Asian Journal of Chemistry; Vol. 23, No. 2 (2011), 857-862

ASIAN JOURNAL OF CHEMISTRY

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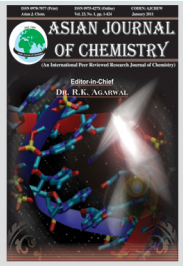

Foliar Phenolic Antioxidant Constitution of *Mallotus stenanthus*

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(*Received*: 28 May 2010; *Accepted*: 14 October 2010) AJC-9182

Cancer remains the second most abundant cause of death globally. Traditional herbal medicines possessing antioxidant potentials offer protection against several chronic and predominantly life-style oriented human diseases, including cancer. *Mallotus stenanthus*, which has been reported to be an anticancer agent against P388 lymphocytic leukaemia in mice, has been evaluated to possess *in vitro* antioxidant ($ABTS^{\cdot+}$ scavenging, FRAP, β-carotene bleaching) capacities and metal chelating abilities. The antioxidant capacities correlated well with the phenolic and flavonoid constitutions. The EtOAc fraction exhibited highest antioxidant capacity, 7-O-β-D-glucopyranosides of diosmetin and chrysoeriol together with ellagic acid, phloroglucinol and its derivatives have been isolated and characterized, substantiating the reported antitumour properties of the leaf extract.

Key Words: *Mallotus stenanthus*, Antioxidant capacity, Polyphenol, Flavone glycosides, Ellagic acid, Phloroglucinol.

INTRODUCTION

Traditional herbal medicines (TM) are regaining significant attention in global health debates. In China, traditional herbal medicines has played a prominent role in the strategy to contain and treat severe acute respiratory syndrome (SARS)[1]. Up to 80 % of the African populations use some form of traditional herbal medicines for health care needs[2,3]. In Asia and Latin America, people continue to use traditional herbal medicines as a consequence of historical circumstances and cultural beliefs and in China, traditional herbal medicines accounts for around 40 % of all health care delivered. In many parts of the world, expenditure on TM/CAM (complementary and alternative medicine) is not only significant, but growing rapidly. In Malaysia, an estimated US$ 500 million is spent annually on this type of health care, compared to about US$ 300 million on allopathic medicine[2] and it is hoped that the traditional herbal medicines research will continue to play a critical role in global health.

Mallotus stenanthus Mueller-Argoviensis (Fam. Euphorbiaceae; Vernacular: Solai karupichchi, Kaattupulasai, Karuvalichchi) is a dioecious tree of 10-15 m high, endemic to the sholas of the evergreen and semi-evergreen forests of the Western Ghats and Kalrayan Hills of the Eastern Ghats between 350 and 1800 m[4-6]. The aqueous ethanolic extract of the aerial parts of the medicinal taxon is reported to be a diuretic and an anticancer agent against P388 lymphocytic leukaemia in mice[7]. Oxidative stress and associated mechanisms involving inflammation, aberrant signaling pathways and gap junction intercellular communication have been reported to be increasingly associated with the pathogenesis of various chronic degenerative disorders, particularly, carcinogenesis, atherosclerosis, age-related neurodegenerative disorders, inflammation and immune disorders[8-10]. Oxygen is of vital importance to aerobic organisms and under controlled circumstances, say, inside mitochondria, endoplasmic reticulum, or peroxisomes, oxygen generally serve a metabolic purpose. However, even under normal circumstances, the electrons passing through the mitochondrial electron transport chain can leak out and combine with molecular oxygen to produce reactive oxygen species (ROS). Reactive oxygen species can cause damage at various sites in the cell, such as the membranes, the cytoplasm and the nucleus. Though human system has evolved with mechanisms to counteract these ROS and maintain a balance, numerous factors can adversely affect the balance in favour of excess ROS production, which results in oxidative stress[11]. Over the past three decades, there has been a significant increase in public and scientific interest to understand the beneficial effects of plant derived antioxidants that can effectively scavenge ROS. Phytophenols are the widespread class of antioxidants that have been studied extensively for their potential chemopreventive and therapeutic properties. Accumulating biochemical, clinical and epidemiological evidences support the chemoprotective potentials of phenolic antioxidants against oxidative stress-mediated disorders. Furthermore, cellular damage, as a result of ROS, is believed to be a causative factor in the process of ageing. The pharmacological actions of phenolic antioxidants stem mainly from their free radical scavenging and metal

