Journal of Research in Biology

An International Scientific Research Journal

ISSN No: Print: 2231 –6280; Online: 2231- 6299

Original Research

Physicochemical, phytochemical and antioxidant studies on leaf extracts of *Mallotus tetracoccus* (Roxb.) Kurz

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ABSTRACT:

In this study quantitative determination of active phytochemicals, physicochemical properties and antioxidant studies of various extracts of *Mallotus tetracoccus* leaves were studied. The *Mallotus tetracoccus* leaf powder was extracted with different organic solvent such as petroleum ether, chloroform, ethyl acetate, acetone and aqueous ethanol. The total phenols and flavonoids content of various organic extracts were determined by folin-cicalteu method and aluminium chloride method. Other antioxidant activities were determined by DPPH free radical scavenging assay, FTC, TBA and phosphomolybdenum methods. Among the various extracts of *Mallotus tetracoccus* leaves, ethanol extract possessed high total phenolic contents (175.67±1.1 mg GAE/g). The reducing power assay values were in the range of 0.013±0.002 to 1.766±0.017 for the extracts with increasing concentrations. Thus the antioxidant activity of different extracts was in the order of ethanol > acetone > ethyl acetate > chloroform > petroleum ether extract. The findings showed that, among the various extracts of *Mallotus tetracoccus*, the organic polar extracts possessed high phenols and antioxidant activities.

Keywords:

Mallotus tetracoccus, Antioxidant, DPPH, Total phenols, Phosphomolybdenum activity.

Article Citation:
Ramalakshmi Subbiah and Muthuchelian Krishnaswamy
Physicochemical, phytochemical and Antioxidant studies on leaf extracts of *Mallotus tetracoccus* (Roxb.) Kurz
Journal of Research in Biology (2018) 8(5): 2518-2528

Dates:
Received: 04 May 2018 **Accepted:** 17 June 2018 **Published:** 23 July 2018

Web Address:
http://jresearchbiology.com/documents/RA0661.pdf

Journal of Research in Biology

INTRODUCTION

Medicinal plants due to the presence of different active phytochemical compounds effectively controls various diseases and also in scavenging free radicals (Cragg and Newman, 2000). *Mallotus tetracoccus* (Roxb.) Kurz. In-habitant of Western Ghats of India, commonly known as "*vatta kanni*" in Tamil. There has been several reports with reference to the activities of the genus *Mallotus* such as antipyretic (Chattopadhyay *et al*., 2002), anti-inflammatory, hepatoprotective (Kim *et al*., 2002) and antioxidant and radical scavenging activities (Arfan *et al*., 2007). Active compounds reported for activities are phloroglucinols, tannins, terpenoids, coumarins, benzopyrans and chalcones (Amakura and Yoshida, 1996; Tanaka *et al*., 1998; Cheng *et al*., 1999; Ma *et al*., 2004)

The GC-MS analysis of *Mallotus tetracoccus* leaf extract revealed bis(2-ethylhexyl) phthalate (46.78 %) as a predominant component (Ramalakshmi and Muthuchelian, 2011). The bark extract of *Mallotus tetracoccus* was reported for higher phenolics, flavonoids and significant antioxidant potential (Ramalakshmi and Muthuchelian, 2012). Recently reports on nanodrug for treatment of breast cancer has been developed from the leaf extract of *Mallotus tetracoccus* (Ramalakshmi and Muthuchelian, 2016). Thus the objective was to study the antioxidant activities of different extracts of *Mallotus tetracoccus* leaf extract.

MATERIALS AND METHODS

Collection of plant materials

Healthy leaves of *Mallotus tetracoccus* were collected from Agathiyar malai Reserve Forest, Western Ghats, South India. It was then authenticated by Director, centre for biodiversity and forest studies, Madurai Kamaraj University, and the voucher specimens were deposited in the herbarium at the centre for biodiversity and forest studies of Madurai Kamaraj University (No.AM-03).

Extraction of plant material

The leaves were shade dried, powdered and packed in small packets and extracted with different solvents of increasing order of polarity namely petroleum ether (Pet ether), chloroform, ethyl acetate, acetone and 70% ethanol using soxhlet apparatus. The solvent extracts were filtered, concentrated, vacuum dried at 45°C for solvent removal and refrigerated in sterile bottles until use.

Physicochemical analysis

The determination of total ash, acid insoluble ash, water soluble ash, sulphated ash, loss on drying at 110°C were done following the procedure recommended in Indian Pharmacopoeia (1996).

