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Elemental analysis, antimicrobial and radical scavenging activity of *Lagerstroemia speciosa* (L.) flower

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ABSTRACT

The present study was conducted to estimate the mineral elements and to determine antimicrobial and radical scavenging efficacy of flower of Lagerstroemia speciosa (L.) belonging to the family Lythraceae. Estimation of mineral elements present in microwave digested sample of flower was done by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES). Antimicrobial activity of methanol extract of flower was determined against 5 isolates of Streptococcus mutans, 5 isolates of Staphylococcus aureus, Candida albicans and Cryptococcus neoformans by Agar well diffusion assay. Radical scavenging activity was performed by ABTS radical scavenging assay. The content of potassium and sodium was highest and least among major elements respectively. In case of minor elements, content of iron was highest whereas the content of nickel was least. The extract was effective in inhibiting all test microorganisms. Among bacteria, high susceptibility was observed in case of S. aureus isolates. C. neoformans was inhibited to high extent than C. albicans. The extract scavenged ABTS radicals in a dose dependent manner. The flower of L. speciosa can be used as a source of various mineral elements having key role in the normal physiology of an individual. The flower may also be used against diseases caused by infectious agents and oxidative damage caused by free radicals.

Key words: *Lagerstroemia speciosa*, Elements, ICP-OES, Antimicrobial, ABTS

INTRODUCTION

Lagerstroemia speciosa (L.) belonging to the family Lythraceae is popularly known as Banaba and is an ornamental plant growing widely in Philippines, India and South East Asian countries. In India, the plant is distributed in tropical Himalaya, Assam, Western and Eastern Ghats. Seed is narcotic. Root is astringent, stimulant, febrifuge. Fruit is used for aphthae of the mouth. Leaves are used as purgative, diuretic and deobstruent. An infusion of bark is given in diarrhoea and abdominal pain. A decoction of the leaves, also of dried fruits, is used like tea for diabetes mellitus in Philippines [1,2,3]. Extracts and purified compounds from various parts of the plant have shown to possess hypoglycemic [1,4,5,6,7], antibacterial [8,9,10], antinociceptive [11,12], antidiarrhoeal [12], cytotoxic [12], antioxidant [13,14], hepatoprotective [15].

Edible flowers are becoming more popular and people use flowers for garnishing meal or as ingredients in salads, soups, desserts, and drinks. Flowers have traditionally been used as medicine and for cooking in various parts of the world. Flowers have medicinal properties as well as nutritional value and it is believed that consumption of these flowers can cure illness and diseases. Natural antioxidants *viz*., phenolic acids, flavonoids, anthocyanins and other phenolic compounds are found in flowers. Flowers are shown to possess biological activities *viz*., antimicrobial, acaricidal, antioxidant, anti-HIV, cytotoxic, antidepressant, anti-inflammatory etc. The high antioxidant activity of flowers may be attributed to the level of polyphenol compounds including flavonoid. They are also promising

sources of various minerals which have key role in the normal health of an individual [16-25]. On searching literatures, it was found that elemental analysis and bioactivities of flowers of *L. speciosa* remain unexplored. Hence, in the present study, we have estimated the mineral elements by ICP-OES and antimicrobial and antioxidant activity of flower of *L. speciosa*.

EXPERIMENTAL SECTION

Collection and identification of plant material

Flowers were collected at college campus during March 2013 and authenticated by Prof. D. Rudrappa, Department of Botany, S.R.N.M.N College of Applied Sciences, Shivamogga. The flowers were shade dried and powdered in a blender. The powdered flower material was stored in air-tight container until use.

Elemental analysis of dried flower

1gm of powdered flower material was digested in 10ml of ultrapure metal free nitric acid in a microwave digester followed by diluting the content to 25ml with distilled water. The digested sample was aspirated into ICP-OES (Agilent Technologies 700series, US) to estimate macroelements*viz*., Calcium (Ca), Potassium (K), Sodium (Na) and Magnesium (Mg) and microelements *viz*., Manganese (Mn), Iron (Fe), Zinc (Zn), Nickel (Ni), Chromium (Cr), Lithium (Li) and Copper (Cu) present in the flower material. The calibration standards were prepared by diluting the stock multi-elemental standard solution in nitric acid [26]. Table 1 shows the instrument configuration and experimental conditions.