chelating properties as well as their effects on cell signaling pathways and on gene expression. A survey of the literature has revealed only an isolated report of the characterization of an unsaturated triterpenoid, *viz*., 24,24-dimethyllanosata-7,25-dien-3-α-ol (mallotin) and sitosterol from the aerial parts of *M. stenanthus*[12]. This has prompted the investigation of the antioxidant capacity of the aqueous ethanolic extract of the leaves and to correlate the antioxidant capacity to its polyphenolic constitution, as an attempt to explore the potentially probable chemical constituents that may be responsible for the reported anticancer activity.

EXPERIMENTAL

Fresh leaves of *M. stenanthus* (600 g), were collected from the Vellimalai forest of the Kalrayan hills, located in the Villupuram District, India, during the month of June and the identity established. The leaves were dried in the shade, crushed and refluxed with 90 % EtOH (aqueous alcohol, 4.0 × 4.5 L, 6 h). The combined extracts were concentrated under reduced pressure. The aqueous alcoholic concentrate was then quantitatively fractionated into C_6H_6 (4.0 × 0.5 L), Et_2O (5 × 0.5 L) EtOAc (4 × 1.0 L) and EtCOMe (4 × 1.0 L) soluble residues. Leaving aside the deeply coloured non-phenolic C_6H_6 fraction, the other fractions were analyzed for their antioxidant/metal chelating capacities and the phenolic and flavonoid constituents contributing to the observed activities, as described in the latter sections of this report. A precisely weighed amount of the residues (*ca.* 50-100 mg), dissolved in MeOH and made up to the mark in 100 mL volumetric flasks, were used for the quantitative determinations.

2,2'-Azino*bis*(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) diammonium salt, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), 3-(2-pyridyl)-5,6-di(4-phenylsulphonic acid)-1,2,4-triazine (ferrozine) sodium salt, anhydrous ethanol (EtOH), ferrous chloride ($FeCl_2$) and β-carotene were obtained from Sigma-Aldrich Inc., acetone and methanol for chromatography/spectroscopy were procured from Merck Specialities Private Limited. All other chemicals/reagents were of analytical/laboratory grades from Himedia/Merck/Loba Chemie. Shimadzu UV-160 spectrophotometer was used for electronic spectral measurements and NMR spectral recordings were performed on Bruker DRX-500 spectrometer, using DMSO-d_6 solutions.

Determination of *in vitro* antioxidant capacity

ABTS radical cation scavenging activity: Determination of $ABTS^{\cdot+}$ scavenging activity was performed according to the improved $ABTS^{\cdot+}$ decolourizing assay of Re *et al.*[13]. Briefly, the chromogenic radical reagent ABTS (7.0 mM) was prepared by dissolving 0.1920 g of the compound in water and diluting to 50 mL. To this solution was added 0.0331 g $K_2S_2O_8$ such that the final persulphate concentration in the mixture was 2.45 mM. The resulting $ABTS^{\cdot+}$ solution was kept in a stoppered flask to mature at room temperature in the dark for 16 h. Prior to use in the assay, the concentration of the solution was adjusted by dilution with EtOH, so that the $ABTS^{\cdot+}$ blue-green chromogen with a characteristic absorption at 734 nm had an absorbance of 0.650 ± 0.020. The solution was then equilibrated at 30° C. Aliquots of the sample solutions and standard L-ascorbic acid solutions were diluted appropriately to provide 20-80 % inhibition of the blank [consisted of 50 % aqueous EtOH (20 µL) and diluted $ABTS^{\cdot+}$ (980 µL)] absorbance. The reaction was then initiated by the addition of the diluted $ABTS^{\cdot+}$ (980 µL) to each sample/standard solution (20 µL) and the mixture was allowed to stand at room temperature for 10 min, at the end of which the absorbance was measured immediately at 734 nm. Determinations were repeated three times for each sample solution.

