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Research Article

TOTAL PHENOLIC CONTENT, FLAVANOID CONTENT, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF CLERODENDRUM SERRATUM, LINN LEAF EXTRACTS

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A R T I C L E I N F O

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A B S T R A C T

Background: The aim of the present study is to assess the total Phenolics, total flavanoid, antioxidant and antimicrobial activities of different extracts of *Clerodendrum serratum* L.
Methods: Subsequent extractions were done by using socklet apparatus with solvents with increasing polarity petroleum ether, chloroform, methanol and water for estimation of phenols, flavanoids antioxidant and antimicrobial activity.
Result: Methanolic extract has the highest amount of total phenolic (7.95±0.09 mg GAE/g) total flavanoid (39.11±0.32 µg QE/g). The greatest scavenging capacity was found in methanolic extract (IC_{50}=15.96 µg/mL), the total antioxidant capacity was high in the methanolic extract (IC_{50}=19.86 µg/mL). *S. aurenus, M. lutens, E. faecium, L. monocytogenes, S. typi* showed the highest zone of inhibition in methanolic extract where as *E. coli* and *P. aeruginosa* in water extract.
Conclusion: selective extraction of bioactive molecules from the natural sources with appropriate solvent can provide compounds with high biological activity.

INTRODUCTION

Taxonomically *Clerrodendrum serratum*, L belongs to verbinaceae family, is slightly woody shrub with bluntly stem and branches. It's about 2-8 ft high, usually aromatic, stem quadrangular, leaves three at a node, flowers are blue to purple many in a long cylindrical thyrsus. The plant is widely distributed in Western Ghats of India[1]. The genus belongs to tropical and warm temperature regions of the world[2]

Clerrodendrum serratum, L is known as Bharangi in Ayurveda and Siretekku in Sidda system of medicine, which is used in treating pain, inflammation, rheumatism, respiratory disease and malarial fever[3].

Medicinally the roots are used in curing diseases like syphilis, cancer, snakebites, inflammation, epilepsy, malaria, ulcer, wound and hypertension[4], asthma and rheumatism[5]. The roots also shown the significant role in Alzheimer's disease in mice[6,7]. The root bark contains sapogenins[8] and is reported to be one of the ingredients of the ayurvedic drug 'Kasa daman' an effective expectorant and antitussive remedy[9] and are mainly investigated for its anti-inflamatory, analgestic, antipyretic properties[10], hepatoprotective properties[11]. The aqueous extracts of the leaves posses bronchodilator property[12]. Many phytochemical constituents reported in the leaves such as carbohydrates, phenoics, flavanoids, terpenoids and steroids. Carbohydrates which includes the mixture of glucose, arabinose and glucuronic acid. Phenolic acids such as caffeic acid and ferulic acid. Flavanoids include catechin, α-spinasterol, luteolin, apigenin, baicalein, scutellarein, 6-OH-luteolin, luteolin-7-0-β-D-glucuronide[13].

The aim of the present study were to determine the amount of total phenolic, total flavanoid, antioxidant capacity and antimicrobial activity of crude extracts of *C. serratum* leaves in different solvents as the polarity of solvents determines the biologically active compounds extracted from the plants. The use of solvents with higher polarity has proved to be effective in isolation of biologically active compounds compared to the use of water[14].

MATERIAL AND METHODS

Collection of plant material and preparation of extracts

The plant (leaves) were collected from in and around different localities of Karnatak University, campus, Dharwad. Karnataka, India. The materials were collected in polythene bags and brought to the laboratory. Plants were thoroughly washed under running tap water to remove the dust particles, air dried in shade at room temperature and made into fine powder and stored in sealed plastic container for further analysis. 20 gram of plant powder was extracted with subsequently with petroleum ether, chloroform, methanol and water solvent using soxhlet extractor for 18 – 20 hours at 30-60 ºC depending on the solvent used.

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Determination of total phenolics

The amount of total phenolics was determined by with slight modification of Follin-Ciocatteu method[15]. Briefly, 100µL of aliquots of extracts were mixed with 2.5 mL of distilled water than with 1.0 mL of 2N of Follin-Ciocatteu reagent and allowed to stand for 5 min followed by 0.5ml of 20% sodium bicarbonate solution. The mixture is allowed to incubate at room temperature for about 30 min and absorbance is measured at 760nm on spectrophotometer. The phenolic content in mixture is measured as Gallic acid equivalent (mg GAE/g of dry weight)

Determination of total flavanoids

The total flavanoid contain is determined by spectophotometrically[16], briefly 0.25mL of extracts was mixed with 1.25 mL of distilled water followed by the addition of 75µL of 5% sodium nitrate solution, after 6 min 0.15mL of 10% aluminium chloride solution was added followed by 0.5 ml of 1 M of sodium hydroxide and the mixture is allowed to stand for another 5 min. the absorbance is measured immediately at 510 nm on the spectrophotometer. The flavanoid contain were reported as querencetin equivalent (µg QE/g of dry weight).

