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Extraction and *in vitro* biological screening of bioactive compounds from leaves of *Lagerstroemia speciosa* and detection of metals from whole plant

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Abstract : The present research paper reports extraction of the bioactive compounds from leaves of *L. speciosa* (*Lythraceae* - Family). The Soxhlet extraction was performed for various solvents like, water, chloroform, petroleum ether etc. and screened for its phytochemical analysis. Tannins, terpenoids, and carbohydrate were found to be the major secondary metabolites. The leaves were subjected to nitric acid digestion – conventional method followed by the detection of metals by Atomic Absorption Spectroscopy and Flame Photometry. Magnesium, zinc and chromium were found to be predominant, while iron, sodium and potassium were also found in minimum amounts. The detection of metals was performed for whole plant. The leaf extracts were subjected to *in vitro* antibacterial and hemolytic assay.

Keywords : Natural products, bioactive compounds, metals and *in vitro* biological screening.

Introduction

In recent years scientist put their effort to isolate the bioactive compounds from plant biodiversity, in order to cure the diseases via traditional healing methods. According to WHO 80% of world's population depends on traditional medicine. Even toxic plants also possess some pharmacological activity[1]. Diabetes is a disease carried from 2nd century AD and it is characterized by excess of sugar in blood and urine[2]. In the present medicine era, there are some patients who are shifting to alternative medicines. Lot of etho-botanical survey is being carried out in different parts of the world and few have been reported for antidiabetic. In addition, few bioactive compounds like alkaloids, tannins, glycosides, terpenoids are being isolated as lead molecules and possess predominant activities[3]. To possess synergistic effect, even metals play a vital role in the treatment of various diseases, e.g. researcher's found that iron, copper, zinc etc. act on the brain's degenerative diseases like Alzheimer's and Parkinson's[4,5]. Similarly zinc, cadmium and magnesium are used to cure diabetes. In this present paper an attempt has been made to extract the bioactive compounds, detect the eco-friendly/toxic metals and preliminary biological screening like antibacterial and hemolytic assay has been performed.

Results and discussion

All leaf extracts showed good yield of 70–80%. The preliminary phytochemical analysis showed terpenoids; tannins were found to be predominant and tabulated below Table 1.

Table 1. Phytochemical screening of leaf extracts of *L. speciosa*

Test for	Aqueous extract	Chloroform extract	Pet. ether extract
Proteins	–	–	–
Carbohydrates	++	++	+
Glycosides	–	+++	+
Alkaloids	++	+	+
Terpenoids	+++	+++	+++
Saponins	+	–	–
Tannins	+++	+++	+++
Phytosterols	+	–	–
Flavanoids	+	++	++
Gums and mucilage	–	–	–

(+++) - present, (–) - absent.

The conventional methods showed good metal extraction rate than microwave method, where magnesium and zinc was found to be predominant in leaves than in other parts of the plant is tabulated in Table 2. In case of mi-

Table 2. Detection of metals from *L. speciosa*

Conventional	Samples	Zn	Fe	Cu	Mn	Na	K	Cd	Mg
	Leaf	0.837	2.422	0.021	0.0121	0.47	3.09	BDL	32.6
	Stem	0.521	0.492	0.037	0.0132	4.4	17.4	0.004	22.6
	Trunk	0.0527	0.231	0.011	0.0164	88.8	28.1	0.007	4.779
	Soil	0.1934	3.434	0.072	0.0633	226	4.2	0.090	1.150
Microwave	Leaf	0.154	0.0791	0.076	0.0110	5.5	30.7	0.004	1.821
	Stem	0.2098	0.162	0.017	0.0127	165.7	66.8	0.008	3.531
	Trunk	0.0469	0.115	0.016	0.0200	164.8	8.1	0.009	2.71
	Soil	0.0867	6.669	0.015	0.0507	241	6.5	0.009	3.08

BDL: Below Detectable Limit.

crowave method potassium and sodium was found to be more, because it is due to the fertilizer which is used as manure for the plant.

The bioactive compound of leaves of *L. speciosa* was extracted in different polarity solvents like aqueous, chloroform and petroleum ether extracts were screened for antibacterial activity against test organisms by agar well diffusion method. In this study aqueous extract showed good activity against all test organisms, whereas the chloroform extract showed better activity against *B. cereus* and *K. pneumoniae*. While petroleum ether extract didn't show activity against *S. aureus* (Table 3). All the leaf extracts in different concentrations showed non-hemolytic activity against human erythrocyte cells. Hence the plant was found to be non-toxic.