Ferric-reducing/antioxidant power (FRAP) assay: The FRAP assay was carried out according to the procedure of Benzie and Strain[14]. The FRAP reagent was prepared from 300 mM sodium acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM hydrochloric acid and 20 mM ferric chloride solution in proportions of 10:1:1 (v/v/v), respectively. The FRAP reagent was prepared fresh daily and was warmed to 37 ºC in a water bath prior to use. An aliquot of the sample solution (50 µL) was added to the FRAP reagent (1.5 mL) and the absorbance of the reaction mixture was recorded at 593 nm after exactly 6 min against a blank, which consisted of 120 µL water and 900 µL reagent. The assay was carried out in triplicate for each determination.

Determination of vitamin C equivalent antioxidant capacity (VCEAC): The *in vitro* antioxidant capacity of the extract, determined by the above two assays, was expressed as vitamin C equivalent antioxidant capacity[15] (VCEAC). Vitamin C standard curves that relate the concentration of L-ascorbic acid (1-20 mg L^{-1}) and the amount of absorbance reduction (for $ABTS^{\cdot+}$ scavenging) or absorbance increase (for FRAP) caused by it were plotted. The absorbance reduction/increase produced by each fraction of the extract was then correlated to that of L-ascorbic acid standards using the standard curve and the results were expressed in mg vitamin C equivalents (VCE) 100 g^{-1} of the fresh leaves. All data were recorded as mean ± SD, computed from three replications.

Inhibition of β-carotene bleaching: The antioxidant activity, based on coupled oxidation of β-carotene and linoleic acid, was measured by the method of Pratt[16]. Linoleic acid (20 mg) and Tween 20 (200 mg) were taken in flasks and then solutions of β-carotene (2 mg in 10 mL of chloroform) were added. After removal of chloroform, distilled water saturated with oxygen (50 mL) was added. Aliquots (200 µL) of the extract fractions, dissolved in EtOH to give 15 µg mL^{-1} solutions, were added to the flasks with shaking. A blank without the addition of the extract and a control, *viz*., 2,6-di-*tert*-butyl-4-methylphenol (BHT), added as standard, were also prepared in separate flasks. Samples were subjected to oxidation by placing them in an oven at 50 ºC for 2 h. The absorbances were read at 470 nm at regular intervals. The antioxidant activity (AA) was expressed as inhibitory ratio, calculated using the relation, $AA = [1-(A_0 - A_t)/(A_{00} - A_{0t})] \times 100$, where, A_0 and A_t, respectively corresponded to the absorbances at the beginning of the incubation and at time t, with the sample, while A_{00} and A_{0t} similarly corresponded to the absorbance at the beginning of the incubation and at time t, without the sample.

Transition metal ion chelating capacity: Chelation of transition metal ions by the polyphenolic constituents of the extract fractions and standard were evaluated by determining

the chelating activity of Fe(II) by the method of Dinis *et al.*[17]. In brief, extract fractions containing 25 and 50 μg mL^{-1} (0.4 mL each)/standard were added to a solution of 2 mM $FeCl_2$ (0.05 mL). The reaction was initiated by the addition of 5 mM of ferrozine (0.2 mL) and the total volume was adjusted to 4.0 mL with MeOH. The mixture was then shaken vigorously and allowed to stand at room temperature for 10 min. Absorbance of the solutions were measured spectrophotometrically at 562 nm. All tests and analyses were run in triplicate. The percentage inhibition of the ferrozine-Fe(II) complex formation was calculated using the relation, Fe(II) chelating capacity (%) = $[(A_0 - A_1)/A_0] \times 100$, where, A_0 is the absorbance of the blank and A_1 is the absorbance in the presence of sample. The blank contained only $FeCl_2$, ferrozine and MeOH. The water soluble versatile hexadentate chelating ligand, disodium salt of ethylenediaminetetraacetic acid (Na_2EDTA), was used as positive standard.

