

Full Length Research Paper

Evaluation of antioxidant potential and antimicrobial studies of bark of medicinal plant, *Mallotus tetracoccus* (Roxb.) Kurz.

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The present study was aimed to investigate the total phenols, flavonoids, carotenoids, antioxidant activity and antimicrobial activity of the bark extract of *Mallotus tetracoccus* (Roxb.) Kurz. Total phenols, flavonoids and carotenoids in the extract were found to be 55.35 ± 0.13 mg GAE, 260.3053 ± 1.413 mg QE and 0.182 ± 0.005 mg/g, respectively. The reducing power and metal chelating activity of the bark extract was said to be increasing with increasing concentration. The metal chelating activity (IC_{50}) of the *M. tetracoccus* bark extract was found to be 647.4 ± 0.321 μ g/ml, which was higher compared to the standard, ethylenediaminetetraacetic acid (EDTA; 1.89 ± 0.03 μ g/ml). Antioxidant activity was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric thiocyanate (FTC) and thiobarbituric acid (TBA) methods. The antioxidant activity (IC_{50}) of the bark extract was said to be 0.504 ± 0.002 μ g/ml by (DPPH) method. The total antioxidant activity of *M. tetracoccus* ethanolic bark extract was found to be 275.21 ± 0.21 mg equivalent of ascorbic acid/100 g of plant extract as determined by phosphomolybdenum method. The antibacterial studies of the ethanolic bark extract was also tested at different concentration of extracts, where 250 mg/ml concentration of extract showed good inhibitory activity against all the clinically important disease pathogens compared with standard antibiotics like streptomycin and penicillin. The highest inhibition was noted in order of *Klebsiella pneumonia* (25.33 ± 0.37 mm), *Staphylococcus aureus* (22.83 ± 0.31 mm), *Vibrio cholerae* (20.00 ± 0.31 mm) and *Escherichia coli* (20.22 ± 0.26 mm). Thus, the bark extract of *M. tetracoccus* was said to possess significant antioxidant and antimicrobial activity, which suggests its use in traditional medicine.

Key words: *Mallotus tetracoccus*, antioxidant, 2,2-diphenyl-1-picrylhydrazyl (DPPH), reducing power, phenols, phosphomolybdenum activity, ferric thiocyanate (FTC), thiobarbituric acid (TBA), biological activity.

INTRODUCTION

Medicinal plants are still used as radical scavengers to fight against many important diseases, such as cancer. The active phytochemical compounds present in the plants such as carotenoids, terpenoids, flavonoids, polyphenols, alkaloids, tannins and saponins, possess various activities which help in scavenging the free radicals produced during initiation or progression of diseases (Cragg and Newman, 2000). *Mallotus tetracoccus* (Roxb.) Kurz, found in Western Ghats of

India, is one of the medicinally important plants belonging to the family Euphorbiaceae. It is commonly known as "vatta kanni" in Tamil. *M. tetracoccus* (Roxb.) Kurz, are found in evergreen forests up to 1600 m. The common names include *Thavatta*, *Vatta*, *Vatta kumbil*, *Vetta kumbil* (malayalam) and *Uppale mara* (kannada). The trees grow up to 5 - 15 m tall, leaf blades are triangular-ovate or ovate, sometimes 1- or 2-lobate, 10 to 25 × 9 to 20 cm, leathery, abaxially brownish tomentose, adaxially glabrous, base obtuse or truncate (biotik.org).

Several species of the genus *Mallotus* are a rich source of biologically active compounds such as phloroglucinols, tannins, terpenoids, coumarins, benzopyrans and chalcones (Amakura and Toshida, 1996; Tanaka et al.,

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1998; Huang et al., 1999; Cheng and Chen, 1999; Wei et al., 2004; Ma et al., 2004; Likhitwitayawuid and Supudompol, 2005). The reported bioactivities of the extracts or the individual chemical constituents isolated from this genus include antipyretic (Chattopadhyay et al., 2002), anti-inflammatory, hepatoprotective (Kim et al., 2000), antioxidant and radical scavenging activities (Arfan et al., 2007). The active compounds present in the *M. tetraococcus* ethanolic leaf extract was studied by Ramalakshmi and Muthuchelian (2011). The gas chromatography-mass spectroscopy (GC-MS) analysis showed the presence of various chemical constituents such as bis(2-ethyl hexyl) phthalate (46.78%), 3-methyl-2-(2-oxopropyl) furan (13.31%), E-8-methyl-9-tetradecen-1-ol acetate (6.63%), octadecanoic acid, 2-oxo (4.46%) and longiborneol (2.39%). Thus, the leaf extract of *M. tetraococcus* have been classified as compounds of nature such as diesters (50%), alcohols (15%), alkanes (3%), sesquiterpenes (5%), terpenoids (13%), fatty acid (5%) and sugars (2.6%) (RamaLakshmi and Muthuchelian, 2011).

Although researches have reported on *M. tetraococcus* (Roxb.) Kurz. leaves, a literature search revealed no references to investigation on its bark. Thus, the objective of the study was to study the antioxidant and antimicrobial property of *M. tetraococcus* bark extract.

