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Research Article

Elemental Analysis (ICP-OES), Antibacterial and Antioxidant Activity of *Maesa indica* (Roxb.) A.DC

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ABSTRACT

Maesa indica (Roxb.)A.DC (Myrsinaceae) is a small tree or a large shrub. The present study aimed to estimate major and minor elements in powdered leaf and to determine antibacterial and radical scavenging activity of petroleum ether, chloroform, ethyl acetate and methanol extracts of leaf of *M. indica*. The elements in leaf powder were estimated using ICP-OES technique after microwave digestion. Antibacterial activity of solvent extracts was performed by Agar well diffusion assay. Radical scavenging effect of solvent extracts was determined by DPPH and ABTS radical scavenging assay. Total phenolic and flavonoid content of solvent extracts was estimated by Folin-Ciocalteau reagent and Aluminium chloride colorimetric estimation method respectively. Among major elements, the content calcium and sodium was highest and least respectively. In case of minor elements, the content of iron and chromium was highest and least respectively. The solvent extracts showed inhibitory potential against Gram positive and Gram negative bacteria but to a varied extent. All solvent extracts were found to scavenge both DPPH and ABTS radicals. Scavenging effect was superior in case of methanol extract followed by ethyl acetate, chloroform and petroleum ether extracts. The content of total phenolics as well as total flavonoids was higher in case of methanol extract than others. A direct correlation was observed between the phenolic and flavonoid content of extracts and the radical scavenging potential of extracts.

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INTRODUCTION

Maesa indica (Roxb.) A.DC, belonging to the family Myrsinaceae, is a small tree or large shrub. Leaves ovate-lanceolate, acuminate or even caudatate-acuminate, coarsely dentate or serrate, glabrous with long petioles; the flowers white, very small, in axillary racemes, bracteates, bisexual, calyx tube adnate to base of ovary, lobes imbricate, corolla companulate, lobes imbricate, stamens 5 inserted on corolla tube, ovary half inferior, stigmas 3; the berries white; bark thin, reddish brown, lenticellate; wood brownish white, soft; flowering and fruiting between October-August. Root and fruits are used in syphilis and as an anthelmintic [1, 2].

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Maesol (dimeric phenol) isolated from seeds was inactive when tested for inhibition of the mutagenic activity of several mutagens [3]. Kiritiquinone, a new benzoquinone was isolated from the fruits [4]. The plant has been used traditionally in various parts of the world. In Chakma community of Arunachal Pradesh, the decoction made from leaves is used for bathing in fever [5]. The plant is used as fodder for feeding cattle in order to obtain high output of milk in Meghalaya [6]. In Lotha-Naga tribes in Wokha district of Nagaland, the fruits are eaten to expel intestinal worms [7]. The roots are used to treat joint and body pain in Kumar parvatha, Kukke Subramanya, Mangalore, Karnataka [8]. In Mao Naga folk medicine of Manipur, young leaves are boiled with water and taken for treating hoarse voice [9]. The fruits are eaten raw by the local communities in Nilgiri hills of the southern

Western Ghats of Tamil Nadu, India [10]. The methanol extract of leaves was found to exhibit inhibitory activity against clinical isolates of *Streptococcus mutans*[11]. Cow urine extract of leaves was found to possess inhibitory activity against pathogens causing rhizome rot of ginger [12]. Ethyl acetate extract of fruit was shown to possess anthelmintic activity against *Caenorhabditis elegans* [13]. The acetone extract of leaves was found to possess larvicidal activity against 4th instar larvae of *Aedes aegypti* [14]. The present study aimed at estimation of major and minor elements in the powdered leaf material and to determine antibacterial and radical scavenging activity of solvent extracts of leaves of *M. indica*.

MATERIALS AND METHODS

Collection and identification of plant

The plant was collected at Hulikal, Karnataka in the month of January 2013 and identified by Dr. Vinayaka K.S. The voucher specimen (SRNMN/MB/Mi-01) was deposited in the department herbaria. The leaves were separated, dried under shade and powdered.

Elemental analysis by ICP-OES

1g of powdered leaf material was added to 10ml of ultrapure metal free nitric acid and digested in a microwave digester (CEM). After digestion, the content was filtered and diluted to 25ml using distilled water. Later, the digested sample was aspirated into ICP-OES (Agilent Technologies 700series, US) to estimate major elements *viz*., Calcium (Ca), Potassium (K), Sodium (Na) and Magnesium (Mg) and minor elements *viz*., Manganese (Mn), Iron (Fe), Zinc (Zn), Nickel (Ni), Chromium (Cr), Lithium (Li) and Copper (Cu). The calibration standards were prepared by diluting stock multi-elemental standard in nitric acid [15]. Instrument configuration/experimental conditions are shown in Table 1.