Analysis of the polyphenolic constitution

Determination of total phenolics and flavonoids: The amount of the total phenolics in each of the fractions was determined by the spectrophotometric method[18] with minor modifications. An aliquot (1 mL) of appropriately diluted extract fractions/standard solutions of gallic acid monohydrate (10-100 mg L^{-1}) were added to 25 mL volumetric flasks, each containing 9 mL of distilled water. A reagent blank was prepared using distilled water. Folin-Ciocalteu phenol reagent (FCR, 1 mL) was added to the mixture and shaken. After 5 min, 7 % Na_2CO_3 solution (10 mL) was added with mixing. The solution was immediately diluted to volume (25 mL) with distilled water, mixed thoroughly and then allowed to stand for 90 min. The absorbance of the solutions *versus* prepared blank was measured at 765 nm. Total phenolic content of each fraction was determined from the standard curve and was expressed as mg gallic acid equivalent (GAE) 100 g^{-1} fresh leaves. The determination was repeated three times and the mean ± SD of the three measurements was taken. The total flavonoid concentration was measured using the colorimetric assay[19]. Appropriately diluted extract fractions (1 mL)/standard solutions of quercetin (10-100 mg L^{-1}) were added to 10 mL volumetric flasks, each containing 4 mL distilled water. A reagent blank was also prepared using distilled water. At time zero min, 5 % $NaNO_2$ (0.3 mL) was added to each flask. After 5 min, 10 % $AlCl_3$ (0.3 mL) was added and at time 6 min, 1 M NaOH (2 mL) was added to the mixture. The solutions were diluted to volume (10 mL) immediately with distilled water and then thoroughly mixed. The absorbances of the pink solutions were measured at 510 nm against the prepared blank and the samples were analyzed in triplicate. Flavonoid content of each fraction was expressed on a fresh weight basis as mg quercetin equivalent (QE) 100 g^{-1} using a standard quercetin calibration curve.

Isolation and characterization of the predominant polyphenolic metabolites: Phenolics are widely distributed in plants and constitute the most abundant secondary metabolites. Plant materials, in general, contain complex mixture of phenolics and these have been classified according to their structure as simple phenols and phenolic acids, phenylpropanoids, flavonoids and tannins. The antioxidant potentials of these plant phenolics have been shown to be superior to those of the other well-known antioxidants, such as vitamins C and E and β-carotene. As a result of the innumerable studies that have been concluded, which has established the abilities of the antioxidants to counteract oxidative stress in biological systems and thereby ameliorate several chronic human illness. There is not only considerable interest in the quantification of the antioxidants but also in determining the specific phenolic constituent that potentially contributes to the antioxidant capacities of medicinal plants. The Et_2O fraction, upon keeping in a refrigerator for a week, yielded a dark brown solid, characterized, as described in detail[20], as 1,3,5-trihydroxybenzene (phloroglucinol). From the mother liquor, two flavones were also isolated and have been characterized as 5,7,3'-trihydroxy-4'-methoxyflavone (diosmetin) and 5,7,4'-trihydroxy-3'-methoxyflavone (chrysoeriol). From the EtOAc fraction that was stored in the ice chest for two days, pale yellow crystals got separated and the identity was established as 2,3,7,8-tetrahydroxy-[1]benzopyrano[5,4,3-cde][1]-benzopyran-5,10-dione (ellagic acid/EA). The fraction also contained two flavone glycosides, characterized as 5,3'-dihydroxy-4'-methoxy-7-O-β-D-glucopyranosylflavone (diosmetin 7-O-β-D-glucopyranoside) and 5,4'-dihydroxy-3'-methoxy-7-O-β-D-glucopyranosylflavone (chrysoeriol 7-O-β-D-glucopyranoside). The latter two flavone glycosides were also identified as the major polyphenols of the EtCOMe fraction. The isolated metabolites have been characterized based on their chemical properties, chromatographic mobilities, fluorescence characteristics, analysis of the products of hydrolyses, UV/Vis and ^1H and ^{13}C NMR spectral features and direct comparison with authentic compounds, as described elsewhere[20].

