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Spectroscopic study of antioxidant property and trace elements of *Meyna spinosa* Roxb. ex Link leaves

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Antioxidant property of *Meyna spinosa* Roxb. ex Link leaf was determined by using UV-Visible and Electron Paramagnetic Resonance (EPR) spectroscopy. Further the trace elements present in it were determined by using Atomic Absorption Spectroscopy (AAS). The concentration of the antioxidant activity, IC_{50} was found to be 563.23 µg/mL and the trace elements detected were Fe, Zn, Cu, Mo, Cr, Mn. Role of antioxidants and trace elements were discussed with reference to the traditional knowledge.

Keywords: *Meyna spinosa*, Spectroscopy, Antioxidant, Trace elements.

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Introduction

Man has been dependent upon nature for survival since time immemorial. Plants and plant derived products are part of health care since human civilization. Prior to the development of modern medicine, the traditional systems of medicine that have evolved over the centuries within various communities are still maintained as a great traditional knowledge base in herbal medicines[1]. Many workers had been engaged in the extraction of biochemicals, structure analysis and antioxidant property of them. Antioxidants are useful for their anti-ageing and anti-disease properties. There is relation among free radicals and quality of life and health but some foodstuffs have paramagnetic properties naturally and they show a single Electron Paramagnetic Resonance (EPR) spectral signal[2]. So, the natural antioxidant properties of herbs are recommended strongly now-a-days. Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Antioxidants may diminish the energy of the free radical, stop the free radical from forming in the first place and minimize the damage caused by free radicals[3] A free radical is any chemical species capable of independent existence that possesses on or more unpaired electrons. Due to their unsaturation with electrons, they are extremely reactive. They can be generated by photolysis, thermolysis, radiolysis, in oxido-reduction processes, enzymatic processes *in vivo*, by the influence of ozone, etc. In addition free radicals are formed in human cells during normal metabolism and take place in many biological reactions[4]. Uncontrolled and abnormal free radical reactions can cause deterioration of physical, biological and health characteristics of food, pharmaceutical and cosmetic products. Excessive quantity of free radicals in human body can produce oxidative stress and lead to many pathological states such as mutagenesis and carcinogenesis, aging, rheumatism, arthritis, arteriosclerosis, necrosis, diabetes, etc. The deficiency or excess of trace elements leads to various complications and metabolic disorders in human beings[5].

Family Rubiaceae plants are trees, shrubs or infrequently herbs comprising about 450 genera and

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6500 species, including lianous forms. Leaves are simple and usually entire and are opposite or sometimes whorled; stipules are present and interpetiolar. *Meyna spinosa* Roxb. ex Link belongs to family Rubiaceae and its local name (Manipuri) is *Lam Heibi*. This herb is popular among the people of Asia for its curability of hepatic disorder, gastrointestinal problems, skin infection; it is refrigerant and abortifacient[6]. Fruits of this medicinal plant are reported to contain sugar, gum and tannic acid whereas seeds contain esters of palmitic acid, stearic and oleic acids. Leaves are used in bone fracture and in the treatment of diphtheria[7]. The Ayurvedic and plant based remedies are a part of cultural heritage of India and so is in the North-Eastern region[8,9].

In Manipur, North-Eastern state of India, leaf of this plant is used as traditional salad, particularly by the Meitei community. Decoction of its leaves is also used for traditional treatment of inflammation, and as a hair lotion. Fruit is used in traditional skin lotion and as an edible item by the Manipuris. So, the present objectives of this study are to evaluate the trace elements by Atomic Absorption Spectroscopy (AAS) and the antioxidant property by using EPR and UV-visible spectrometer of the *M. spinosa* leaf.

Materials and Methods

Plant materials

M. spinosa (Plate 1) leaves were collected from Imphal West District, Manipur and washed with distilled water to avoid from possible environmental contaminants like dust and other unwanted substances. Plant sample was identified by The Department of Botany, D.M. College of Science and deposited (Acc. No. OC-00103) at bio-hub Centre, Oriental College, Imphal.