DPPH assay

The electron donating ability of the obtained extracts was measured by bleaching a purple solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical[17]. Briefly the extracts (0.1 mL, 5, 10, 50 and 100 mg/mL) were added to 0.5 mL of 0.2 mmol/L DPPH–methanol solution. After incubation for 30 min at room temperature, the absorbance was determined against a blank at 517 nm. The percentage inhibition of free radical DPPH was calculated as

% radical scavenging = [(Ac-As)/Ac] × 100

Where Ac is absorbance of control, As is absorbance of the sample. The concentration of extract that caused 50% inhibition (IC_{50}) was calculated from the regression equation for the concentration of extract and percentage inhibition. Butylated hydroxy-toluene was used as a positive control. Samples were analyzed in triplicate.

Total antioxidant capacity

The assay is based on the reduction of Mo(VI) to Mo(V) by the extract and subsequent formation of a green phosphate–Mo(V) complex at acid pH by the method described by Prieto et al. An aliquot (0.1 mL) of plant extract was added to1.0 mL of reagent solution (0.6 mol/L sulphuric acid, 28 mmol/L sodium phosphate and 4 mmol/L ammonium molybdate). The tubes were incubated in a thermal block at 95◦C for 90 min, the mixture was than cooled to room temperature and the absorbance of each solution was measured at 695 nm against a blank. Antioxidant capacity was expressed as mg gallic acid equivalent per gram dry weight (mg GAE/g DW). The calibration curve range was 0–500 µg/mL. All samples were analyzed in triplicate.

Antimicrobial activity

Microorganisms

We screened antimicrobial activity against seven human pathogenic bacteria, four Gram-positive bacteria namely (*Staphylococcus aureus* ATCC 25923, *Micrococcus luteus* NCIMB8166, *Enterococcus faecium* ATCC 29212 and *Listeria monocytogenes* ATCC 19115) and three Gram-negative bacteria namely (*Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853 and *Salmonella typhi* LT2)

Preparation of test discs

The extracts were dissolved in dimethyl sulfoxide to final concentrations of 50 and 12.5 mg/mL. Sterile discs (6 mm in diameter) were impregnated with 20 µL of the extracts (50 mg/mL and 25 mg/mL, respectively). Negative controls were prepared on discs impregnated with dimethylsulfoxide (solvent control). Gentamicin (10 UI) was used as the positive reference for all bacterial strains.

Disc diffusion assay

Inoculation was done as per the method described by Freney *et al*[19], the bacterial strains were prepared from 18-h cultures, and suspensions were adjusted to 0.5 at 570 nm with a spectrophotometer. Petri dishes were prepared with 20 mL of Mueller Hinton agar; the inoculation were spread on top of the solidified medium and allowed to dry for 60 min. The discs with extract were then applied and the plates were left for 30 min at room temperature to allow diffusion of the extract, before incubation for 24 h at 37 ^{0}C. The diameter of the inhibition halo was evaluated in millimetres. Each assay was repeated in triplicate.

Statistical analysis

Data were analyzed with statistical software. Analysis of variance (ANOVA) and Duncan's multiple range method were used to compare any significant differences between solvents and samples. Values were expressed as means ± standard deviations. Differences were considered significant at $p < 0.05$.

RESULTS

Total phenolics and total flavaoid content

The recovery of phenolics from the methanolic extract was higher from the other extracts (Table 1). The efficiency of solvents for the polyphenols extracts were in descending order methanol followed by water than chloroform and petroleum ether. The content of the phenolics is three times higher in methanolic extract (7.95±0.09 mg/g) and two times higher in water extract (4.93±0.01 mg/g) than in petroleum ether extract (2.43±0.52 mg/g). The chloroform extract contains slightly higher amount of phenolics (3.81±0.31 mg/g) than Petroleum ether. Methanolic extract has the greatest amount of flavanoid (39.11±0.32 µg QE/g) followed by water (22.48±0.02 µg QE/g), chloroform (12.18±0.02 µg QE/g) and petroleum ether (8.29±0.38 µg QE/g)