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)

Extraction of powdered flower

For extraction, about 25g of dried and powdered flower material was taken and extracted with methanol in Soxhlet apparatus. The extract was filtered over Whatman No. 1 filter paper, concentrated in vacuum under reduced pressure and dried in the desiccator [27].

Antimicrobial activity of flower extract

In order to determine antimicrobial activity, we have performed Agar well diffusion assay. Antibacterial activity was tested against 5 isolates of *Streptococcus mutans* (isolated previously from dental caries subjects) and 5 isolates of *Staphylococcus aureus* (isolated from burn specimens). Antifungal activity was determined against *Candida albicans* NCIM-3466 and *Cryptococcus neoformans*NCIM-3378. The test bacteria and fungi were inoculated into sterile Nutrient broth (HiMedia, Mumbai) tubes and Sabouraud dextrose broth (HiMedia, Mumbai) tubes respectively. The broth cultures of bacteria and fungi were then aseptically swabbed on sterile Nutrient agar (HiMedia, Mumbai) and Sabouraud dextrose agar (HiMedia, Mumbai) respectively using sterile cotton swabs. Wells of 6mm diameter were punched in the inoculated plates using sterile cork borer. 100µl of flower extract (20mg/ml of 25% DMSO), standard antibiotic (1mg/ml of sterile distilled water) and DMSO (25%, in sterile water) were filled in labeled wells. Streptomycin and Fluconazole were used as standard antibacterial antifungal antibiotics. The plates were incubated at 37^{o}C for 24 hours (for bacteria) and 48 hours (for fungi) and the zone of inhibition (cm) was measured [27].

Radical scavenging activity of flower extract

The efficacy of flower extract to scavenge free radicals was determined using ABTS radical scavenging assay with minor modification [28]. The ABTS radical was generated by reacting 7mM ABTS stock solution with 2.45mM potassium persulfate and the mixture was left at room temperature in the dark for 12–16 hours. The resulting

solution was diluted with distilled water to an absorbance of 0.70 at 730 nm. 1ml of different concentrations of flower extract was added to 4ml of ABTS solution and the tubes were incubated for 30 minutes followed by measuring the absorbance at 730 nm. Ascorbic acid was used as reference standard. The radical-scavenging activity of the extract was calculated using the formula:

Scavenging activity (%) = ($A_{control} - A_{test} / A_{control}$) x 100, where $A_{control}$ is the absorbance of the ABTS solution without extract/standard and A_{test} is the absorbance of the test sample.

RESULTS

Estimation of elements present in the dried and powdered flower of *L. speciosa* was performed by using ICP-OES technique after acid digestion of the powder. The content of elements was expressed as ppm. Among major elements, the content of potassium (11715.50ppm) and sodium (64.63ppm) was highest and least respectively. In case of minor elements, iron (216.15ppm) and nickel (1.99ppm) were present in high and least concentration **(Table 2).**

Table 2: Elemental contents of *L. speciosa* flower

Element	Content (ppm)
Potassium	11715.50
Magnesium	2648.24
Calcium	2149.46
Sodium	64.63
Iron	216.15
Zinc	32.24
Copper	18.79
Lithium	7.80
Chromium	2.38
Manganese	2.09
Nickel	1.99