MATERIALS AND METHODS

Collection of plant material

The fresh bark of *M. tetraococcus* (Roxb.) Kurz. were collected from the Agasthiar Malai Reserved Forest, Western Ghats, South India and authenticated by the Director, Centre for Biodiversity and Forest Studies, Madurai Kamaraj University. The voucher specimens were deposited in the herbarium of Centre for Biodiversity and Forest Studies of our university (No.AM-07).

Chemicals

Folin-ciocalteu reagent, sodium carbonate, gallic acid, quercetin, aluminium chloride, potassium ferricyanide, potassium acetate, linoleic acid, ammonium thiocyanate, ferrous chloride, beta hydroxyl toluene, α -tocopherol, trichloroacetic acid, 2-thiobarbituric acid, 2,2-diphenyl-1-picryl-hydrazyl (DPPH), hydrogen peroxide, diethyl ether, potassium hydroxide, trichloroacetic acid, ferric chloride, ferrozine, ethylenediaminetetraacetic acid (EDTA), ammonium molybdate, ascorbic acid, sodium phosphate and Muller Hinton agar were obtained from Himedia, Merck, Sigma. All other reagents used were of analytical grade.

Preparation of extract

Fresh barks were shade dried, powdered and extracted with ethanol for 6 to 8 h using soxhlet apparatus, at a temperature of 80°C. The extract was then filtered through muslin, evaporated under reduced pressure and vacuum dried to get the viscous residue. The ethanolic extract of the bark was used for antioxidants and antimicrobial studies.

Determination of total phenols and flavonoids

The total phenolic content was determined according to the method described by Siddhuraju and Becker (2003). Aliquots of each extracts were taken in test tubes and made up to the volume of 1 ml with distilled water. Then 0.5 ml of Folin-ciocalteu phenol reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 40 min and the absorbance was recorded at 725 nm against the reagent blank. Total phenol contents were expressed in terms of gallic acid equivalent (mg/g of dry mass), which is used as a reference compound.

Flavonoid content was determined according to the aluminium chloride colorimetric method by Chang et al. (2002) with some modifications. Quercetin was used as a standard to make the calibration curve. The sample solution (0.5 ml) was mixed with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminium chloride hexahydrate, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. After incubation at room temperature for 40 min, the absorbance of the reaction mixture was measured at 415 nm. The same amount (0.1 ml) of distilled water substituted for the amount of 10% aluminium chloride as the blank and a seven point standard curve (0 to 500 μ g/ml) was obtained.

Determination of carotenoids

Total carotenoids were determined by the method of Jensen (1978). One gram sample was extracted with 100 ml of 80% methanol solution and centrifuged at 4000 rpm for 30 min. The supernatant was concentrated to dryness. The residue was dissolved in 15 ml of diethyl ether and after addition of 15 ml of 10% methanolic KOH, the mixture was washed with 5% ice-cold saline water to remove alkali. The free ether extract was dried over anhydrous sodium sulphate for 2 h. The ether extracts were filtered and its absorbance was measured at 450 nm using ether as blank.

Antioxidant assay

The antioxidant activity of the plant extracts was tested using two methods: ferric thiocyanate (FTC) and thiobarbituric acid (TBA) methods.

Ferric thiocyanate (FTC) method

The standard method described by Kikuzaki and Nakatani (1993) was used. A mixture of 4.0 mg plant extract in 4 ml absolute ethanol, 4.1 ml of 2.5% linolenic acid in absolute ethanol, 8.0 ml of 0.05 M phosphate buffer (pH 7.0) and 3.9 ml of water was placed in a vial with a screw cap and then placed in an oven at 40°C in the dark. To 0.1 ml of this solution was added 9.7 ml of 75% ethanol and 0.1 ml of 30% ammonium thiocyanate. Precisely 3 min after addition of 0.1 ml of 0.02 M ferrous chloride in 3.5% HCl to the reaction mixture, the absorbance of red colour was measured at 500 nm each 24 h until the day after absorbance of control reached maximum. Beta hydroxyl toluene (BHT) and α -tocopherol were used as positive controls, while the mixture without plant sample was used as the negative control.

Thiobarbituric acid (TBA) method

The method of Ottolenghi (1959) was referred. Briefly, Two ml of 20% trichloroacetic acid and 2 ml of 0.67% 2-thiobarbituric acid was added to 1 ml of sample solution, as prepared in FTC method. The

Table 1. Total phenols, flavonoids and carotenoids contents of *Mallotus tetracoccus* bark extract.

Determination of Phyto-constituent	<i>Mallotus tetracoccus</i> bark extract
Total phenols	55.35 ± 0.13 mg GAE
Flavonoids	260.3053 ± 1.413 mg QE
Carotenoids	0.182 ± 0.005 mg/g

mixture was placed in a boiling water bath and after cooling was centrifuged at 3000 rpm for 20 min. Absorbance of supernatant was measured at 552 nm. Antioxidant activity was based on the absorbance on the final day of FTC method.

