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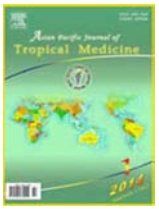

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Phytochemical, antioxidant and antibacterial potential of *Elaeagnus kologa* (Schlecht.) leaf

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ABSTRACT

Objective: To screen different solvent extracts of *Elaeagnus kologa* (*E. kologa*) leaf to determine the phytochemicals, potent antioxidant and antibacterial activity to find out the possible source of applied pharmaceutical formulations.

Methods: Solvent extracts of leaf material were prepared using the Soxhlet apparatus. A study was performed on antioxidant activity of methanolic extract of leaf by 1-1-diphenyl-2-picrylhydrazyl method. The phenolic and flavonoid content of all the fractions were determined using high performance liquid chromatography. Leaves were also subjected to protein and carbohydrate test.

Results: The total phenols, flavonoids were found to be high in petroleum ether as compare to other solvent fraction. The IC_{50} value of methanolic extract of the sample was 62.20 μg/mL which showed significant antibacterial activity against *Bacillus subtilis* (Gram-positive).

Conclusions: The present study suggests that the methanolic extract of *E. kologa* leaf possesses antioxidant and antibacterial properties. Such properties may be of great use in mitigating the detrimental effects of oxidative stress and reducing susceptibility to bacterial infection. Notably, extracts of *E. kologa* leaf also contain proteins and carbohydrates which add to its nutritional value.

1. Introduction

The medicinal properties of plants have been investigated in several countries with a focus on identifying key phytochemicals with potential therapeutic effects. The more important bioactive components found in plant materials are alkaloids, tannins, flavonoids, phenols, proteins and carbohydrates[1]; these may be derived from different parts of plant including the bark, leaves, flowers, roots, seeds, fruits, and others. Major groups of phytochemicals are now widely used to treat degenerative diseases, such as cancers, diabetes, Alzheimer's disease, and Parkinson's disease[2,3], which have been associated with reactive oxygen species that damage cellular biomolecules. Medicinal plants, which are often rich in phenols and flavonoids, are known to have potent antioxidant properties that can decrease oxidative stress and offer valuable health benefits[4-6]. A large number of phytochemicals belonging to several chemical groups having inhibitory effects on several types of pathogenic microbes are drawing attention of scientist to search for potential drug. Thus, the potential of the plant and their ability to combat infectious disease justifies the continued identification and characterization of medicinal plants to quantify their antioxidant or antimicrobial potentials[7,8].

Elaeagnus kologa (*E. kologa*) (Schlecht.) belongs to family Elaeagnaceae. It is a large thorny shrub with elliptic obovate leaves and prominent silvery scale. It is endemic and generally distributed in shola forest of Nilgiri district of Tamil Nadu, India. Populations of these plant species are declining due to shifts in cultivation practices and

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habitat destruction. Within the tribal communities of this region, this plant is traditionally used in treating of a broad spectrum of common ailments since the time immemorial[9]. The tribes of Nilgiris including the Todas and Kotas use this plant decoction against cutaneous infections, dropsy, venereal sores, deafness, anasarca and dysentery[10]. Motivated by the ethano medical history of the plant, the present study was focused on evaluating antioxidants and antibacterial properties of different solvent extracts of the *E. kologa* leaf.

2. Materials and methods

Fresh and matured leaves of *E. kologa* were collected from Nilgiris district of Tamil Nadu, India, during the month of July 2012. The plant material was identified and authenticated[11]. The voucher sample was prepared and deposited in the herbarium of Botany Department, Bangalore University, Bangalore, with voucher number KE 118. The freshly collected leaves were washed, shade dried at room temperature, ground to fine powder, and stored in air tight polythene bags until use[12].

2.1. Preparation of extracts

Twenty grams of powdered leaf sample was used for extraction via a Soxhlet system using 200 mL of different solvents like petroleum ether, chloroform, and methanol for 16-20 h. Extracts were subsequently concentrated under reduced pressure. The resulting residue was then filtered and stored at 4 °C. For water extracts, 20 g of powdered leaf sample was kept in water bath for 8 h at 40 °C. Extract was filtered, centrifuged, concentrated and stored at 4 °C until use. All extracts were dissolved in respective solvents prior to analysis and subjected to quantitative determination for the presence of primary and secondary metabolites[13].

2.2. Quantitative phytochemical analysis

Quantitative phytochemical analysis was performed with focus on testing different chemical groups present in different solvent extracts of *E. kologa* leaf.

2.2.1. Determination of phenols

The total phenolic content in various solvent extracts of *E. kologa* leaf was determined according to method employed by using catechol as standard[14]. A volume of 1 mL of different aliquots of plant extract was mixed with 1mL of FC reagent and 3 mL of 20% Na_2CO_3 solution. The mixture was incubated for 40 min at room temperature and absorbence was measured at 760 nm.