Determination of total phenols and flavonoids

Siddhuraju and Becker (2003) method was followed for total phenolic content. To each extracts, folin-ciocalteu phenol reagent and sodium carbonate solution (20 %) were added, vortexed, placed in dark for 40 min and absorbance was measured at 725 nm against the reagent blank. Total phenol contents are expressed in terms of gallic acid equivalent (mg/g of dry mass).

Aluminium chloride colorimetric method Chang *et al*. (2002) was carried for flavonoid analysis. Quercetin was used as a standard to make the calibration curve (0-500 µg/mL). To test samples 95 % ethanol, 10 % aluminium chloride hexahydrate, 1 M potassium acetate and water was added, incubated for 40 min and absorbance measured at 415 nm.

Determination of carotenoids

Jensen (1978) method was done for determination of total carotenoids. The extracts were treated with 80% methanol, dissolved in diethyl ether; 10% methanolic KOH, finally the mixture was rinsed with 5% ice-cold saline water to remove alkali. The free ether extract was dried, filtered and absorbance at 450 nm read by using ether as blank.

Antioxidant assay

Ferric Thiocyanate (FTC) method

In this method the plant extracts were mixed with 2.5 % linolenic acid, 0.05 M phosphate buffer (pH 7.0) and water was placed in a vial placed in an oven at 40˚C in the dark (Kikuzaki and Nakatani, 1993). To this solution, 75% ethanol and 30% ammonium thiocyanate were added and after 3 min, 0.02 M ferrous chloride in HCl was added and red colour was measured at 500 nm. BHT and α- tocopherol were used as positive controls.

Thiobarbituric Acid (TBA) method

To the plant extracts, 20 % trichloroacetic acid and 0.67 % 2-thiobarbituric acid was added, placed in a boiling water bath, cooled, centrifuged at 3000 rpm for 20 min and read at 552 nm (Ottolenghi, 1959). Antioxidant activity was based on the absorbance on the final day of FTC method.

DPPH free radical scavenging activity

The stable 2,2-diphenyl-2-picrylhydrazyl (DPPH) free radical was used for analysing the radical scavenging activity (Braca *et al*., 2002). To plant extracts, 0.004 % methanol dissolved DPPH was added, and after 30 min read at 517 nm, and the percentage inhibition activity was calculated using the formulae,

$[(A_0–A_1)/A_0] \times 100$

where, A_0 is the absorbance of the control and A_1 is the absorbance of the extract/ standard. The IC_{50} value for each plant extracts were obtained by plotting the percent inhibition values versus concentration curve.

Reducing power assay

In this assay, to each of plant extracts, 0.2 M phosphate buffer, 0.1% potassium ferric cyanide (Siddhuraju *et al*., 2002) was added, incubated at 50°C for 20 min and 10 % trichloroacetic acid was added and centrifuged. To this solution water and 0.1% ferric chloride solution were added and absorbance was read at 700 nm against reagent blank. The reducing power of the plant extracts were studied by their absorbance values.

Metal chelating activity

To plant extract, 2 mmol/L $FeCl_2$ and 5 mmol/L ferrozine solution was added, mixed and absorbance read at 562 nm. The metal chelating activity of the plant extracts were denoted as milligrams of EDTA equivalent/g extract (Dinis *et al*., 1994).

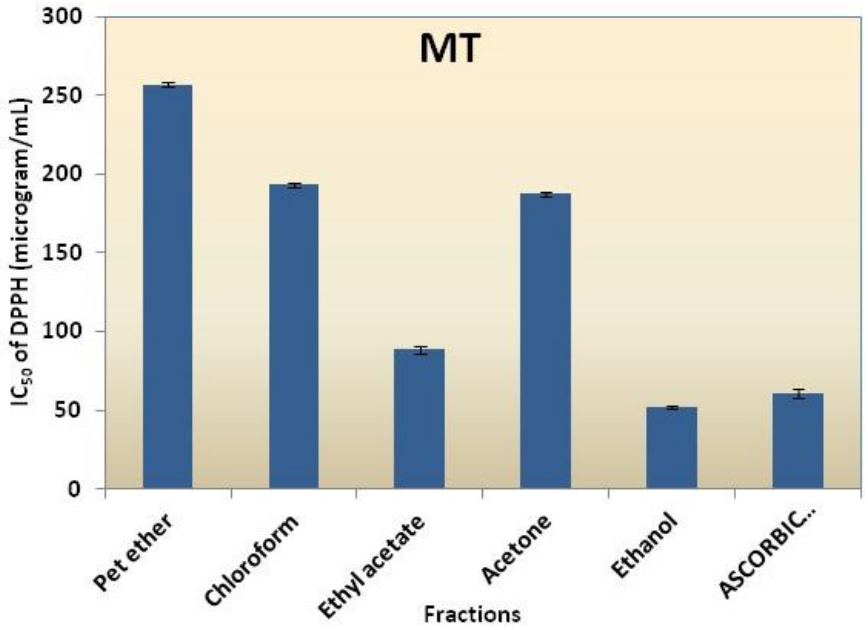

Figure 1. Radical scavenging activity (IC_{50}) values of different extracts of *Mallotus tetracoccus* leaf extracts