DPPH free radical scavenging activity

The free radical scavenging activity of the extracts, based on the scavenging activity of the stable 2,2-diphenyl-2-picrylhydrazyl (DPPH) free radical was determined by the method described by Braca et al. (2002). Plant extract (0.1 ml) was added to 3 ml of a 0.004% methanol solution of DPPH. Absorbance at 517 nm was determined after 30 min, and the percentage inhibition activity was calculated as $[(A_0 - A_1)/A_0] \times 100$, Where A_0 is the absorbance of the control and A_1 is the absorbance of the extract/ standard. A blank is the absorbance of the control reaction (containing all reagents except the test compound). A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was determined and represented as IC_{50} value for each of the test solutions.

Reducing power assay

Aliquots of each extracts were taken in test tubes and dissolved in 1 ml of 0.2 M phosphate buffer in a test tube to which was added 5 ml of 0.1% solution of potassium ferric cyanide (Siddhuraju et al., 2002). The mixture was incubated 50°C for 20 min. Subsequently, 5 ml of trichloroacetic acid (10%) (w/v) solution was added and the mixture was then centrifuged at 7000 rpm for 10 min. A 5 ml of aliquot of the upper layer was combined with 5 ml of distilled water and 1 ml of ferric chloride solution (0.1%) and absorbance was recorded at 700 nm against reagent blank. A higher absorbance of the reaction mixture indicates greater reducing power of the sample.

Metal chelating activity

The chelating of ferrous ions by *M. tetracoccus* (Roxb) Kurz. ethanolic bark extract was estimated by the method of Dinis et al. (1994). Briefly, the extract samples (250 µL) were added to a solution of 2 mmol/L $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mmol/L ferrozine (0.2 ml) and the mixture was shaken vigorously and left standing at room temperature for 10 min, after which the absorbance was measured spectrophotometrically at 562 nm. The chelating activity of the extracts was evaluated using EDTA as standard. The metal chelating activity of the extract is expressed as mg EDTA equivalent/g extract.

Phosphomolybdenum activity

The antioxidant activity of samples was evaluated by the green phosphomolybdenum complex formation according to the method of Prieto et al., 1999. An aliquot of 100 µl of sample solution was

combined with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in a 4 ml vial. The vials were canned and incubated in a water bath at 95°C for 90 min. After the samples have cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank.

The results were reported (ascorbic acid equivalent antioxidant activity) as mean values expressed in g of ascorbic acid equivalents/100g extract.

Test organisms

The ethanolic bark extract of *M. tetracoccus* (Roxb.) Kurz. was screened against four bacterial pathogens. The clinical isolates used for study are *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia* and *Vibrio cholerae*.

Antibacterial Screening

The different concentrations of the bark extracts (50, 100 and 250 mg/L) were tested for antibacterial activity using agar disc diffusion assay according to the method of Bauer et al. (1966). The strains of microorganisms obtained were inoculated in conical flask containing 100 ml of nutrient broth. These conical flasks were incubated at 37°C for 24 h and were referred to as seeded broth. Media were prepared using Muller Hinton Agar (Himedia, Mumbai, India), poured on Petri dishes and inoculated with the test organisms from the seeded broth using cotton swabs. Sterile discs of 6 ml width had been impregnated with 20 µL of test extract and introduced onto the upper layer of the seeded agar plate. The plates were incubated overnight at 37°C. Antibacterial activity was assigned by measuring the inhibition zone formed around the discs. The experiment was done three times and the mean values were presented. Streptomycin (10 µg/disc) and penicillin (10 µg/disc) were used as standards.

RESULTS

Determination of total phenols and flavonoids

The percentage yields of the ethanolic bark extract of *M. tetracoccus* (Roxb.) Kurz. were found to be 14.89%. Total phenolic content of the ethanolic extract of *M. tetracoccus* (Roxb.) Kurz. bark extract was 55.35 ± 0.13 mg gallic acid equivalent per gram of plant extract (Table 1). The flavonoid contents of the *M. tetracoccus* ethanolic bark extract was 260.305 ± 1.413 mg Quercetin equivalent per gram of plant extract (Table 1).

While the carotenoids contents of the *M. tetracoccus* ethanolic bark extract were found to be 0.182 ± 0.005 mg/g (Table 1).