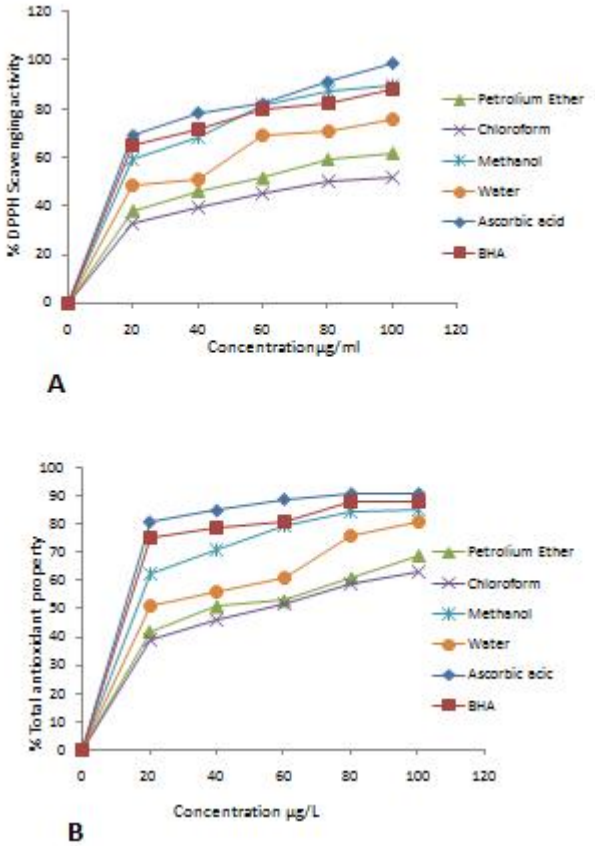
Table 1 Concentration of Total Phenolics and Flavanoids in solvent extracts C. serratum, L leaves

solvent	Total phenolics (mg GAE/g)	Favanoids (µg QE/g)
Petroleum ether	2.43±0.52d	8.29±0.38d
Chloroform	3.81±0.31c	12.18±0.02c
Methanol	7.95±0.09a	39.11±0.32a
water	4.93±0.01b	22.48±0.02b

Data are Mean±SD; mean separation within columns by Duncan's test p<0.05

Antioxidant activity

The DPPH radical scavenging activity of the leaf extracts of C. serratum increased with the increase in the concentration (Figure 1). Greater ability to scavenging the free radicals was show by the methanolic extract (IC_{50}=15.96 µg/mL), followed by water extract (IC_{50}=21.58 µg/mL), chloroform (IC_{50}=27.52 µg/mL) and then by petroleum ether (IC_{50}=29.83 µg/mL). The IC_{50} value for the standard used Ascorbic acid is 4.96 µg/mL and for BHA (Butylated hydroxy anisole) 11.09 µg/mL. The total antioxidant capacity (TAC) of the leaf extract was measured by phopsho molybdenum method where Mo(IV) is reduced to Mo(V) to form phosphate/Mo (V) complex which is usually green in color. TAC is directly prepositional to the concentration where, increase in the concentration showed increase activity (Figure 2). The methanolic extract (IC_{50}=19.86 µg/mL), followed by water extract (IC_{50}=25.48 µg/mL), chloroform (IC_{50}=28.96 µg/mL) and then by petroleum ether (IC_{50}=30.25 µg/mL). The IC_{50} value for the standard used Ascorbic acid is 12.52 µg/mL and for BHA (Butylatedhydroxy anisole) 14.95 µg/mL.



Figure, **A.** % DPPH Scavenging activity, **B.** % Total antioxidant activity of *C. serratum*.

Antimicrobial activity

Antimicrobial activity is represented as zone of inhibition of tested bacteria. Zone of inhibition for both gram positive and gram negative bacteria is represented in table 2. Methanolic extract showed the highest zone of inhibition for *S. aurenus* (16.25±012 in 500 µL), *M. lutens* (15.82±0.26 in 250 µL), *E. faecium* (9.98±0.14 in 250 µL) *L. monocytogenes* (13.58±0.21 in 250 µL), S. typi (15.21±0.25 in 250 µL) followed by water and chloroform (except for *E.faecium* did not show the response). In water extract *E. coli* (14.56±0.09 in 250 µL) and *P. aeruginosa* (16.29±0.14 in 250 µL) followed by methanolic extract and then choloform. Petroleum ether extract did not show any response against the tested pathogens.