Table 1: Total phenols, flavonoids and carotenoids content, TBA inhibition values, metal chelating activity and total antioxidant activity of *Mallotus tetracoccus* leaf extracts

S. No	Plant extracts of *Mallotus tetracoccus*	Total phenolics (mg GAE/g)	Total Flavonoids (mg QE/g)	Carotenoids (mg/g)	TBA Percentage inhibition	Metal chelating activity IC_{50} (µg/mL)	Total antioxidant activity (mg AAE/ g extract)
1	**Pet ether**	56.99 ± 1.56^{a}	27.25 ± 1.49^{a}	16.93 ± 1.87^{a}	46.31 ± 0.716^{a}	257.1 ± 3.38^{a}	97.31 ± 1.84^{a}
2	**Chloroform**	82.55 ± 2.29^{b}	43.64 ± 0.79^{b}	52.08 ± 1.26^{b}	46.99 ± 2.010^{a}	288.9 ± 1.29^{b}	112.71 ± 1.92^{b}
3	**Ethyl acetate**	102.16 ± 1.39^{c}	52 ± 1.35^{c}	23.39 ± 1.31^{c}	48.49 ± 0.332^{b}	352.4 ± 1.54^{c}	137.2 ± 1.78^{c}
4	**Acetone**	112.36 ± 1.68^{d}	78.17 ± 2.06^{d}	14.49 ± 1.66^{a}	47.28 ± 0.529^{b}	305.97 ± 1.77^{d}	208.1 ± 1.66^{d}
5	**Ethanol**	175.67 ± 1.1^{e}	55.78 ± 1.57^{c}	11.86 ± 1.66^{a}	58.52 ± 0.856^{c}	416.71 ± 1.93^{e}	141.34 ± 1.08^{c}

Phosphomolybdenum activity

To the plant extracts, 1 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) were added, incubated at 95°C for 1.5 h cooled and read at 695 nm against a blank (Prieto *et al*., 1999). The phosphomolybdenum values are expressed as g of ascorbic acid equivalents/100g extract.

Statistical analysis

The values are presented as mean ± SD (standard deviation) of triplicate measurements. Multiple comparisons between more than two groups were performed by one way ANOVA supplemented with Duncan's multiple range post hoc tests. Values at ($P<0.05$) were considered to indicate statistical significance. The Pearson correlation analysis was performed to determine the correlation between various factors (SPSS, 2017).

RESULTS

The extracts of petroleum ether, chloroform, ethyl acetate, acetone and ethanol of *Mallotus tetracoccus* were (25.3, 13.72, 12.87, 21.01 and 27.01 %) respectively. The maximum yield was obtained in aqueous ethanol compared to other solvents which can be due to the presence of high level of polar compounds than non polar compounds.

Physicochemical studies

The moisture content of *Mallotus tetracoccus* was found to be 0.063±0.004 g, whereas the water, acid insoluble ash and total ash were found to be 0.104±0.005 g, 0.046±0.003 g and 0.195±0.002 g respectively. Thus the powder of *Mallotus tetracoccus* were found to contain high ash content. The purity and quality of the crude drug is studied based on the ash values. Important parameters in pharmacognostic evaluation of drug includes inorganic radicals such as silicates of sodium, potassium, calcium etc., and inorganic components such as oxalates, silica etc., in the