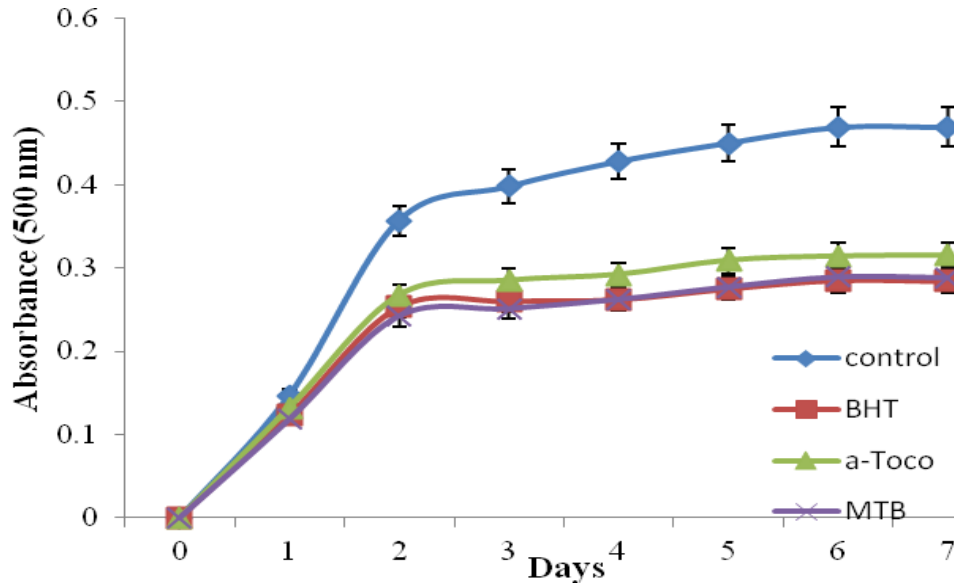


Figure 1. Antioxidant activity of the standards (α -tocopherol and beta hydroxy toluene) and *Mallotus tetraococcus* bark extract as measured by FTC method. Each experiment was executed in triplicate and repeated three times.

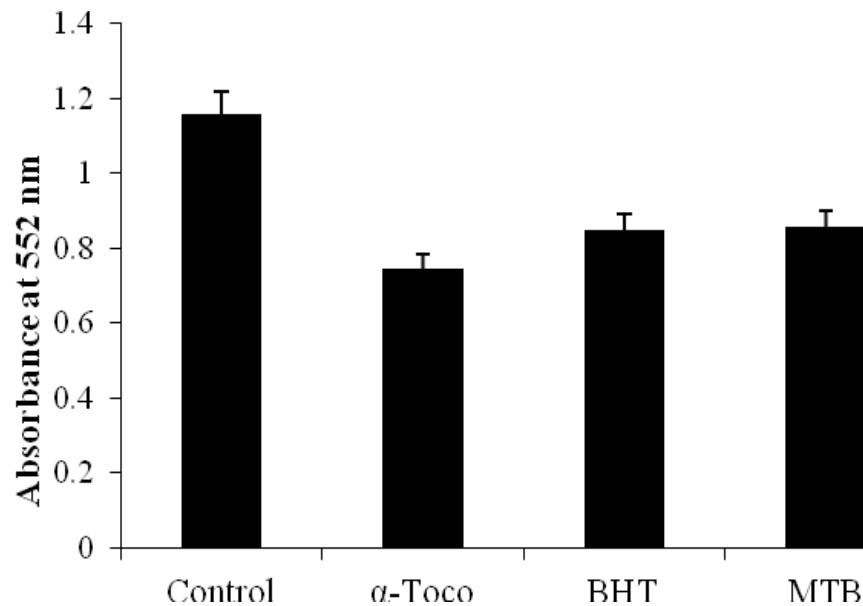


Figure 2. Total antioxidant activity of samples by TBA method.

Antioxidant assay

Ferric thiocyanate (FTC) method

As shown in Figure 1, the absorbance of the *M. tetraococcus* bark extract and beta hydroxyl toluene (BHT) at day 6 and 7 was significantly ($P < 0.05$) decreased when compared with the absorbance of standard (α -

tocopherol) at day 6 and 7. The control had the highest value throughout the study.

Thiobarbituric acid (TBA) method

Figure 2 shows the absorbance of standards and bark extract. The absorbance values of bark extract and

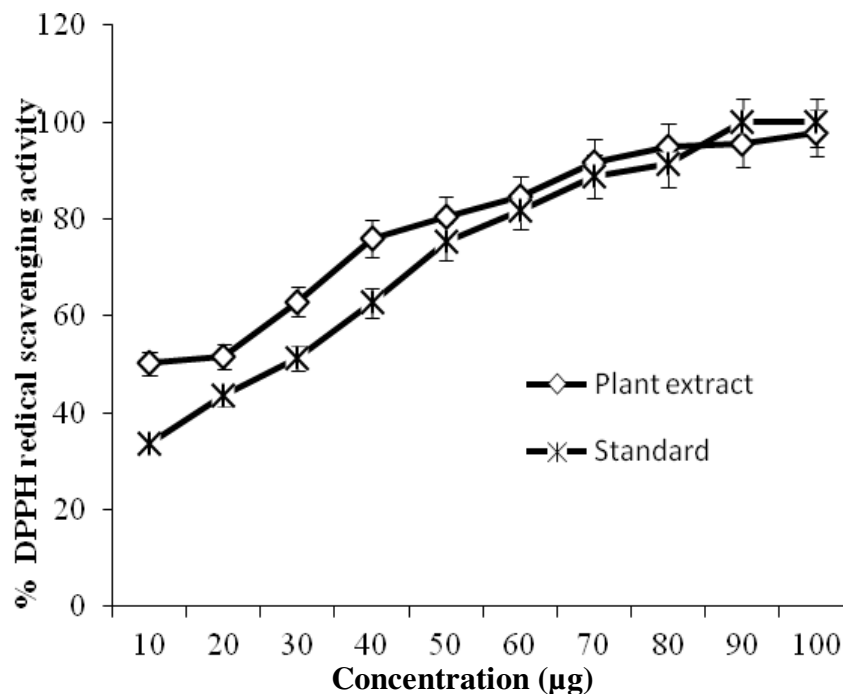


Figure 3. DPPH radical scavenging activity of *Mallotus tetracoccus* (Roxb.) Kurz. ethanolic bark extract.

standard BHT, where significantly higher than the α -tocopherol.