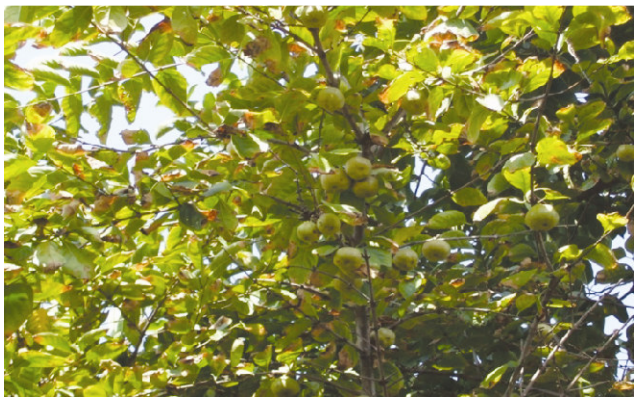

Plate 1—*Meyna spinosa* Roxb. ex Link

Plant extract

200g of shade-dried and pulverized leaves were subjected to extraction in a Soxhlet apparatus with one litre of methanol boiling it at 48°C for 72 h. Extraction was conducted until no more coloured matter was extracted. Solvent extracted mixture was evaporated to dry state using a rotary evaporator under reduced pressure at 40°C. Dried extract was then kept in tightly fitted stopper bottles and stored at -4°C.

DPPH radical scavenging assay

Scavenging activity of extracts on DPPH was determined using the method followed by Okada and Okada[10]. This method depends on the reduction of purple DPPH to a yellow coloured diphenyl picrylhydrazine. Determination of the disappearance of free radicals was done using UV-visible spectrometer (Model: Cary 100 Bio). The remaining DPPH which showed maximum absorption at 517 nm was measured. Methanol extract sample's stock solution (1.0 mg/mL) was diluted to final concentrations of (0.6 .0.5, 0.4, 0.3, 0.2, 0.1, 0.05 and 0.01) mg/mL in ethanol. One millilitre of a 0.3 mM DPPH ethanol solution was added to 2.5 mL of sample solution of different concentrations. These are test solutions. One mL of ethanol was added to 2.5 mL of sample solution of different concentrations. These are blank solutions. One mL DPPH solution plus 2.5 mL of ethanol was used as a negative control. The blank for this solution is ethanol. As DPPH is sensitive to light, it iwas exposed to the minimum possible light. These solutions were allowed to react at room temperature for 30 minutes. The radical scavenging activity, expressed as percentage of inhibition was calculated using the equation:

Inhibition Concentration (%) =

$$100 - [(\text{absorbance of sample} - \text{absorbance of blank}) \times 100/\text{absorbance of control}].$$

Tests were done in triplicate and IC_{50} values were calculated by linear regression of plots, where the abscissa represents concentration of the tested plant extract and ordinate as the average percent of scavenging capacity. Concentration of sample required to scavenge 50% of DPPH (IC_{50}) was determined.

Electron Paramagnetic/Spin Resonance spectrometer (EPR) analysis

This extract was used for analysis of antioxidant property by using Electron Paramagnetic/Spin

Resonance spectrometer (Model: JEOL, JES-FA 200 ESR spectrometer, X-band microwave unit; TEMPOL was used as field marker). All EPR measurements were performed at room temperature on Varian X Band EPR Spectrometer, operating at a microwave frequency of 9443.847 MHz, with 100 kHz field modulation.

Atomic Absorption Spectrometer (AAS) analysis

Leaves were dried at 65°C in an oven for 24 h and subsequently ground by an agate mortar. Powders were converted into liquid form with acid digestion process and used for elemental analysis by Graphite Furnace - Atomic Absorption Spectrometer (Model: Analytic Jena vario-6).

Results and Discussion

As free radicals are responsible for diseases in the human body, presence of it in our day to day edible items has been found very interesting for the researchers all around the world. On the other hand search for antioxidants in the food items as well as in medicinal plants had been pursued for many decades. Detection of the EPR signal in the EPR spectrum of *M. spinosa* leaf powder emphasises the presence of free radicals in it. The weak signal was found to be with g = 2.03871. Origin of this singlet line may be due to free radicals of semi-quinones, lignin or due to phenols especially flavonoids[11-15].

The sharp peak in the spectrum is due to the effect of quartz tube holding the powder sample. From this experiment it is concluded that some free radicals are present in the leaves of *M. spinosa*, containing antioxidants. The EPR spectrum of 2, 2-diphenyl-1-picrylhydrazyl ($C_{18}H_{12}N_5O_6$), abbreviated as DPPH, a stable free radical, was taken. For taking the EPR spectrum, an amount of 12 mg of DPPH is dissolved in 10 mL of methanol which is also used as stock solution and kept in a dark chamber as DPPH is very sensitive to light. The spectrum was taken within 15 minutes for 1.5 mL stock solution.