2.2.2. Determination of flavonoids

An aluminum chloride colorimetric method was used to determine the flavonoids content[15]. The plant extracts in different solvents were mixed with 0.5 mL of aluminum chloride (1.2%) and 0.5 mL of 120 mmol/L potassium acetate. The mixture was allowed to stand for 30 min at room temperature. The absorbance was measured at 415 nm. Flavonoid content was expressed in terms of rutin equivalent.

2.2.3. Determination of carbohydrates

Different aliquots of leaf extracts were prepared and final volume was made up to 1 mL with distilled water. About 4 mL of anthrone reagent, prepared in ice cold H_2SO_4 was added, incubated for 10 min in boiling water and absorbance was measured at 630 nm. A standard curve was prepared using glucose[16].

2.2.4. Determination of proteins

Different aliquots of the sample were prepared and final volume was made to 1 mL by distilled water. A total of 5 mL of copper reagent was added and incubated for 10 min, 0.5 mL of Folin's reagent was mixed and incubated at room temperature for 30 min and absorbance was measured at 660 nm. BSA was used to prepare the standard curve[17].

2.2.5. High performance liquid chromatography (HPLC)

Phenols and flavonoids analyses for each of the four solvent extracts of *E. kologa* leaf were conducted with HPLC by Waters HPLC (model 510, column C_{18}). The phenols and flavonoids were detected at 254 nm and 215 nm, respectively using mobile phase 7:3 acetonitrile and water, eluted isometrically. Phenols were compared with catechol and flavonoids by using rutin as standard. All samples and the mobile phase were filtered through a 0.4 μm membrane filter (Millipore) and degassed by ultrasonic bath prior to use. Quantification was performed in comparison to linear calibration curves using standard compounds.

2.3. Antioxidant activity

Antioxidant activity of different solvent extracts of *E. kologa* leaf was estimated using 1-1-diphenyl-2-picrylhydrazyl (DPPH*)[18]. About 1 mL of each solvent extract prepared in multiple concentrations was mixed with 3 mL of DPPH* prepared in methanol. The mixture was incubated at 37 °C for 30 min and absorbance was measured at 517 nm, using a spectrophotometer. A decrease in DPPH* solution absorbance indicates an increase in radical scavenging activity. The percent of DPPH* radical scavenging activity of the sample was calculated according to the formula:

$$\text{Antiradical activity} = A_{control} - A_{sample} / A_{control} \times 100$$

Assays were performed in triplicate for each sample and at each concentration.

2.4. Antibacterial activity

Antibacterial activity of solvent extracts was determined by the agar well diffusion method on nutrient agar media[19]. Bacterial cultures including Gram-positive (*Bacillus subtilis*) and Gram-negative (*Pseudomonas aeruginosa*) were used in this study. On the agar plates, 6 mm diameter wells were punched and filled with 100 μL of sample extract. The plates inoculated with test pathogens were incubated at 37 °C for 24 h and zone of inhibition around the wells were measured (in millimeters). The solvent used for extraction was tested as a control. Effects were compared with that of standard antibiotic amoxicillin at concentration of 1 mg/mL[20]. All assays were performed in triplicate.

2.5. Statistical analysis

The experiment was conducted in triplicates and data were expressed as mean±SEM. The difference between the means was analyzed by ANOVA test. The level of significance was set at $P<0.05$

3. Results

Table 1 summarizes the results of quantitative assessment of phytochemical composition of *E. kologa* with a focus on phenols, flavonoids, carbohydrates and proteins of different solvent extracts from leaf material. These results indicate that carbohydrate concentration is higher in petroleum ether extract (0.98 mg/g), moderate in water and chloroform extracts (0.75 and 0.73 mg/g, respectively) and lower in methanol extracts (0.50 mg/g). The data indicated that protein was higher in water extract (0.94 mg/g) and low in chloroform extract (0.25 mg/g). Methanol and petroleum ether extract showed moderate protein concentrations. HPLC analyses resulted in a total phenol content range of 9.42 mg/g (in chloroform extract) to 23.11 mg/g (in petroleum ether extract). Flavonoid content was low in chloroform extract (5.20 mg/g) and high in petroleum ether extract (21.19 mg/g). The HPLC chromatogram indicating total phenol and flavonoid content across all extracts and compared to controls are shown in Figures 1 and 2. Phenol and flavonoids were determined to be highest in petroleum ether extract followed by methanol, water and chloroform.

Table 1

Quantitative phytochemical estimation of *E. kologa* leaf (mg/g).