DPPH free radical scavenging activity

The radical scavenging activity of the *M. tetracoccus* ethanolic bark extract was tested using stable free radical DPPH (deep purple colour), as DPPH has the advantage of being unaffected by certain side reactions. Figure 3 shows the DPPH radical scavenging activity of *M. tetracoccus* with ascorbic acid as reference, where the IC_{50} values for the *M. tetracoccus* ethanolic bark extract ($0.504 \pm 0.002 \mu\text{g/ml}$) which was said to be significant than the standard ascorbic acid ($IC_{50} = 30.31 \pm 0.01 \mu\text{g/ml}$).

Reducing power assay

Figure 4 shows the reducing power of the *M. tetracoccus* ethanolic bark extract using potassium ferricyanide reduction method. The absorbance value of the extract shows higher increase with increase in concentration, when compared to standard ascorbic acid.

Metal chelating activity

Figure 5 shows the metal chelating activity of the *M.*

tetracoccus bark extract, where IC_{50} of plant extract was said to be $647.4 \pm 0.321 \mu\text{g/ml}$, which was higher compared to the standard, EDTA ($1.89 \pm 0.03 \mu\text{g/ml}$).

Phosphomolybdenum activity

The total antioxidant activity of *M. tetracoccus* (Roxb.) Kurz. ethanolic bark extract was found to be $275.21 \pm 0.21 \text{ mg}$ equivalent of ascorbic acid/100g of plant extract as determined by phosphomolybdenum method.

Antibacterial screening

The *M. tetracoccus* (Roxb.) Kurz. ethanolic bark extract showed good inhibition against both Gram positive and Gram negative organisms at higher concentration of 250 mg/L (Table 2). The highest inhibition was noted in order of *K. pneumonia* ($25.33 \pm 0.37 \text{ mm}$), *S. aureus* ($22.83 \pm 0.31 \text{ mm}$), *V. cholerae* ($20.00 \pm 0.31 \text{ mm}$) and *E. coli* ($20.22 \pm 0.26 \text{ mm}$).

DISCUSSION

The phenolic compounds present in natural products have higher antioxidant activity than synthetic antioxidants, also by acting as free radical terminators (Lu and Foo, 2000). The total phenolic content of the stem

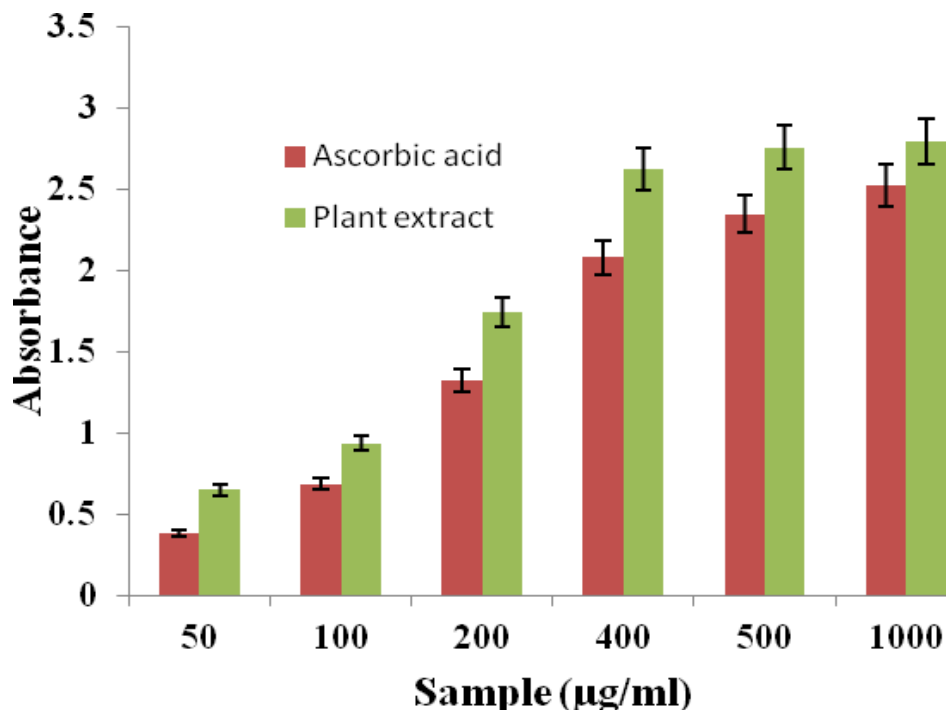


Figure 4. Reducing power of the *Mallotus tetracoccus* (Roxb.) Kurz. ethanolic bark extract.