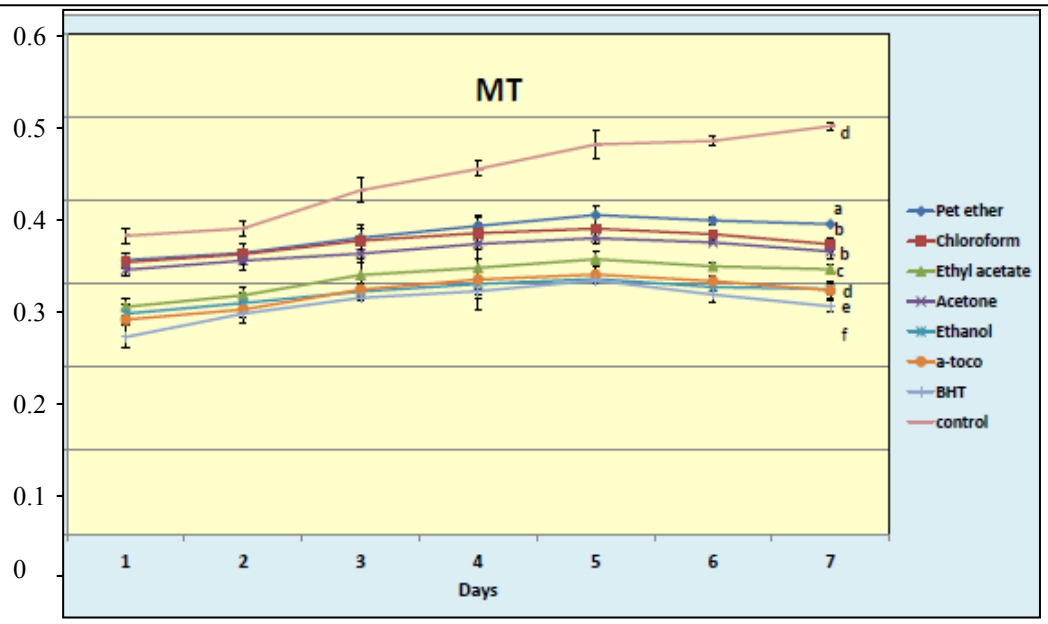

Figure 2. Antioxidant activity of various extracts of *Mallotus tetracoccus* leaf by ferric thiocyanate method

ash.

Determination of total phenols and flavonoids

Among the five extracts of *Mallotus tetracoccus*, ethanolic extract (175.67±1.1 mg GAE/g) showed the highest TPC, followed by acetone (112.36±1.68 mg GAE/g), ethyl acetate (102.16±1.39 mg GAE/g), chloroform (82.55±2.29 mg GAE/g) and pet ether (56.99±1.56 mg GAE/g) (Table 1). The total flavonoid contents of the various extracts of *Mallotus tetracoccus* varied from 27.25±1.49 to 78.17±2.06 mg/g (Table 1).

Determination of carotenoids

The carotenoids content of *Mallotus tetracoccus* are given as mg/g (Table 1). Among the carotenoids values of *Mallotus tetracoccus* extracts, chloroform extract (52.08±1.26 mg/g) possessed high value followed by extracts of ethyl acetate (23.39±1.31 mg/g), petroleum ether (16.93±1.87 mg/g), acetone (14.49±1.66 mg/g) and ethanol (11.86±1.66 mg/g) respectively (Table 1).

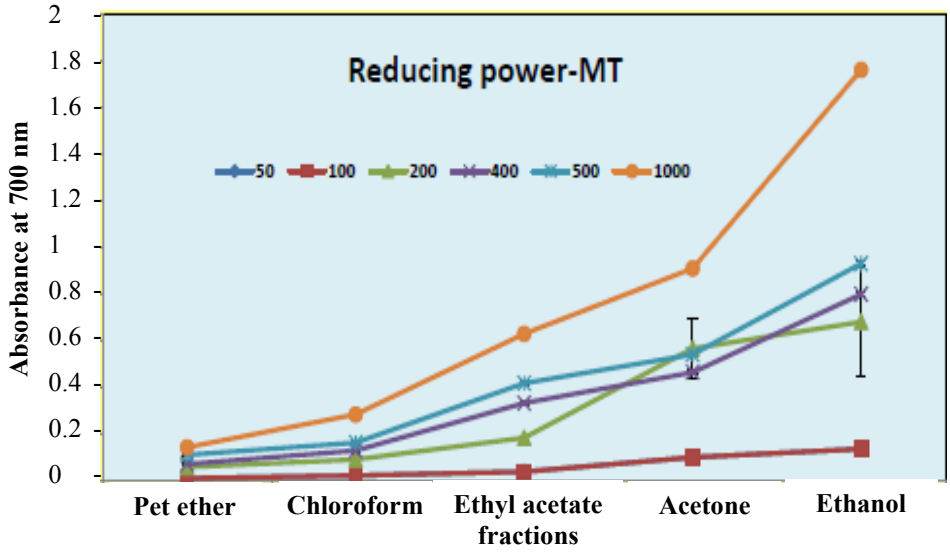

Figure 3. Reducing power activity of different extracts of *Mallotus tetracoccus* leaf

Table 2. Showing correlation (r) values between various parameters of *Mallotus tetracoccus*

