antioxidants. From the evaluation of total antioxidant potential commonly used medicinal plants and concluded that the phenolic compounds play a vital role in scavenging of ABTS (Pietta et al., 1998). It is revealed that radical scavenging activity increased as the concentration of extract of *P. marsupium* increased and the extract exhibited strong ABTS radical scavenging activity. Nitric oxide (NO) is an important chemical media to regenerated by endothelial cells, macrophages, neurons etc. and involved in the regulation of various physiological processes. Excess concentration of NO is associated with several diseases. Oxygen reacts with excess nitric oxide to generate nitrite and peroxynitrite anions, which act as free radicals (Siddhuraju et al., 2003). Nitric oxide scavenging method of extract showed moderate scavenging activity.
Table 2. TLC analysis of phytoconstituents using ethyl acetate extract of *Pterocarpus marsupium*

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Solvent system</th>
<th>Detecting agent</th>
<th>Solvent front</th>
<th>No. of spots</th>
<th>R_f value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Tolune: Ethyl acetate: Diether amine (7:2:1)</td>
<td>Ultraviolet spectroscopy</td>
<td>3.4</td>
<td>1</td>
<td>0.142</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>0.323</td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td>3</td>
<td>0.5</td>
</tr>
<tr>
<td>Flavanoids</td>
<td>Tolune: Ethyl acetate (93:7)</td>
<td>Ultraviolet spectroscopy</td>
<td>4.5</td>
<td>1</td>
<td>0.355</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>0.555</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>3</td>
<td>0.688</td>
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<td>4</td>
<td>0.844</td>
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<tr>
<td>Steroids</td>
<td>Petroleum ether: Ethyl acetate (7:3)</td>
<td>Ultraviolet spectroscopy</td>
<td>5.3</td>
<td>1</td>
<td>0.264</td>
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<td>2</td>
<td>0.433</td>
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<td></td>
<td></td>
<td></td>
<td>3</td>
<td>0.660</td>
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<td>Tryterpinoids</td>
<td>Chloroform: Methanol (19:1)</td>
<td>Ultraviolet spectroscopy</td>
<td>6.1</td>
<td>1</td>
<td>0.114</td>
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<td>2</td>
<td>0.213</td>
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<td>3</td>
<td>0.491</td>
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<td>4</td>
<td>0.704</td>
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<tr>
<td>Glycosides</td>
<td>Benzene: Ethanol (19:2)</td>
<td>Ultraviolet spectroscopy</td>
<td>3.5</td>
<td>1</td>
<td>0.257</td>
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<td>0.371</td>
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<td>4</td>
<td>0.571</td>
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</tbody>
</table>

**Fig. 1.** 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity

The highly reactive hydroxyl radicals can cause oxidative damage to DNA, lipids and proteins. The effect of *P. marsupium* on the inhibition of free radical mediated deoxyribose damage was assessed by means of the Iron (II)-dependent DNA damage assay. The Fenton reaction generates hydroxyl radicals (OH) which degrade DNA deoxyribose, using Fe^{2+} salts as an important catalytic component. Oxygen radicals may attack DNA either at the sugar or the base, giving rise to a large number of products. Extent of hydroxyl radical scavenged was determined by the decrease in intensity of pink coloured chromophore was determined at 532 nm. In the present study, radical scavenging activity of the extract is moderate when compared to standard (ascorbic acid). The peroxyl radical scavenging activity was determined and the results were compared with standard (ascorbic acid). In the present study, plant extract scavenged the free peroxyl radicals produced by AAPH solution. The scavenging activity was mild at lower concentration of extract and increased as the concentration of extract of *P. marsupium* increased. Hydrogen peroxide is a weak oxidizing agent that inactivates a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. It can cross cell membranes rapidly; once inside the cell, it can probably react with Fe^{2+}.

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and possibly Cu²⁺ ions to form hydroxyl radicals and this may be the origin of many of its toxic effects. Thus, removing hydrogen peroxide well as O₂⁻is very important for protection of food systems.

**Fig. 2.** Ferric reducing/antioxidant power (FRAP) scavenging activity

![FRAP Graph](image)

**Fig. 3.** Antioxidant activity by the 2, 2 azinobis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay

![ABTS Graph](image)

Dietary polyphenols have also been shown to protect mammalian and bacterial cells from cytotoxicity induced by hydrogen peroxide. Especially compounds with the orthodihydroxy phenolic structure quercetin, catechin, gallic acid ester, caffeic acid ester. Therefore, the phenolic compounds of the *P. marsupium* extracts are involved in removing the hydrogen peroxide (Kumaran et al., 2007). In the present study, the activity of the extract was mild at lower concentration (20 μg/ml) when concentration increased, free radical scavenging activity also increased. The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. The reducing power increased with increasing amount of the extract. Increased absorbance of the reaction mixture indicated increased reducing power (Guptha et al., 2007). In present study, the ethyl acetate leaf extract showed the highest reducing ability. However, the activity was less than the standard, ascorbic acid.

**Conclusion**

Based on above results it is concluded that Ethyl Acetate leaf extract of *Pterocarpus marsupium* showed strong *in vitro* free radical scavenging effect in free system. Phytochemical and TLC studies showed that the leaf extract contain free radical scavenging molecules, such as amino acids, terpenoids, tannins, glycosides, flavonoids, alkaloids, amines, and other metabolites, which are rich in antioxidant activity.

**References**