Further, an amount of 3 mL of stock solution is mixed with 50 mg of plant extract and EPR spectrum was taken again. Obviously no signal was found to be lost. This is due to fact that the free radicals of DPPH molecules are absorbed by antioxidants of the *M. spinosa* extract by chemical reaction leading to the conclusion that this plant is a good scavenger of free radicals due to the presence of antioxidants.

Total antioxidant activity was observed with the help of the UV-visible spectrometer and inhibition concentration 50, IC_{50} value was found to be 563.23 ± 0.22 µg/mL which is responsible for 50 % inhibition of free radicals. Scavenging activity is 2.45 ± 0.12 µg/mL. IC_{50} value of methalonic extract of leaves was reported to be 16.4 ± 0.41 µg/mL by earlier workers[16]. The difference is attributable to the collection of samples from different geographical locations. Protection of UV radiations with the help of antioxidants[17-19] corroborates idea of traditional use of *M. spinosa* as a skin lotion.

Some of the essential trace elements detected so far in *M. spinosa* leaf are: Fe, Zn, Cu, Mo, Cr, Mn and corresponding concentrations were found to be 1.02±0.005, 0.434±0.014, 2.30±0.02. 0.011±0.001, 4.27±0.097, 0.014±0.002 ppb, respectively. These elements can have their definite nutritive roles as well as antioxidant activity. Zinc is essential to all organisms and it is an important trace element having a definite role in metabolism, growth and development. It is an essential component of over 200 enzymes, viz. cytosolic superoxide dismutase, having both catalytic and structural roles. Zinc deficiency is characterised by recurrent infection, lack of immunity and poor growth[20]. Low intake of zinc leads to coronary artery disease[21]. Clinical materials prove that Zn can have good effect on eliminating ulcer and promoting healing of wounds[22,23]. Iron is essential for human body in production of haemoglobin, in oxygenation of red blood cells. It is needed for healthy immune system and for energy production. Severe iron deficiency results in anaemia and red blood cells that have a low haemoglobin concentration. It is responsible for the catalase enzyme.

Copper is involved in the oxidation of Fe^{+2} to Fe^{+3} haemoglobin formation and it is an important catalyst for iron absorption. Copper deficiency may be a risk factor for cardiovascular disease. When copper deficiency occurs symptoms include neutropenia, cardiac disorders, osteoporosis and anaemia[24]. It is responsible for cytochrome oxidases and cytosolic superoxide dismutase enzymes. Chromium is essential functioning as a glucose tolerance factor. It is also used for insulin signalling for biological role and thus sugar metabolism and diabetes[22].The functional values of manganese are as Lewis acid and catalyst for oxidation. It is essential to all organisms, activates numerous enzymes, viz. mitochondrial superoxide dismutase. Molybdenum is used as nitrogenase and nitrate reductase enzymes by plants in

nitrogen fixation. Out of the above mentioned six elements, elements like copper, manganese, zinc are reported to be antioxidants[25]. Further, iron is treated as antioxidant[26]. Now-a-days chromium is also used in antioxidant formulation[22]. The antioxidant property of the elements may be attributed due to existence of them as various enzymes in the leaves of *M. spinosa.* Lastly, daily intake requirements of some of the trace elements by our human body is given as follows[28]: Iron, 10 mg/ day for male and 15 mg for female; Zn, 15 mg/ day; Mn, 2.5 – 5.0 mg/ day; Cr, 0.05-0.2 mg/ day; Cu, 2-3 mg/day. So the leaves can provide such nutritive elements at some extent as it is used as salads in Manipur.

Conclusion

Antioxidant activity of leaves of *M. spinosa* were analysed with the help of UV-visible and EPR spectrometer. Traditional use of this plant as skin lotion for protection from UV radiation by the Manipuris, might be due to the presence of antioxidants in it. Moreover, essential trace elements like Fe, Zn, Cu, Mn, Mo and Cr, having nutritive values, are also present in leaves and contribute antioxidant property at some extent. Hence, such trace elements would have taken roles to scavenge free radicals as done by phenols and flavonoids. Presence of zinc is attributable for traditional treatment of inflammation and hair lotion as it is effective in wound healing and protecting hair loss, respectively.

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