bark of *Gmelina arborea* was $356 \pm 1.4 \text{ mg g}^{-1}$ (Patil et al., 2009). The bark extract of *Acacia confusa* showed total phenolic content of 470.6 mg/g , where the antioxidant activity by DPPH possessed IC_{50} values ranging from 1 to 5 µg/ml (Chang et al., 2001). *Chamaecyparis obtusa* various fractions of bark extract was also evaluated for antioxidant activity, and the total phenolic content varied from 27.71 to $102.86 \text{ mg GAE g}^{-1}$ dry weight for fractions (Marimuthu et al., 2008). The flavonoids possess antioxidant activity acting through scavenging or chelating process, thereby having considerable effect on human health and nutrition (Kessler et al., 2003). Since the bark extract of *M. tetracoccus* showed higher amount of phenolic compounds, this suggests their usage as a good source of natural antioxidant, preventing free radical-mediated oxidative damage.

In addition, carotenoids have a positive role on the epithelisation process and influence the cell cycle progression of the fibroblasts (Stiavala et al., 1996). Carotenoids act as photo-protective agents, reducing skin cancer, skin related diseases, photo-allergy and sun burns (Lee et al., 2000). The methanolic and aqueous bark extracts of *Mespilus germanica* L. exhibit 287.24 ± 14.5 , $457.07 \pm 22.3 \text{ mg g}^{-1}$; 33.73 ± 2.1 , $34.12 \pm 1.9 \text{ mg g}^{-1}$; 11.4 ± 0.8 , $10.7 \pm 0.6 \text{ gml}^{-1}$; 28.4% , 504 ± 34.5 (IC_{50}) for total phenol, total flavonoid, DPPH free radical scavenging and Fe^{2+} chelating ability, respectively (Nabavi et al., 2011).

Ferric thiocyanate (FTC) method

The ferric thiocyanate (FTC) results showed that *M. tetracoccus* bark extract had greater antioxidant activity when compared with α -tocopherol. The absorbance values for all the samples were maximal on the 6th day and then dropped at the 7th day due to malondialdehyde (MDA) compounds from linoleic acid oxidation, in which peroxide reacts with ferrous chloride to form a reddish ferric chloride pigment. The control had increasing absorbance values from 0 day until the absorbance reached the maximal level at 6 day and the absorbance value dropped at 7 day.

The reaction in the FTC method is due to the MDA compounds from the linoleic acid oxidation in which peroxide reacts with ferrous chloride to form a reddish ferric chloride pigment (Al-Naqeeb et al., 2009). Peroxides are slowly decayed to lower molecular compounds during the oxidation course (Behbahani et al., 2007). The bark and leaf extracts of *M. germanica* L. also exhibited higher antioxidant activity in the ferric thiocyanate (FTC) method showing higher peroxidation inhibition than vitamin C and BHA at the 96th hour ($p < 0.05$) (Nabavi et al., 2011).

Thiobarbituric acid (TBA) method

The TBA results were in agreement with the FTC results.