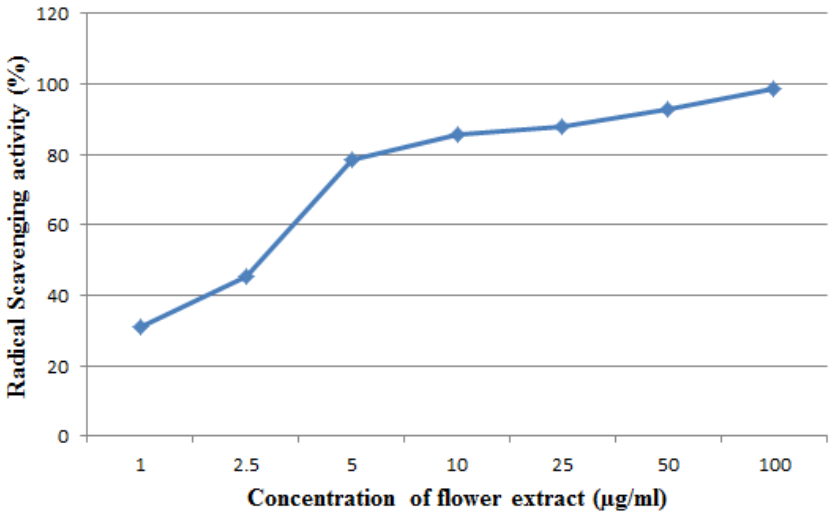
Antimicrobial activity of flower extract of *L. speciosa* was determined against a panel of 10 bacterial isolates and two fungi. Agar well diffusion method was employed and the presence of zone of inhibition formed around the well was considered positive for antimicrobial activity. All bacterial isolates were shown to be susceptible to flower extract with zone of inhibition ranging between 1.8 to 2.5 and 2.3 to 2.8cm in case of *S. mutans* and *S. aureus* respectively. Among fungi, sensitivity to flower extract was higher in case of *C. neoformans* than *C. albicans*. Inhibitory activity of reference antibiotic was higher than that of flower extract. DMSO (25%) did not cause inhibition of bacteria and fungi **(Table 3)**.

Table 3: Antimicrobial activity of flower extract of *L. speciosa*

Test microbes	Zone of inhibition in cm	
	Extract	Standard
Sm-01	2.5	3.7
Sm-02	1.8	3.9
Sm-03	2.1	3.6
Sm-04	2.5	3.7
Sm-05	2.3	3.4
Sa-01	2.6	4.1
Sa-02	2.8	3.9
Sa-03	2.3	3.9
Sa-04	2.8	4.2
Sa-05	2.6	3.9
C. albicans	1.8	3.9
C. neoformans	2.1	4.1

Radical scavenging potential of flower extract was determined by ABTS radical scavenging assay and the result is shown in **Figure 1**. A dose dependent scavenging of radical was observed with >90% scavenging activity at extract concentration 50µg/ml and higher.

Figure 1: ABTS radical scavenging activity of flower extract of *L. speciosa*



DISCUSSION

In order to live, organisms in particular humans consume a huge number of organic and inorganic compounds. Carbohydrates, fats and proteins form the major part of the diet and are consumed in greater quantity whereas mineral elements and vitamins form comparatively smaller part and are consumed in smaller quantities. Elements are found very essential for the normal functioning of the human body and their absence leads to ill effects. From the physiological point of view, the mineral elements can be distinguished into essential elements that are required for metabolism and life processes and non-essential elements being considered toxic and harmful. Further, these essential elements can be categorized as major and minor elements on the basis of their daily requirements. These elements perform several functions such as components of enzymes, regulation of cellular energy transduction, gas transport, antioxidant defense, membrane receptor functions, second-messenger systems and integration of several physiological functions. Some 25 elements are identified as important for maintenance of health and therefore, the estimation of elements in food and plants is of great interest [29-33].