S. No	Correlation between important activity parameters	*Mallotus tetracoccus*
1.	Total phenols and total antioxidant activity	+0.3894
2.	Total phenols and reducing power	+0.9898
3.	Total phenols and TBA lipid peroxidation	+0.9244
4.	Total flavonoids and total antioxidant activity	+0.9736
5.	Total flavonoids and reducing power	+0.5266
6.	Total flavonoids and TBA lipid peroxidation	+0.1715

Antioxidant assays

DPPH radical scavenging activity

The IC_{50} of standard (61.22±3.03 µg/mL), ascorbic acid was compared with extracts, where the ethanolic extract (51.74±0.97 µg/mL) showed higher antioxidant activity Figure 1 showing its effectiveness as radical scavenger. With high value of IC_{50} denotes lower antioxidant activity. The active compounds such as antioxidants reduce the DPPH free radicals to colourless product (2,2 -diphenyl-1- picrylhydrazyl, or a substituted analogous hydrazine) giving low absorbance read at 517 nm. The study reports on various extracts of *Mallotus tetracoccus* shows that the polar fraction has high DPPH activity.

Ferric Thiocyanate (FTC) method

The ability of the plant extracts to inhibit Lipid Per-Oxidation (LPO) was studied by FTC and TBA methods. The Ferric Thiocyanate (FTC) results for *Mallotus tetracoccus* are shown in Figure 2. The absorbance values of the ethanolic (0.296±0.005; 0.294±0.007) extract of *Mallotus tetracoccus* at 6th and 7th days were similar. During linoleic acid oxidation, at 5th and 6th day's. The values were found to be reduced which can be because of the malondialdehyde and peroxide reacts with ferrous chloride to form a reddish ferric chloride pigment. The control (0.489±0.003) had the highest value throughout the study.

Thio Barbituric Acid (TBA) method

The absorbance values of the ethanolic (0.753±0.016) extract were significantly lower than the standard BHT (0.871±0.004) (Table 1). The standard α-tocopherol had the highest percent inhibition value of (58.85±0.434%) compared to BHT (53.27±0.189%), ethanolic extracts (58.52±0.856 %) and ethyl acetate extracts (48.49±0.332 %) (Table 1).

Reducing power assay

Concentration dependency of antioxidant activity was found as a function of reducing power (Figure 3). The reducing capacities of various extracts of *Mallotus tetracoccus* were found to be in a dose-dependent manner (50 to 1000 µg/mL). The reducing power values of ethanolic extract were found to be highest when compared to the other extracts of *Mallotus tetracoccus*. The antioxidants present in the plant extracts reduce the Fe^{3+}/ Ferric cyanide complex resulting in the formation of green and blue colour indicating the high reducing power activity. Among various extracts of *Mallotus tetracoccus*, polar extracts possessed high absorbance values than others.

Metal chelating activity

The metal chelating activity of the various extracts of *Mallotus tetracoccus* leaves were shown in Table 1. The results for all the extracts showed an increase in metal chelating activity with increase in concentration. Among the extracts studied, the ethanolic extract (416.71±1.93 µg/mL) and ethyl acetate fraction (352.4±1.54 µg/mL) of *Mallotus tetracoccus* displayed high metal chelating activity (Table 1). The metal chelating activity was observed in the order of petroleum ether < chloroform < acetone < ethyl acetate < ethanol respectively.

Phosphomolybdenum activity

The acetone extract of *Mallotus tetracoccus* possessed the highest antioxidant activity 208.1±1.66 mg AAE (Table 1). BHT showed the value of 265.89±1.26 mg AAE. Highest antioxidant activities exhibited in order of acetone > ethanol > ethyl acetate > chloroform > petroleum ether respectively (Table 1). In this assay there is formation of green phosphate/Mo (V) complex due to the reduction of Mo (VI) to Mo (V) by the activity of plant extracts measured at an intensity of 695 nm.

Correlation between antioxidants and other assays

Table 2 shows the various correlation values (r value) between total phenolics, total flavonoids and various antioxidant activities exhibited by the samples. If the r value is between 0-0.2, it indicates very low or no correlation; 0.2-0.4 indicates low correlation; 0.4-0.6 indicates moderate correlation; 0.6-0.8 indicates high correlation and 0.8-1.0 values indicates very high correlation between the parameters analysed. If the values are positive there is positive correlation and when the values are negative the correlation is said to be negative between the two parameters studied.