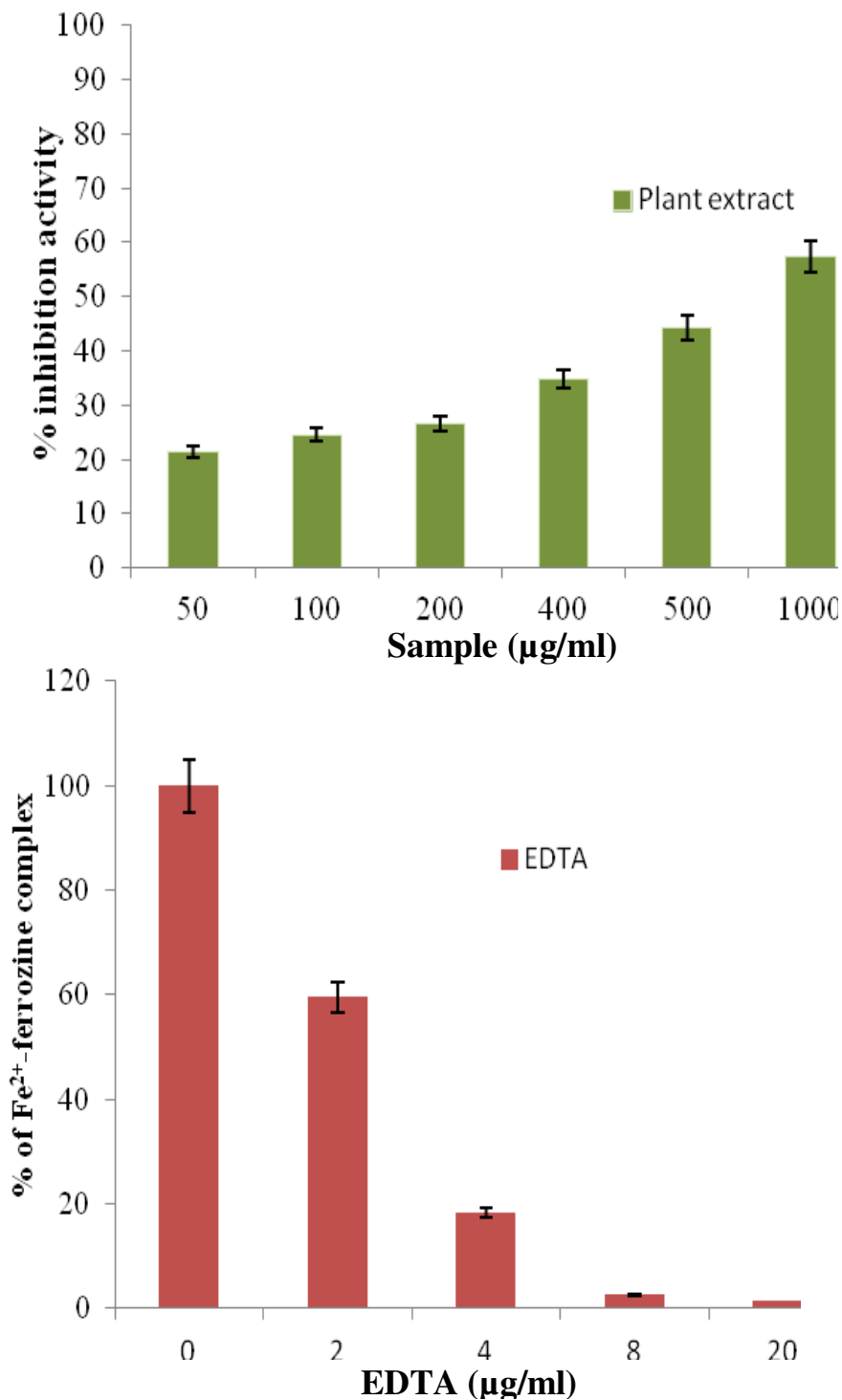


Figure 5. Metal chelating activity of *Mallotus tetracoccus* (Roxb.) Kurz. ethanolic bark extract.

After sometime, malonaldehyde a secondary product of lipid peroxidation changes into alcohol and acid. The alcohol and acid cannot be detected with a spectrophotometer (Rahmat et al., 2003). The FTC method

indicates the amount of peroxide in the initial stages of lipid peroxidation (Saha et al., 2004; Rahmat et al., 2003), whereas the thiobarbituric acid method shows the amount of peroxide in the secondary stage of lipid

Table 2. Antibacterial activities of ethanolic bark extract of *Mallotus tetracoccus*.

Microorganisms	Inhibition zone in diameter (mm)				
	Streptomycin	Penicillin	50 mg/L Extract	100 mg/L Extract	250 mg/L Extract
<i>Vibrio cholerae</i>	16.80 ± 0.87	19.34 ± 0.38	8.00 ± 0.35	19.21 ± 0.48	20.00 ± 0.31
<i>Escherichia coli</i>	14.70 ± 0.60	10.10 ± 0.25	17.00 ± 0.42	18.65 ± 0.33	20.22 ± 0.26
<i>Klebsiella pneumonia</i>	12.10 ± 0.25	17.60 ± 0.71	17.12 ± 0.43	20.34 ± 0.26	25.33 ± 0.37
<i>Staphylococcus aureus</i>	22.80 ± 0.35	23.10 ± 0.45	15.15 ± 0.34	20.55 ± 0.37	22.83 ± 0.31

Values are inhibition zone (mm), and an average of triplicate.

peroxidation (Rahmat et al., 2003). Therefore, the higher antioxidant activity found from the ferric thiocyanate method indicated that the amount of peroxide in the initial stage of lipid peroxidation was greater than the amount of peroxide in the secondary stage (Rezaeizadeh et al., 2011).

DPPH free radical scavenging activity

The antioxidants present in the extract quenches the DPPH free radicals (by providing hydrogen atom or by electron transfer, conceivably via a free radical attack on the DPPH molecule) and convert them to a colourless product (2, 2-diphenyl-1-picrylhydrazyl, or a substituted analogous hydrazine), resulting in a decreasing absorbance at the 517 nm (Yamaguchi et al., 2002). The antioxidative effect of the bark extract in these studies may be due to the presence of thiocyanic acid, furfural and 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl compounds (unpublished results). In addition, antioxidative activities observed in the extracts could be the synergistic effect of more than two compounds that may be present in the plant. It has been reported that most natural antioxidative compounds often work synergistically with each other to produce a broad spectrum of antioxidative activities that creates an effective defence system against free radical attack (Lu and Foo, 1995).

Stem bark extract of *M. germanica* L. (aqueous and methanol) showed best activity in 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity with $IC_{50} = 10.7 \pm 0.6$ and $11.4 \pm 0.8 \mu\text{gml}^{-1}$, respectively (Nabavi et al., 2011). The alcoholic extract of stem bark of *Terminalia arjuna* showed potent antioxidant activity with EC_{50} value of 2.491 ± 0.160 (Viswanatha et al., 2010). Stem bark of *Gmelina arborea* studied for antioxidant activity also possessed IC_{50} values of extract and standard were 46.2 ± 1.2 and 28.80 ± 0.50 , respectively (Patil et al., 2009). Ethyl acetate extract of *Tectona grandis* bark showed very high activity with 98.6% inhibition against DPPH and ABTS+ free radicals (Krishna et al., 2010). Moreover, the Ethyl acetate soluble fraction of *Chamaecyparis obtusa* bark was found to be the best antioxidant-rich fraction in terms of DPPH (0.15 to 0.26 mmol L⁻¹ Trolox equivalents) and reducing power

assays (Marimuthu et al., 2008).