DISCUSSION

We have determined the total phenolic, total flavanoid content, antioxidant and antibacterial activities of leaf extracts of *Clerodendrum serratum* L. Phenolic and flavanoid content is depends on the polarity of the solvent used. Methanolic extract gave the highest yield of total phenolis and flavanoids. Phenolic compounds have redox properties which act as antioxidant[20]. Where, their hydroxyl groups act as free radical for scavenging activity. On the other hand flavanoid which is the secondary metabolite act as antioxidant agent due to the presence of 3-OH group and has antioxidant activity in both *in vivo* and *in vitro*[21,22]. In the previous studies ethanol and water is used for extraction, where ethanol has the higher content of phenols and flavanoids[23]. Solvent polarity plays important role increasing phenolic solubility[24]. Therefore it is difficult to define a suitable procedure for the extraction of phenols, flavanoids and other secondary metabolites in the plant. Every plant contains different types of antioxidant depending on the compounds present in them and it is difficult to measure the scavenging activity of each and every compound associated with it.

Antioxidant capacity is associated with high phenolic and flavanoid. Structurally these compounds content anaromatic ring with one or more hydroxyl groups which helps to donate hydrogen atoms or electrons or chelate metal cations which in turn helps to scavenging the free radicals. In our study it is methanol and the aqueous extracts which showed the higher scavenging activity than chloroform and petroleum ether. In the present study both gram positive and gram negative bacteria were used where, methanolic extract showed the highest zone of inhibition for *S. aurenus*, *M. lutens*, *E. faecium*, *L. monocytogenes*, S. typi. While *E. coli* and *P. aeruginosa* has the highest zone of inhibition in water extract. The antibacterial activity plays important role as these bacteria's are resistant to number of antibiotics and produce toxins that causes many types of enteritis and septicaemia.

Table 2 Antimicrobial activity of leaf extracts of *C. serratum* against human pathogenic bacteria.

Solvent	Concentration µL/disc	S.*aureus*	M.*luteus*	*E.faecium*	*L.monocytogenes*	*E.coli*	*P.aeruginosa*	*S.typhi*
Petroleum ether	250	-	-	-	-	-	-	-
	500	-	-	-	-	-	-	-
Chloroform	250	8.21±0.05d	6.21±0.52d	-	13.52±0.12ab	8.56±0.32ds	7.62±0.24c	10.25±0.06bc
	500	9.26±0.09d	7.23±0.14d	-	12.58±0.34b	6.94±0.14d	8.39±0.12c	12.69±0.01b
Methanol	250	15.62±0.05b	15.82±0.26b	9.23±0.05b	16.78±0.28b	13.58±0.21b	12.25±0.31b	15.21±0.25ab
	500	16.25±012b	14.62±0.14b	9.98±0.14b	14.59±0.14ab	10.28±0.04bc	14.52±0.52b	14.85±0.09ab
water	250	12.25±0.08c	10.33±0.08c	7.52±0.02b	11.32±0.25b	14.56±0.09b	16.29±0.14a	12.95±0.13b
	500	13.62±0.14bc	9.85±0.15c	6.12±0.01b	9.25±0.07c	12.36±0.14bc	13.69±0.52b	11.25±0.25b
Gentamicin	10UI	20.21±0.08a	28.85±0.3a	20.09±0.18a	2.19±0.02d	26.58±0.32a	18.64±0.31a	22.29±0.14a

–, no antimicrobial activity; The inhibition zone of the control, gentamycin (10 UI), was >15 mm for all bacteria; the diameter of disc was 6 mm. Each experiment was done in triplicate.

Earlier studies have shown that extracts of *C. serratum* is effective against *Staphylococcus aurens* and *Bacillus subtilis*[23]. Many species of *Clerodendrum* have shown the antioxidant property where ethanol and methanol extracts have shown the greater affinity to induce antioxidant property than water such as ethanolic extract of *C. inerme*[25], C. colebrookianum[26]. Methanolic extract of *Clerodendron inerme*[27], *C. trichotomum*[28] have shown similar results to that of present study on *C. serratum.* The biological activities in the leaf extract observed in the present study were due to bioactive compounds present in the extraction solvent.

CONCLUSION

The leaf extracts of *C. serratum* in methanol and water were more effective than petroleum ether and chloroform. The result indicate that selective extraction of bioactive molecules from the natural sources with appropriate solvent can provide compounds with high biological activity that could be used in food and pharmaceutical industries.

DECLARATION

The authors report no conflict of interest

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