DISCUSSION

The phenolics and flavonoids have got much attention in the day to day life due to their anti-mutagenic, anti-tumor and anti-oxidant activities. The present study findings showed that polar extracts possess high TPC and flavonoids similarly reported by other studies too. The Indonesian medicinal plant, *Phyllanthus niruri* studied showed that the ethanolic fraction (863.3 mg GAE/g) had highest TPC than water and methanolic extracts (Nurcholis *et al*., 2012). Of the *Synadium grantii* fractions, the TPC content was highest in the order of methanolic (40.21±0.1 mg GAE/g), ethyl acetate (35.62±0.55 mg GAE/g), ethanolic (33.15±0.25 mg GAE/g) and hexane (9.1±0.25 mg GAE/g) fractions (Dasari *et al*., 2012). Of the *Leea indica* leaf fractions, TPC contents were in the order of water (37.29 mg GAE/g), ethanolic (19.15 mg GAE/g), ethyl acetate (15.61 mg GAE/g) and hexane (1.27 mg GAE/g) (Suba *et al*., 2012). Among the solvents used, aqueous ethanol was found to be proficient to extract phenolics present in plant extracts.

For the extraction of flavonoids from the plant extracts of *Mallotus tetracoccus*, acetone had good solubility. Similarly the *Primula auriculata* leaf methanolic extract possessed high TPC (8.36 mg GAE/ g) and TFC (1.16 mg QE/g) than other fractions (Jaberian *et al*., 2013). Of the *Sonchus asper* fractions, the TFC was in the order of methanolic (11.4±0.45 mg QE/g), chloroform (8.66±1.9 mg QE/g), ethyl acetate (7.57±0.09 mg QE/g) and hexane (5.16±0.9 mg QE/g) (Khan *et al*., 2012).

DPPH radical scavenging activity

Similar reports have been reported by various researchers also showed that polar extracts mostly have high DPPH activity than other extracts. Of the *Synadium grantii* fractions, the DPPH activity was in order of ethyl acetate (211 µg/mL), methanolic (284 µg/ mL), ethanolic (424 µg/mL) and hexane (531 µg/mL) fractions (Dasari *et al*., 2012). The DPPH activity of *Leea indica* leaf fractions showed that highest activity was observed in water (48 µg/mL) and ethanolic extracts (60 µg/mL) than hexane extracts (1285 µg/mL) (Suba *et al*., 2012). Of the *Phyllanthus acidus* fractions, the high DPPH activity was in order of methanolic (86±1.03 µg/mL), ethyl acetate (28.6±0.72 µg/mL) and pet ether (117.4±1.33 µg/mL) respectively (Raja *et al*., 2012). Of the *Sonchus asper* fractions, the DPPH activity was in order of methanolic (2.5±0.05 µg/mL), chloroform (3.8±0.2 µg/mL), ethyl acetate (4.1±0.32 µg/mL) and hexane (12.2±1.43 µg/mL) (Khan *et al*., 2012).

Antioxidant assays

The ability of the plant extracts to inhibit lipid peroxidation was indicated by their low absorbance

values, particularly ethanol as solvent exhibited strong antioxidant activity. The quantity of peroxide in the primary and secondary stages of lipid peroxidation are measured by ferric thiocyanate and thiobarbituric acid methods respectively (Ramalakshmi and Muthuchelian, 2012). Thus the plant extracts of *Mallotus tetracoccus* results showed that the peroxides are formed low and stable in secondary stage of lipid peroxidation.

Metal chelating activity

Iron, catalyst in important oxidation reactions generates free hydroxyl radicals, peroxides, resulting in diseases (Halliwell, 1997). The oxidation reactions are stopped by iron chelators by forming soluble and stable complexes with available iron. The presence of chelating agents in the plant extracts interrupt with ferrozine, an iron chelator which complexes with Fe^{2+} effecting formation of red color. Thus among the various extracts of *Mallotus tetracoccus*, polar extracts exhibited high metal chelating activity, which is supported by study reports of other researchers. In *Phyllanthus acidus* extracts, the highest metal chelating activity was in the order of methanolic (121.7±1.39), ethyl acetate (178.3±2.01) and pet ether (159.7±1.98) respectively (Raja *et al*., 2012). In *Sonchus asper* extracts, the metal chelating activity was in order of methanolic (64±2.12 µg/mL), chloroformic (87.8±2.56 µg/mL), ethyl acetate (100.4±2.21 µg/mL) and hexane extracts (110.6±1.67 µg/mL) (Khan *et al*., 2012). Metal chelators such as presence of 7-quinolinol in the *Mallotus tetracoccus* extract might be responsible for metal chelating activity (Ramalakshmi and Muthuchelian, 2011).