Solvent extracts	Phenols	Flavonoids	Carbohydrate	Protein
Petroleum ether	23.11±0.31	21.19±0.45	0.500±0.038	0.980±0.051
Chloroform	9.42±0.43	5.20±0.06	0.250±0.012	0.730±0.033
Methanol	18.99±0.24	19.99±0.29	0.800±0.029	0.500±0.042
Water	20.04±0.09	18.79±0.53	0.940±0.018	0.750±0.088

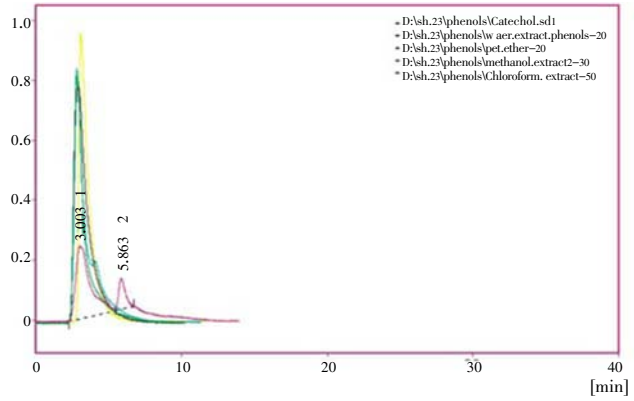

Figure 1. The HPLC chromatogram of total phenol content.

Based on the above results, methanol extract was chosen for the antioxidant studies. Free radical scavenging of methanol leaf extract was measured using DPPH*, which is frequently used for the evaluation of the antioxidation potential of plant extracts. The DPPH* radical is a stable organic free radical with absorption maxima at 517 nm. It loses this optimal absorption when accepting an electron, resulting in color variation from purple to yellow. The degree of discoloration indicates the scavenging potential of antioxidant compounds[21]. Table 2 summarizes radical scavenging activity of methanol extract of *E. kologa* leaf compared to a standard (*i.e.*, ascorbic acid). Concentration of the sample necessary to decrease initial concentration of DPPH* (IC_{50}) under the experimental condition was calculated. The IC_{50} values of the sample extract were found to be 62.20 μg/mL and ascorbic acid was 49.30 μg/mL. The extract was found to have less antioxidant activity than the standard. Scavenging activity of DPPH* ranged from 2.21% at 20 μg/mL concentration to 60.14% at 100 μg/mL concentration. Ascorbic acid was able to scavenge 85.35% at 100 μg/mL concentration. Radical scavenging activity is likely to be related to the nature of phytochemicals and their hydrogen donating ability to reactive free radicals, converting them into more stable non reactive species as previously reported[22].

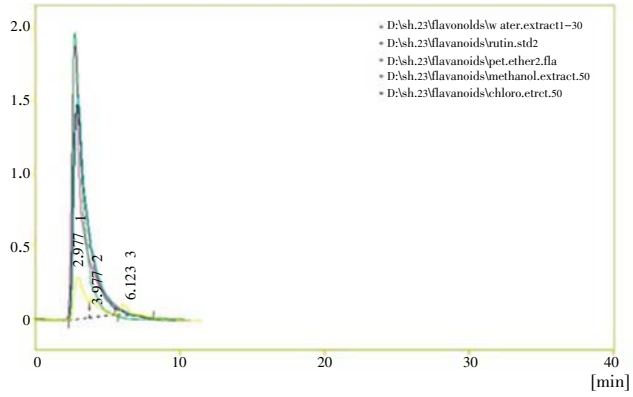

Figure 2. The HPLC chromatogram of flavonoid content.

Table 2

DPPH* radical scavenging activity of methanol extract of *E. kologa* leaf.

Concentration μg/mL	Methanol extract		Ascorbic acid	
	% Inhibition	IC_{50} (μg/mL)	% Inhibition	IC_{50} (μg/mL)
20	2.21±0.35		4.37±0.15	
40	11.14±0.29		20.76±0.54	
60	16.72±0.15	62.20	27.65±0.27	49.50
80	33.22±0.24		50.60±0.10	
100	60.14±0.77		85.35±0.51	

Methanol extract exhibited greater antibacterial activity when compared to petroleum ether, chloroform and water extracts, which did not show any significant zones of inhibition when compared with control. Methanol extracts showed significant antibacterial activity against *Bacillus subtilis* (Gram-positive) when compared with *Pseudomonas aeruginosa* (Gram-negative). The antibiotic control (*i.e.*, amoxicillin), significantly inhibited both of the tested bacteria compared to the examined extracts. The antimicrobial properties of plant extracts have recently attracted more attention. However, comprehensive evaluation of the biological or pharmacological properties has only been reported for a few plant groups[23]. Here, we demonstrate that leaf extracts of *E. kologa* possess antibacterial activity. The molecular basis for this activity remains to be elucidated. It has been observed that plants generally survive from microbial attack through the presence or accumulation of antimicrobial metabolites.

4. Discussion

Phenols, widely distributed in plants have gained much attention due to their free radical scavenging ability which has beneficial effects on human health[24]. The present study clearly indicates that methanol extracts of *E. kologa* leaf material are not only rich in proteins and carbohydrates but also possess significant antioxidant and antibacterial activity. It is well known that carbohydrates and proteins add nutritional value required for plant growth and for animals that consume plants. Studies have shown that many antioxidant compounds posses antibacterial or antiviral activity to a greater or lesser extent[25]. This study suggests that methanol extract from *E. kologa* leaves are a rich source of antioxidants that may be developed as a functional ingredient in nutraceutical or pharmaceutical products.

Conflict of interest statement

We declare that we have no conflict of interest.

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