RESULTS AND DISCUSSION

Determination of *in vitro* antioxidant capacity: It is well established that oxidative stress and oxidative damage of biomolecules in mammalian system are involved in several pathological or degenerative processes, including cancers, coronary heart diseases and stroke, age-related neurodegenerative disorders and immune dysfunctions. Traditional herbal medicines of the East have been shown to offer protection against these predominantly life-style oriented diseases of the present time because of their rich antioxidant constitution. Notable among these natural antioxidants are the ubiquitous plant phenolics that are commonly encountered in all plant organs. The antioxidant capacities of the plant extracts largely depend both on the composition of the extracts and on the conditions of the test system and are influenced by scores of factors that cannot be fully described by one single method. Consequently, more than one type of antioxidant capacity measurement is necessary to take into account the various mechanisms of antioxidant action[21]. In the present investigation, the antioxidant capacity of the leaf extract has been evaluated based on the measurement of the abilities of the various fractions (fractionated on the basis of the polarities of the chemical constituents) of the extract (i) to scavenge stable $ABTS^{\cdot+}$ radicals, (ii) to reduce Fe(III)-Fe(II), (iii) to inhibit the autoxidation of β-carotene-linoleic acid aqueous emulsion and (iv) inhibit Fe(II)-ferrozine complex formation.

It may be inferred from Fig. 1 that the leaf extract was found to possess as much as 886.493 mg vitamin-C equivalent

antioxidant capacity 100 g^{-1} of fresh leaves. More than 60 % of this (536.078 ± 6.926 mg) was concentrated in the EtOAc fraction and about 20 % (178.775 ± 2.906 mg) and 13 % (116.186 ± 2.176 mg), respectively in the Et_2O and EtCOMe fractions. The FRAP assay also measured the highest capacity for the EtOAc fraction (358.111 ± 8.269), which amounted to 64.6 % of the total capacity of 553.994 mg VCEAC measured for 100 g of fresh leaves. Et_2O and EtCOMe fractions followed this with 101.220 ± 3.923 mg (18.3 %) and 69.555 ± 2.872 mg (12.6 %), respectively. The residual capacities remained in aqueous mother liquor. Radical scavenging and ferric reducing antioxidant capacities of the various fractions of the leaf extract correlated well with their total phenolic and flavonoid contents (Figs. 2 and 3). EtOAc fraction that exhibited highest antioxidant capacities accounted for 55.4 % of the total 706.879 mg polyphenols 100 g^{-1} fresh leaves and 66.6 % of the total 394.968 mg flavonoids 100 g^{-1} fresh leaves, while the Et_2O fraction accounted to 15.6 % phenols and 12.4 % of the flavonoids. The contribution of the EtCOMe fraction was marginally higher with 21.2 and 18.6 %, respectively whereas in the aqueous mother liquor, the proportion was almost equal (7.8 and 7.4 %).