Table 3. *In vitro* antibacterial activity of leaves of *L. speciosa*

Samples	Zone of inhibition (mm)			
	S. aureus	*B. cereus*	*E. coli*	*K. pneumoniae*
Aqueous extract	18 ± 0.2	20 ± 0.1	17 ± 0.3	20 ± 0.1
Chloroform extract	–	19 ± 0.1	–	17 ± 0.2
Pet ether extract	–	8 ± 0.1	10 ± 0.2	12 ± 0.4
Chloramphenicol (standard)	22 ± 0.2	18 ± 0.1	24 ± 0.2	27 ± 0.1

Materials and methods :

Collection and processing :

The leaves of *Lagerstroemia speciosa* were collected from the Garden of VIT University, Vellore, Tamilnadu, India, in the month of May-July. It was authenticated by Dr. P. Jayaraman, PARC, Tambaram, Tamilnadu. The matured leaves of the plant were identified, collected and stored in a dry place; care should be taken by avoiding contact with the sunlight (shade dried for 20–25 days). Finally the leaves were crushed into powder by blender.

Extraction :

Hot continuous percolation method was used to extract the secondary metabolites using different solvents like water, chloroform and petroleum ether. Excess of solvent was removed by simple distillation technique.

Phytochemical analysis :

The crude extracts were subjected to preliminary phytochemical screening like alkaloids, flavonoids, glycosides, tannins etc. by Harbone[6].

Detection of metals :

Eco-friendly and toxic metals were extracted by both conventional and microwave methods[7]. 1 g of leaves was weighed into 250 ml digestion tube and add 10 ml of nitric acid. The sample was heated at 90 °C for 1 h and the temperature was raised to 150 °C. The process is continued until the clear solution was obtained. During the digestion process nitric acid was added in regular intervals and it was continued until the volume was reduced to 1 ml. In order to prevent the loss of sample the inner walls of the tube is washed with distilled water. After cooling 5–6 ml of 1% nitric acid was added to the sample and filtered in Whatmann filter paper. The sample was made upto 25 ml with distilled water, subjected to flame photometry and Atomic Absorption Spectroscopy.

In vitro Biological screening :

Test organism : Bacterial cultures were collected from Microbial type culture collection (MTCC), IMTECH, Chandigarh, India. Test organism includes *Staphylococcus aureus* (MTCC 9760), *Bacillus cereus* (MTCC 9411), *Escherichia coli* (MTCC 1679) and *Klebsiella pneumoniae* (MTCC 9401). The test organism are maintained in glycerol stock and stored at –20 °C.

Antibacterial assay – Agar well diffusion technique and antihemolytic activity :

Antibacterial screening was performed by agar well diffusion method on Mueller Hinton agar[8–10] for aqueous, chloroform and pet. ether extracts. The clinical isolates were inoculated in nutrient broth and incubated for 24 h at 37 °C. The turbidity of the broth was adjusted at 0.5 (optical density) using spectrophotometer. The bacterial cultures such as *S. aureus, B. cereus, E. coli* and *K. pneumoniae* were inoculated on mueller hinton agar plates using sterilized cotton swabs. In each of these plates, wells were cut out using a sterilized gel borer. The 100 µL of sample were loaded into each well. Plates were incubated at 37 °C for 24 h. After incubation, all plates were examined for the presence of zone of inhibition around the Wells.

Preparation of erythrocyte suspension :

Five milliliter of blood was collected from healthy individual (Blood group - O positive) into a tube containing heparin. The blood was centrifuge at 1500 rpm for 3 min at the laboratory centrifuge. Plasma (supernant) was discarded and the serum was collected, washed with phosphate buffer saline solution (pH – 7.2 ± 0.2) by centrifugation at 1500 rpm for 5 min. The cells were resuspended in 0.5% normal saline solution. *In vitro* anti-hemolytic activity was performed by spectrophotometer method by using various concentration of samples (125, 250, 500 and 1000 µg/ml in phosphate buffer solution)[11,12].

Conclusion

The leaf extracts showed good yield and the major secondary metabolite which was found to be tannins and terpenoids upon qualitative analysis. The aqueous extract showed good *in vitro* antibacterial activity against all test organisms, also all the extracts were found to be non-hemolytic against human erythrocyte cells, due to the presence of secondary metabolites, friendly metals like zinc and magnesium found in considerable amounts. An *in vitro* antidiabetic and anticancer activities will be performed in near future, since metals and bioactive compounds of the plant, play a vital part in curing such predominant diseases.

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