Extraction

A known quantity (50g) of powdered leaf material was subjected to soxhlet extraction process and extracted sequentially with solvents *viz*., petroleum ether, chloroform, ethyl acetate and methanol. The solvents were filtered through Whatman No. 1, concentrated under vacuum in rotary evaporator [16].

Antibacterial activity of solvent extracts

Agar well diffusion technique was employed to determine susceptibility of test bacteria *viz*., *Staphylococcus aureus* NCIM-2079 and *Bacillus cereus* NCIM-2016, *Pseudomonas aeruginosa* NCIM-2242 and *Shigella flexneri* NCIM-4924 to solvent extracts of leaf[17].

Table 1: ICP-OES Operation conditions

Parameter	Value
Power (kW)	1.2
Plasma flow (L/min)	15.0
Auxiliary flow (L/min)	1.50
Nebulizer flow (L/min)	0.75
Sample flow rate (L/min)	1.5
Replicate read time (s)	3.00
Instrument stabilization delay (s)	15.0
Sample uptake delay (s)	10.0
Pump rate (rpm)	15.0
Rinse time (s)	10.0
Spray chamber	Cyclonic type
Elements, wavelengths (nm)	Ca (422.673), Cu (327.395), Na (589.592) Cr (267.716), Fe (238.204), K (766.491), Mg(279.553), Mn (257.610), Ni (231.604), Zn (213.857), Li (670.783)

The test bacteria were inoculated into sterile Nutrient broth tubes and incubated for 24 hours at 37°C. The broth cultures were swabbed aseptically on the sterile Nutrient agar plates. Using a sterile cork borer, wells of 0.6 cm diameter were punched in the inoculated plates and 0.2 ml of solvent extracts (25 mg/ml of 10% DMSO), standard (Streptomycin, 1mg/ml of sterile distilled water) and control (DMSO, 10%) were filled into the respectively labelled wells. The plates were incubated for 24 hours at 37°C and the zones of inhibition formed around the wells were measured. The experiment was repeated two times and the mean value was obtained.

DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging assay

The efficacy of solvent extracts to scavenge free radicals was determined by DPPH free radical scavenging assay [17]. Here, 2ml of various concentrations (5, 10, 25, 50 and 100 µg/ml of methanol) of extracts and ascorbic acid (reference standard) were added to 2ml of DPPH solution (0.002% in methanol) in clean and labeled tubes. The tubes were incubated in dark at room temperature for 30 minutes. Later, the absorbance was measured at 517nm in UV-Visible spectrophotometer. The absorbance of

DPPH control (2ml of DPPH+2ml of methanol) was also noted. The scavenging activity (%) of each concentration of solvent extracts was calculated using the formula: (A_0-A_1/A_0) x 100, where A_0 is absorbance of control and A_1 is absorbance of test (extract/standard). The concentration of extract required to inhibit 50% of free radicals (Inhibitory concentration, IC_{50}) was calculated for each extract.

ABTS (2, 2'-azino-bis[3-ethylbenzothiazoline-6-sulphonic acid]) free radical scavenging activity

The efficacy of solvent extracts to scavenge free radicals was tested by ABTS radical scavenging assay [18]. The ABTS radical was generated by mixing ABTS stock solution (7mM) with potassium persulfate (2.45mM) and the mixture was left in the dark for 12–16 hours at room temperature. The solution was then diluted using distilled water to an absorbance of 0.70 at 730nm. 1ml of different concentrations of solvent extracts (5, 10, 25, 50 and 100µg/ml) were mixed with 4ml of ABTS solution in labeled tubes and the tubes were incubated for 30 minutes. The absorbance was measured at 730nm. Ascorbic acid was used as standard. The radical scavenging effect (%) of each concentration was calculated using the formula: (A_0-A_1/A_0) x 100, where A_0 is absorbance of control and A_1 is absorbance of test (extract/standard). The concentration of extract required to inhibit 50% of free radicals (Inhibitory concentration, IC_{50}) was calculated for each extract.