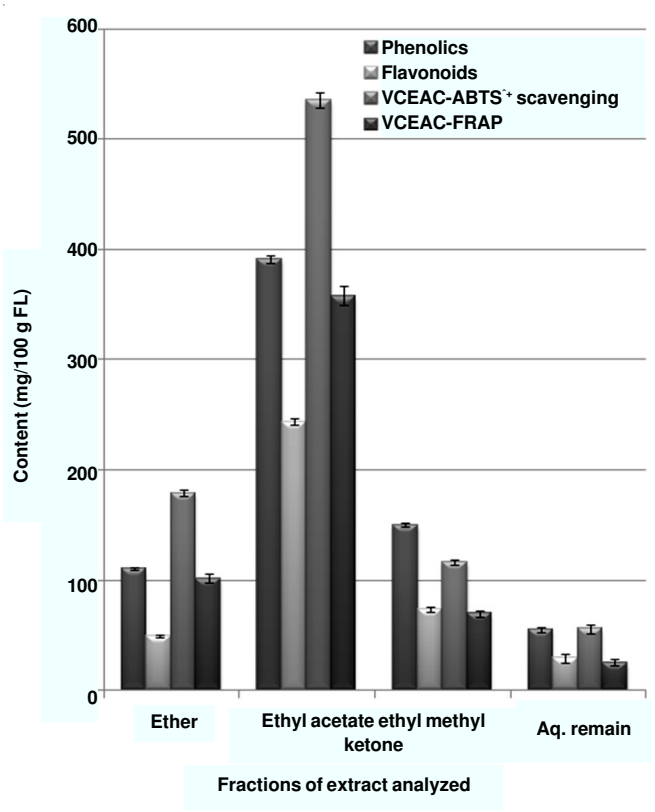

Fig. 1. Polyphenolic antioxidant constitution

The β-carotene bleaching assay measures the ability of a compound or a mixture of compounds to inhibit the oxidation of β-carotene. Therefore, antioxidants co-existing in the system hinder the extent of β-carotene bleaching by neutralizing the hydroperoxide produced by linoleic acid. From Fig. 4 it is clear that the inhibition rates are in the order BHT > EtOAc > Et_2O > EtCOMe. Thus, EtOAc fraction was found to be 77 and 91.8 % more potent than Et_2O and EtCOMe fractions, respectively in inhibiting the bleaching process. EtOAc fraction

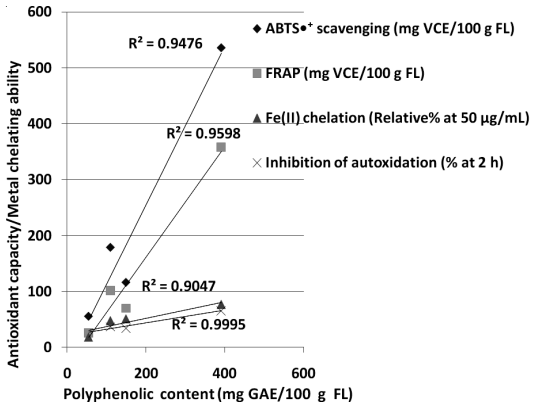

Fig. 2. Correlation between antioxidant and polyphenol content

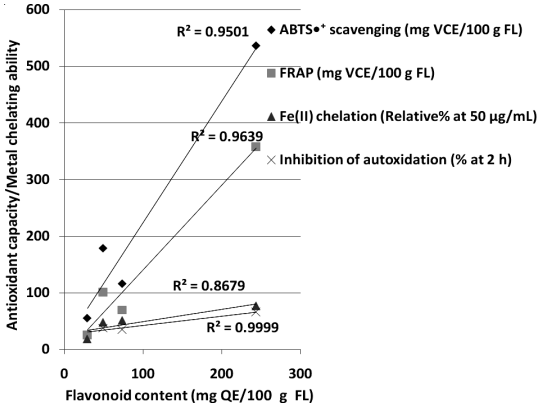

Fig. 3. Correlation between antioxidant and flavonoid content

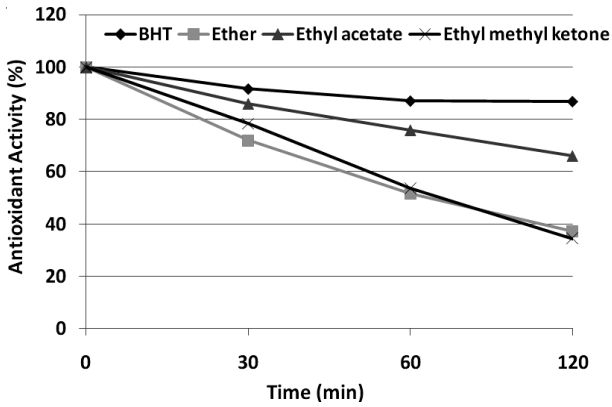

Fig. 4. Inhibition of β-carotene bleaching

also exhibited potent metal chelating properties in the two concentrations studied (Fig. 5). The fraction was evaluated to possess 73.6 and 77 % of the activities of Na_2EDTA.