Correlation between antioxidants and other assays

The correlation results for *Mallotus tetracoccus* extracts revealed high correlation among total phenols and reducing power and lipid peroxidation inhibition values by TBA method of the samples (r=0.9898; 0.9244). The correlation between total phenols and total antioxidant activity for the *Mallotus tetracoccus* plant was said to be low (r=0.3894), which may be due to the variations in the phenolics contents of plant extracts. But there is very high relationship between total flavonoids and total antioxidant activity and reducing power (r=0.9736; 0.5266). Over all the antioxidant of *Mallotus tetracoccus* extracts may be due to flavonoids and reducing activities due to the presence of total phenols present in them. Several authors have reported positive correlation between antioxidant activity and total phenols of the plant materials (Kalaivani and Mathew, 2010). The correlation values for *Sonchus asper* extracts showed that there is a high correlation between DPPH and TPC, TFC activity (r=0.9762; 0.8843). Similarly the correlation values for iron chelating activity with TPC and TFC showed high correlation (r=0.8101; 0.7657) (Khan *et al*., 2012). This study on correlation between different activities of plant extracts clearly reveals the antioxidant activity and presence of active photo chemicals.

Thus the overall activity of *Mallotus tetracoccus* extracts might be due to the presence of volatile terpenoid compounds such as longiborneol (2.39 %); p-menth-8(10)-en-9-ol (a terpene alcohol, 1.49 %) and fatty acid esters (48.11 %) such as bis(2-ethylhexyl) phthalate (46.78 %); di-n-octyl phthalate (1.33 %) analysed by GC-MS (Ramalakshmi and Muthuchelian, 2011). Also high phenolics and flavonoids can add to the antioxidant activities of the plant extracts. This shows that there is also synergistic effect of more than two compounds that are responsible for antioxidant properties (Lu and Foo, 1995).

CONCLUSION

From the various organic extracts of *Mallotus tetracoccus* leaf it may be concluded that the ethanolic extract possess significant phenolics, antioxidant and radical scavenging activity, suggesting it for further active compound isolation studies for application in biomedicine.

ACKNOWLEDGEMENT

The author Subbiah Ramalakshmi is thankful to University Grants Commission for providing meritorious fellowship (UGC-BSR) (Grant No. F.4-1/2006(BSR)/7-121/2007 (BSR)). The author also acknowledges the Dean and management of Karpagam Academy of Higher Education, Coimbatore for support and encouragement.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Amakura Y and Yoshida T. 1996. Tannins and related polyphenols of euphorbiaceous plants. XIV. Euphorbin I, a new dimeric hydrolyzable tannin from *Euphorbia watanabei*. *Chemical and Pharmaceutical Bulletin*, 44: 1293–1297.

Arfan M, Amin H, Karamać M, Kosińska A, Amarowicz R, Shahidi F and Wiczkowski W. 2007. Antioxidant activity of extracts of *Mallotus philippensis* fruit and bark. *Journal of Food Lipids*, 14(3): 280–297.

Braca A, Sortino C, Politi M, Morelli I and Mendez J. 2002. Antioxidant activity of flavonoids from *Licania licaniaeflora*. *Journal of Ethnopharmacology*, 79(3): 379-381.

Chang CC, Yang MH, Wen HM and Chern JC. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis*, 10(3): 178-182.

Chattopadhyay D, Arunachalam G, Mandal AB, Sur TK, Mandal SC and Bhattacharya SK. 2002. Antimicrobial and anti-inflammatory activity of folklore: *Mallotus peltatus* leaf extract. *Journal of Ethnopharmacology*, 82(2-3): 229–237.

Cheng XF, Chen Z and Zeng-Mu M. 1999. Two new diterpenoids from *Mallotus apelta* Muell. Arg. *Journal of Asian Natural Products Research*, 1(3): 319–325.

Cragg GM, Newman DJ. 2000. Antineoplastic agents from natural sources: achievements and future directions. *Expert Opinion on Investigational Drugs*, 9(12): 2783-2797.

Dasari NP, Ganga RB, Sambasiva RE, Mallikarjuna RT and Praneeth D. 2012. Quantification of phytochemical constituents and *in vitro* antioxidant activity of *Synadium grantii*. *Free Radical and Antioxidant*, 2(2): 68-72.

Dinis TC, Madeira VM and Almeida LM. 1994. Action of phenolic derivatives (acetoaminophen, salicilate and 5-aminosalicilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Archives of Biochemistry and Biophysics*, 315(1): 161-169.

Halliwell B. 1997. Antioxidants and human diseases: a general introduction. *Nutrition Reviews*, 55(1 Pt 2): S44 -S52.