Total phenolic content (TPC) of solvent extracts

The TPC of solvent extracts was estimated by Folin-Ciocalteu reagent (FCR) method [17]. A dilute concentration of solvent extracts (0.5 ml) was mixed with 0.5 ml diluted Folin-Ciocalteu reagent (1:1) and 2 ml of sodium carbonate (7%). The mixtures were allowed to stand for 30 minutes and the absorbance was measured colorimetrically at 765nm. A standard curve was plotted using different concentrations of Gallic acid (standard, 0-1000 µg/ml). The concentration of total phenolic compounds was determined as µg Gallic acid equivalents (GAE) from the graph.

Total flavonoid content (TFC) of solvent extracts

The content of total flavonoids in solvent extracts was estimated by Aluminium chloride colorimetric estimation method. A dilute concentration of solvent extracts (0.5ml) was mixed with 0.5ml of methanol, 4 ml of water, 0.3ml of $NaNO_2$ (5%) and incubated for 5 minutes at room temperature. After incubation, 0.3ml of $AlCl_3$ (10%) was added and again incubated at room temperature for 6 minutes. Later, 2ml of 1M NaOH and 2.4ml of distilled water were added and the absorbance was measured against blank (without extract) at 510nm using UV-Vis spectrophotometer. A calibration curve was constructed using different concentrations of Catechin (0-120 µg/ml) and the flavonoid content of LE and FE was expressed as µg Catechin equivalents (CE) from the graph [17].

Statistical Analysis

The experiments were conducted in triplicates. The values are represented as Mean±Standard Deviation (SD). The IC_{50} values of extracts were calculated by Origin 6.0 software.

RESULTS

Table 2 shows the content of elements in leaves of *M. indica*. In case of major elements, the content of calcium (18829.05ppm) and sodium (128.10ppm) was highest and least respectively. In case of minor elements, iron content (290.95ppm) was highest and the content of chromium was least (1.30ppm).

Table 2: Content of major and minor elements in leaves

Element	Quantity (ppm)
Calcium	18829.05±10.25
Potassium	13422.00±5.15
Sodium	128.10±2.14
Magnesium	2295.35±5.23
Iron	290.95±1.13
Manganese	59.70±0.15
Nickel	1.84±0.00
Zinc	20.77±0.05
Copper	4.27±0.00
Chromium	1.30±0.00
Lithium	4.50±0.10

Inhibitory efficacy of solvent extracts of *M. indica* leaf was tested against two Gram positive bacteria *viz*., *S. aureus* and *B. cereus* and two Gram negative bacteria *viz*., *P. aeruginosa* and *S. flexneri* by agar well diffusion method. The presence of zones of inhibition around the well was taken as positive for antibacterial activity.

Table 3: Antibacterial activity of solvent extracts of leaf

Test bacteria	Zone of inhibition in cm					
	PEE	CE	EAE	ME	Standard	DMSO
S. aureus	1.7±0.1	1.6±0.1	1.5±0.1	1.5±0.0	4.1±0.2	0.0±0.0
B. cereus	1.7±0.2	1.7±0.1	1.6±0.1	1.9±0.2	3.9±0.1	0.0±0.0
P. aeruginosa	1.6±0.1	1.4±0.1	1.5±0.1	1.4±0.1	3.6±0.1	0.0±0.0
S. flexneri	1.2±0.0	1.4±0.0	1.4±0.0	1.5±0.0	3.5±0.2	0.0±0.0

The test bacteria have shown varied sensitivity to solvent extracts. Petroleum ether extract showed marked inhibition of *S. aureus* and *P. aeruginosa*. Methanol extract was more active against *B. cereus* and *S. flexneri* when compared to other extracts. Inhibition caused by standard antibiotic was marked when compared with solvent extracts. Overall, Gram positive bacteria have shown higher susceptibility to solvent extracts and standard antibiotic. There was no inhibition recorded in case of DMSO (Table 3).

Figure 1 shows the result of DPPH radical scavenging potential of solvent extracts. The extracts showed dose dependent scavenging of free radicals. Among extracts, highest and least scavenging effect was observed in case of methanol extract and petroleum ether extract respectively. The IC_{50} value for methanol, ethyl acetate, chloroform and petroleum ether extract was found to be 14.04, 24.08, 36.91 and 92.07µg/ml respectively. Scavenging potential of extracts was lesser than ascorbic acid (IC_{50} 2.64µg/ml).