Reducing power assay

The yellow colour of test solution changes to various shades of green and blue due to the reduction of Fe³⁺/ Ferric cyanide complex to ferrous form by the antioxidants present in the extract. Thus, the reducing power of medicinal plants and vegetables are said to be well associated with the antioxidant activity and its phenolic constituents (Siddhuraju and Becker, 2003). The leaves and bark extracts of *M. germanica* L. also showed good reducing power than fruit extract (Nabavi et al., 2011, 2003).

Metal chelating activity

In this metal chelating activity, the presence of chelating agents in the extract of *M. tetracoccus* disrupts the ferrozine-Fe²⁺ complex formation, thus decreasing the red colour. It is reported that chelating agents are effective as secondary antioxidants as they stabilise the oxidised form of the metal ion by reducing the redox potential (Gardner et al., 2000). Iron can generate free radicals from peroxides and may be implicated in human cardiovascular disease (Halliwell and Gutteridge, 1990). Bivalent transition metal ions play an important role as catalysts of oxidative processes, leading to the formation of hydroxyl radicals and hydroperoxide decomposition reactions via Fenton chemistry (Halliwell, 1997). These processes are delayed by iron chelators, which mobilize tissue iron by forming soluble and stable complexes (Nabavi et al., 2011). Ferrozine can quantitatively form complexes with Fe²⁺. In the presence of other chelating agents, the ferrozine complex formation is disrupted, resulting in a decrease in the red color of the complexes (Ebrahimzadeh et al., 2008).

Phosphomolybdenum activity

This method is based on the formation of green phosphomolybdenum complex at 95°C measured at an

intensity of absorbance at 695 nm. In this method, reduction of Mo (VI) to Mo (V) by the antioxidant compounds present in the plant extract, forming green phosphate/Mo (V) complex takes place (Sowndhararajan et al., 2010).

Antibacterial screening

The different fractions of methanolic, ethanolic and aqueous extracts of stem bark of *Acacia leucophloea* was studied for their antimicrobial activity against different bacterial strains and fungal strains. Methanol extract exhibited a broad spectrum activity on all strains also high antioxidant activity due to the presence of various phytochemicals (Anjaneyalu et al., 2010). The isolated compounds of stem bark of *Garcinia malaccensis*, α - and β -mangostin and cycloart-24-en-3 β -ol showed high cytotoxic, antioxidant and antibacterial activity (Taher et al., 2012). In addition, the aqueous and alcoholic extract of stem bark of *Cassia fistula* was tested for their antibacterial activity. Alcoholic extract (7.0 to 12.0 mm) showed greater inhibition against *S. aureus* compared to aqueous extract (7.0 to 11.6 mm) (Vimalraj et al., 2009). The stem bark *Manilkara zapota* was also evaluated for *in vivo* antitumor activity against EAC in Swiss albino mice, and the ethyl acetate fraction was said to possess significant antitumor activity (Osman et al., 2011).

Furthermore, the bark extracts of *Bauhinia purpurea* were phytochemically analyzed and evaluated for antimicrobial and antioxidant activities. Among the different solvent extracts, aqueous and methanolic extract exhibited a broad spectrum of antimicrobial (antibacterial and antifungal) activity and 80 to 90% antioxidant activity in terms of DPPH radical scavenging activity (Avinash et al., 2011). The antimicrobial and phytotoxic activities of the crude methanolic extract and its subsequent solvent fractions of *Pistacia integerrima* bark were investigated. The outstanding activity was shown by the ethyl acetate fraction followed by aqueous fraction against *S. aureus* having zone of inhibition of 19 and 15 mm, respectively (Rahman et al., 2011). The antibacterial activity of all extracts from *Tectona grandis* (Teak) were also checked against important hospital strains by disc diffusion assay, where chloroform extract showed inhibition to growth of *S. aureus* (14 mm) and *K. pneumoniae* (8 mm), and also a high cytotoxicity against chick embryo fibroblast (CEF) and human embryonic kidney (HEK 293) cells with 87 and 95.3% inhibition, respectively (Krishna et al., 2010).

Conclusion

The present study indicates that the ethanolic bark of *M. tetraococcus* (Roxb.) Kurz. is high in phenolic and flavonoid content. Also, the results of scavenging activities observed against DPPH, reducing power, phosphomolybdenum activities, metal chelating shows

that *M. tetraococcus* (Roxb.) Kurz. provide promising natural sources of antioxidants suitable for preventing free radical-mediated diseases. The extract also showed high antibacterial against important clinical bacterial isolates. Further studies are needed to explore the potential phenolics and flavonoid compounds from *M. tetraococcus* bark for application in drug delivery, nutritional or pharmaceutical fields.

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