Analysis of the polyphenolic constitution: Flavonoids constituted 55.9 % of the total phenols determined in the material along with ellagic acid (*ca.* 24 %), phloroglucinol and its derivatives. The fraction that exhibited the highest antioxidant capacity in all the four assays, namely, the EtOAc fraction, was found to contain 61.6 % of the total flavonoids and more than 55 % of the total phenolics. Phloroglucinol and its derivatives were analyzed as the major phenolics of the Et_2O fraction while ellagic acid was five-fold greater in the EtOAc fraction compared to the Et_2O fraction. The concentrations of these phenolics in the EtCOMe fraction was relatively

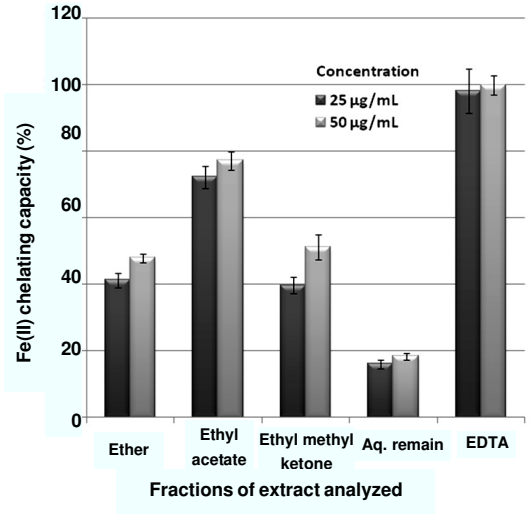

Fig. 5. Metal chelating capacities

low (Fig. 1). The major flavonoids of the EtOAc and EtCOMe fractions were characterized as the 7-O-β-D-glucopyranosides of diosmetin and chrysoeriol and the aglycones were detected only in insignificant quantities from the Et_2O fraction.

Oxidative damage has been implicated in the pathophysiology of many cancers and ROS induced activation of activator protein-1 plays pivotal roles in the development of cancer. Consequently, quenching of activated oxygen species or preventing the cellular damage they cause to proteins and DNA form an important mechanism to potentially prevent diseases like cancer. Several promising chemopreventive polyphenols are known to interrupt or reverse the carcinogenesis process by acting on intracellular signaling network molecules involved in the initiation and/or propagation and some may even arrest or reverse the progression stage[22,23]. Studies have also shown that plant phenolics can trigger apoptosis in cancer cells through the modulation of a number of key elements in cellular signal transduction pathways linked to apoptosis[22-24]. Flavonoids are known to act at the different development stages of malignant tumours by protecting DNA against oxidative damage, inactivating carcinogens, inhibiting the expression of the mutagenic genes and enzymes, responsible for activating procarcinogenic substances and activating the systems responsible for xenobiotic detoxification[25].

A number of papers appeared in the literature on the chemopreventive and therapeutic potentials of ellagic acid. It has been well documented to exhibit antimutagenic and anticarcinogenic activities, in a wide range of assays *in vitro* and *in vivo* and to cause apoptosis in cancer cells[26], associated with antioxidant and antiinflammatory activities in both bacterial and mammalian systems, probably by reducing endogenous oxidative DNA damage by mechanisms that may involve increase in DNA repair[27]. The reported anticancer activity of the aqueous ethanolic extract against P388 lymphocytic leukaemia in mice[7] may be attributed to the presence of ellagic acid, in significant quantities, in the leaf. The mechanisms by which ellagic acid may exert its intracellular actions include the inhibition of DNA topoisomerases, the induction of cell cycle arrest and the activation of apoptotic pathways. The cancer-chemopreventive properties of ellagic acid have been associated with the inhibition and long-term induction of glutathione S-transferases too. Studies have also found that ellagic acid can inhibit the growth of cancer cells of the breast, oesophagus, skin, lung, colon, prostate and pancreas and more specifically prevent the destruction of P53 gene by cancer cells and is also capable of reducing heart disease, birth defects, liver ailments and to promote wound healing[28-30]. In a recent study, the investigators have confirmed *in vivo* antiangiogenic effect of ellagic acid and its inhibition of matrix metalloproteinase-2 activity in human vascular endothelial cells with unchanged mRNA level, recommending its future clinical development as an antitumor agent[31]. Antiplasmodial activity has also been reported and the activity has been linked to the inhibition of plasmepsin II and an impairment of β-hematin formation in the parasites by ellagic acid[32-34]. An Italian research has recorded that ellagic acid appeared to reduce the side effects of chemotherapy in men with advanced prostate cancer, even though it could not retard the disease progression or improve survival[35]. Reports on the chelation of ellagic acid with several divalent cations and the resulting enhancement of its ability to inactivate metalloproteinases such as MMP-1, MMP-2, MMP-9, are also available in literature. Chelation of Zn(II) and Cu(II) are claimed to be associated with antiangiogenic activity[36-38]. A very recent study has revealed that ellagic acid is an inhibitor of human telomerase reverse transcriptase: h*TERT* $\alpha^+\beta^+$ in MCF-7 cells and has suggested that the ellagic acid-induced down-regulation of h*TERT* $\alpha^+\beta^+$ may be a mechanism through which ellagic acid exerts, at least in part, its chemopreventive effects in breast cancer[39].