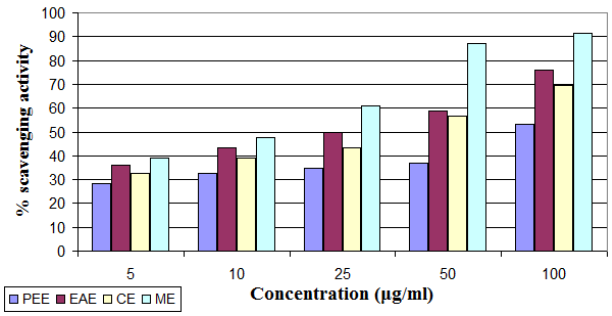

Figure 1: DPPH scavenging activity of solvent extracts of leaf

The result of ABTS radical scavenging potential of solvent extracts is shown in Figure 2. Here also, methanol extract and petroleum ether extract scavenged free radicals to higher and least extent respectively. The IC_{50} value for methanol, ethyl acetate, chloroform and petroleum ether extract was found to be 3.45, 6.42, 10.63 and 45.47µg/ml respectively. Ascorbic acid scavenged radicals with an IC_{50} value of 2.26µg/ml.

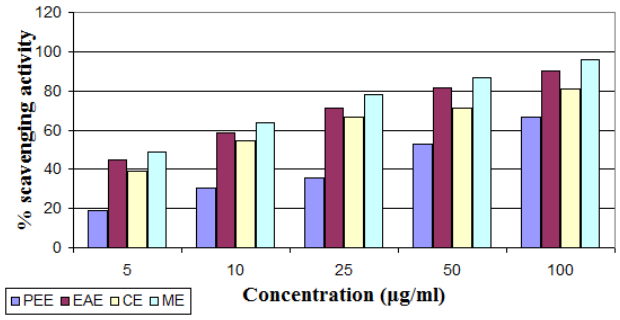

Figure 2: ABTS scavenging activity of solvent extracts of leaf

Table 4 shows the TPC and TFC of solvent extracts of leaf. Both phenolic and flavonoid contents were higher in methanol extract followed by ethyl acetate, chloroform and petroleum ether extract.

Table 4: Total phenolic and flavonoid content of solvent extracts of leaf

Solvent extract	TPC (µg GAE/mg)	TFC (µg CE/mg)
Petroleum ether extract (PEE)	46.25±05.10	18.75±03.13
Chloroform extract (CE)	85.00±10.50	23.14±01.12
Ethyl acetate extract (EAE)	121.25±05.25	27.33±02.33
Methanol extract (ME)	133.75±09.36	43.61±05.06

DISCUSSION

Mineral elements, inorganic substances present in body tissues and fluids, represent comparatively smaller portion of the diet as compared with major nutrients such as carbohydrates, proteins and fats. Although these elements do not yield any energy, they are necessary for several metabolic processes being essential to life. These elements can be broadly categorized into major or minor elements on the basis of their daily requirement. The significance of mineral elements is well studied in human, animal and plant nutrition as their deficiencies in

the nutrition can lead to a variety of diseases/disorders. Plant materials form a major portion of human and animal diet and hence, their nutritive value is important [19-22]. In our study, we estimated major and minor elements in microwave digested leaf of *M. indica* by ICP-OES technique. The content of calcium and iron was highest and least among major and minor elements respectively. ICP-OES is advantageous over other analytic techniques which are used to estimate elements as the technique can estimate many elements at a time. Hence, ICP-OES is now the most widely used technique employed for elemental determination and many studies have been conducted to validate this method for metal analysis in a large variety of sample types including plant samples [15, 18, 22-25].

The discovery of antibiotics from bacteria and fungi is one of the leading health-related events of modern times. Antibiotics have revolutionized the field of medicine and saved countless lives at the time of discovery. However, the successful use of antibiotics is compromised by the development of tolerance or resistance in pathogens. The overuse and abuse of antibiotics often remains the key reason for potential development of resistance among pathogens [26]. Development of resistance, high cost and side effects stimulate interest in plant based antimicrobial agents. Plants have the ability to produce a variety of metabolites such as phenolics, alkaloids, saponins and others having marked biological and pharmacological properties. These plant components are used as chemotherapeutic agents or serve as ingredients for the development of modern drugs. Over 50% of all modern clinical drugs originated from natural sources mainly plants. A vast majority (>80%) of global population depend on traditional medicine for their primary healthcare needs. Ayurveda and other systems of medicine have vast record of medicinal plants being used for the treatment of various types of ailments. A vast number of plants have been screened for their antimicrobial activity [27-31]. In our study, the solvent extracts have shown inhibitory activity against Gram positive and Gram negative bacteria. It was shown that the methanol extract of leaves exhibit inhibitory activity against *Streptococcus mutans* recovered from dental caries subjects [11]. In another study, cow urine extract of leaves was found to inhibit *Ralstonia solanacearum* isolated from rhizome rot of ginger [12].