Indian Pharmacopoeia. **1996.** 4th ed. Ministry of health and family welfare, Govt. of India, New Delhi; Controller of publications: 1996. p. A-80.

Jaberian H, Piri K and Nazari J. 2013. Phytochemical composition and in vitro antimicrobial and antioxidant activities of some medicinal plants. *Food Chemistry*, 136(1): 237–244.

Jensen A. 1978. Chlorophyll and carotenoids. In: hallebust JA, craigie JS. (eds). handbook of physiochemical and biochemical Methods. Cambridge University Press, Cambridge, UK, 5-70 p.

Kalaivani T and Mathew L. 2010. Free radical scavenging activity from leaves of *Acacia nilotica* (L.) wild. ex delile an Indian medicinal tree. *Food and Chemical Toxicology*, 48(1): 298-305.

Khan RA, Khan MR, Sahreen S and Ahmed M. 2012. Evaluation of phenolic contents and antioxidant activity of various solvent extracts of *Sonchus asper* (L.) Hill. *Chemistry Central Journal*, 6: 12.

Kikuzaki H and Nakatani N. 1993. Antioxidant effects of some ginger constituents. *Journal of Food Science,* 58(6): 1407–1410.

Kim HS, Lim HK, Chung MW and Kim YC. 2000. Antihepatotoxic activity of bergenin, the major constituent of *Mallotus japonicus*, on carbon tetrachloride-intoxicated hepatocytes. *Journal of Ethnopharmacology*, 69(1): 79–83.

Lu F and Foo LY. 1995. Phenolic antioxidant component of evening primrose. In: nutrition, lipids, health and disease; Ong, ASH, Niki E, Packer L, Eds, American Oil Chemists Society Press: Champaign, Illinois.

Ma J, Jones SH and Hecht SM. 2004. A coumarin from *Mallotus resinosus* that *mediates DNA cleavage. Journal of Natural Products, 67(9)*: 1614–1616.

Nurcholis W, Priosoeryanto BP, Purwakusumah ED, Katayama T and Suzuki T. 2012. Antioxidant, cytotoxic activities and total phenolic content of four Indonesian medicinal plants. *Journal of Kimia Valensi*, 2(4): 501-510.

Ottolenghi A. 1959. Interaction of ascorbic acid and mitochondrial lipids. *Archives of Biochemistry and Biophysics,* 79: 355–358.

Prieto P, Pineda M and Aguilar M. 1999. Spectrophotometric quantitative of antioxidant capacity through the formation of a phosphomolybednum complex: specific application to the determination of vitamin E. *Analytical Biochemistry*, 269(2): 337-341.

Raja C, Biplab De, Nayakanti D, and Saikat S. 2012. Antiinflammatory, antinociceptive and antioxidant activities of *Phyllanthus acidus* L. extracts. *Asian Pacific Journal of Tropical Biomedicine*, 2(2): S953-S961.

Ramalakshmi S and Muthuchelian K. 2011. Analysis of bio-active constituents from the leaves of *Mallotus tetracoccus* (Roxb.) Kurz by gas chromatography-mass spectrometry. *International Journal of Pharmaceutical Sciences and Research*, 2(6): 1449-1454.

Ramalakshmi S and Muthuchelian K. 2012. Evaluation of antioxidant potential and antimicrobial studies of bark of medicinal plant, *Mallotus tetracoccus* (Roxb.) Kurz. *Journal of Medicinal Plant Research*, 6 (38): 5156-5165.

Ramalakshmi S and Muthuchelian K. 2016. Development of nanodrug for treatment of breast cancer using *Mallotus tetracoccus* leaves - standardisation, synthesis and characterization. *Nano Biomedicine and Engineering*, 8(2): 82-90.

Siddhuraju P, Mohan PS and Becker K. 2002. Studies on the antioxidant activity of Indian Laburnum (*Cassia fistula* L.): a preliminary assessment of crude extracts from stem, bark, leaves and fruit pulp. *Food chemistry*, 79(1): 61-67.

Siddhuraju R and Becker K. 2003. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa olifera* Lam.) leaves. *Journal of Agricultural and Food Chemistry*, 51(8): 2144-2155.

SPSS. 2017. IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.

Suba RN, Navanesan S, Sinniah SK, Wahab NA and Sim KS. 2012. Phenolic content, antioxidant effect and cytotoxic activity of *Leea indica* leaves. *BMC*

Complementary and Alternative Medicine, 12: 128.

Tanaka T, Ito T, Iinuma M, Takahashi Y and Naganawa H. 1998. Dimeric chalcone derivatives from *Mallotus philippensis*. *Phytochemistry*, 48(8): 1423–1427.