Diosmin, the 7-O-rutinoside of diosmetin, has a long history of use around the world as a venotonic and has been investigated in a number of animal models and human cancer cell lines to possess chemopreventive and antiproliferative characters in addition to several other properties[40,41]. Diosmetin has been shown to get metabolized to luteolin[42] *via* an aromatic demethylation reaction from CYP1A1, CYP1B1 and the hepatic isozyme CYP1A2 (CYP = cytochrome P450) and is reported to selectively inhibit proliferation[43] of breast adenocarcinoma MDA-MB 468 and to downregulate inducible nitric oxide synthases expression. Chrysoeriol has also been recently shown to protect cells against cardiotoxicity induced by the potent wide spectrum anticancer drug, doxorubicin induced cardiotoxicity and thereby suggesting its co-administration with doxorubicin[44]. The protection offered is believed to be due to its antioxidant activity. Both diosmetin and chrysoeriol tend to get metabolized by the CYP1 family enzymes[42,43] to the more potent luteolin, which can delay or block the development of cancer cells *in vitro* and *in vivo* by protection from carcinogenic stimuli: by inhibition of tumour cell proliferation, by induction of cell cycle arrest and by induction of apoptosis *via* intrinsic and extrinsic signaling pathways[45].

Conclusion

The present study has attempted to account for the reported anticancer activities of the aqueous ethanolic extract of the aerial parts of *M. stenanthus*. Cancer continues to remain as the second most abundant cause of death in many developed western countries and according to an annual report of the German Cancer Research Centre, over 450,000 new cases have been diagnosed in Germany with 270,000 cancer-related deaths

each year among females alone. The prognosis for a patient with metastatic carcinoma of the lung, colon, breast or prostate remains an issue of concern, accounting for more than half of all cancer deaths[46]. The scenario in the developing countries is not different today. Cancer kills more people than HIV, tuberculosis and malaria combined and make up more than 50 % of the global total, according to WHO[47]. In India, about a million new cases are diagnosed every year and the number is projected to triple in the next 20 years. ICMR's projection estimates an increase from 4.47 lakh in 2008 to 5.34 lakh by 2020 of fresh cases of cancer annually among the male population alone[48]. In such a context, investigations to explore the possibilities of amalgamating traditional herbal medicines (rich in antioxidant phytochemicals) with established anticancer drugs, which are invariably double-edged swords and frequently leads to multi-drug resistance, in devising cancer chemotherapy or their incorporation in the chemopreventive strategies would definitely offer promising results.

ACKNOWLEDGEMENTS

The author is grateful to Dr. K. Kadavul, Department of Botany, Tagore Arts College, Puducherry and Mr. S. Aravajy, Department of Ecology, French Institute of Pondicherry, Puducherry for their help in the location, collection and identification of the plant material and SIF, IISc., Bengaluru, India for the NMR spectral data.

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