To ensure the structural integrity of critical components, cells have to maintain a proper balance between the antioxidant defense and the levels of free radicals. The levels of free radicals when exceeds the antioxidant defense, results in oxidative stress leading to damaging effect on biomolecules such as lipids, proteins and DNA and thereby numerous degenerative chronic diseases develop. The most widely used synthetic antioxidants are butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl galate (PG) and tertiary butyl hydroquinone (TBHQ). However, their use has been suspected to cause or promote negative health effects. Natural antioxidants, in particular from plants have gained a lot of interest nowadays as epidemiological studies have showed that frequent consumption of natural antioxidants is associated with a lower risk of cardiovascular disease and cancer. The protective properties of plant based antioxidants are because of three major groups of substances *viz*., vitamins, phenolics, and carotenoids [32-35].

A number of in vitro assays are available for determining antioxidant nature of plant extracts and purified compounds. DPPH free radical scavenging assay is one of the most popular radical scavenging assays and is used to determine radical scavenging nature of many compounds including plant extracts. DPPH radical is a stable, commercially available organic nitrogen centred free radical. It reacts with compounds capable of donating hydrogen/electron and has a maximum UV–Vis absorption within the range of 515–520 nm. Upon reduction, the radical solution (purple) becomes discoloured (yellow) according to the number of electrons paired and the reaction progress can be conveniently monitored using a spectrophotometer. This assay is simple, sensitive and fairly rapid. The result is often expressed as IC50 value which is defined as the concentration of sample that causes a 50% decrease in the DPPH absorbance [36, 37]. In our study, methanol extract scavenged DPPH radical more efficiently than other extracts as revealed by lower IC_{50} value. Scavenging potential of petroleum ether extract was least among solvent extracts. Although radical scavenging potential of solvent extracts was lesser than ascorbic acid, it is evident that the extracts possess hydrogen donating ability and therefore the extracts could serve as free radical scavengers, acting possibly as primary antioxidants [38].

Like DPPH assay, ABTS radical scavenging assay is another assay used to determine free radical scavenging nature of several types of samples including plant extracts. On interaction with ABTS radical, antioxidants either transfer electrons or hydrogen atoms to ABTS radical and thereby neutralizing its free radical character [18]. In the present study, the solvent extracts have shown a dose dependent scavenging of ABTS radicals. Among extracts, methanol extract showed higher scavenging efficacy followed by other extracts. Least scavenging effect was observed in case of petroleum ether extract. Overall, the extracts scavenged ABTS radicals to higher extent than DPPH free radicals. Even though the scavenging potential of solvent extracts was lesser than ascorbic acid, it is evident that the extracts possess hydrogen donating ability and therefore the extracts could serve as free radical scavengers, acting possibly as primary antioxidants [38].

polyphenolic phytochemicals including flavonoids are present in various parts of the plants. These compounds are of immense importance as they possess a range of bioactivities including antioxidant activity. The presence of hydroxyl groups in these compounds might account for their antioxidant potential. These compounds reduce oxidative damage by scavenging free radicals and other harmful agents and thereby protect the cells in oxidative stress [32, 37]. In the present study, we evaluated total phenolic and flavonoid content of solvent extracts by FCR and aluminium chloride colorimetric estimation method respectively. Methanol extract was found possess higher phenolic and flavonoid contents followed by ethyl acetate, chloroform and petroleum ether extracts. Many studies have shown a direct correlation between the total phenolic and flavonoid content and the antioxidant activity [17, 29, 39-42]. In our study also, the radical scavenging effect was highest and least in case of methanol and petroleum ether extracts as they contained high and low phenolic and flavonoid contents respectively.

CONCLUSION

The leaf of *M. indica* can be used as a source of important elements required for normal physiology. The plant can be used for the treatment of infectious diseases and for prevention and treatment of oxidative damage caused free radicals. The observed bioactivities could be ascribed to the presence of phytoconstituents such as phenolics, flavonoids and others. Further studies are to be carried out to isolate and characterize bioactive principles from the plants and to determine their biological activities.

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