Estimation of mineral elements of plant specimens requires digestion of the specimens with acid or mixtures of concentrated acids. Digestion can done using acids *viz*., hydrofluoric acid, hydrochloric acid, nitric acid, perchloric acid and sulphuric acid in various digestion equipment such as open beakers heated on hot plates, block digesters and digestion units placed in microwave ovens. ICP-OES is one of the sophisticated techniques widely used for the determination of elements. The inductively coupled plasma generates excited atoms which emit electromagnetic radiation at characteristic wavelengths for a particular element. These atomic emission lines are sharp and can be resolved from other elements. Aspects such as accuracy, precision, limit detection and quantification attributes of ICP-OES are superior over mono-element detection systems. In many cases, the technique has replaced flame atomic absorption spectroscopy (with mono-elemental detection) owing to its multi-element estimation capability and relatively high analytical throughput. ICP-OES is employed in various industries *viz*., aerospace, chemical, environment, food and beverage, geological, pharmaceutical and alloy production industries [26,32,34-39].

In the present study, we have estimated the mineral elements present in the flower of *L. speciosa* by ICP-OES technique. A microwave-assisted acid digestion system was used to rapidly extract the elements from the flower. This microwave extraction method was designed to mimic extraction using conventional heating acids such as HNO_3 and HCl. A total of 11 elements were estimated which involved 4 major and 7 minor elements. The content of potassium and sodium was highest and least respectively among major elements whereas the content of iron and nickel was high and least among minor elements. Flowers that have been used as medicine or food are known to possess appreciable quantities of various mineral elements having marked impact on normal physiology of an individual. In a study by Huang *et al*. [40], the medicine-food flowers containedmarked contents of iron, calcium, magnesium and cobalt. Flowers contained high potassium and low sodium. In another study, Fan and Li [41] found high content of potassium followed by calcium, magnesium, sodium and others. Similar results were obtained in our study. Wang *et al*. [42] found rich contents of iron, zinc, manganese and copper in the flower of *Sonchusoleraceus* L. In our study also, the content of iron was highest among minor elements estimated. Niu *et al*. [43] estimated elements in flowers commonly used in Tibetan medicines and found higher content of potassium, magnesium and sodium.

Staphylococcus aureus is the aetiological agent of skin and soft-tissue infections, endovascular infections, pneumonia, septic arthritis, endocarditis, osteomyelitis, foreign-body infections, and sepsis [44]. Despite effective antimicrobial agents and hygiene measures, it remained an important community or hospital-acquired infectious agent. Methicillin resistance among staphylococci is worldwide. However, methicillin-resistant *S. aureus*,usually causing health care-associated infections, also emerged recently as a cause of community-acquired infections [45,46].The extracts of flowers have shown to be promising agents effective against *S. aureus* including clinical isolates of *S. aureus*. In a study of Aqil *et al*. [47], the flower extract of *Delonixregia* showed a broad-spectrum of antibacterial activity against methicillin resistant and methicillin sensitive *S. aureus*. Maneemgalai and Naveen [48] investigated the fresh and dry flower extracts of *Cassiaauriculata* against *S.aureus* and found that the aqueous extract of dry flower and acetone extract of fresh flower inhibited *S. aureus* to higher extent than other solvent extracts. Jain *et al*. [49] observed higher inhibition of clinical isolate of *S. aureus* by solvent extracts of *Tagetes erecta* than *Tagetes patula*. Uddin *et al*. [50] observed higher inhibition of clinical isolate of *S. aureus* by flower extract of *Hibiscus rosa-sinensis* than leaf extract. Similarly, flower extracts of *Lippia nodiflora* [51], *Helenium mexicanum* [52] and *Peltophorum pterocarpum* DC [21] have shown to possess inhibitory activity against *S. aureus*. In our study, the extract of *L. speciosa* flower exhibited marked inhibition of 5 clinical isolates of *S. aureus* recovered from burn specimens.

Dental caries, a common type of dental disease, is associated with microorganisms present on the tooth surface in dental plaque. The disease is infectious and multifactorial. Mutans streptococci, mainly *Streptococcus mutans* and *S. sobrinus* participate in the formation of dental biofilm and play a significant role in the initiation of dental caries. *S. mutans* and *S. sobrinus* are frequently isolated from humans. There is an increasing interest on the effect of natural compounds against cariogenic microorganisms due to the development of resistance in cariogenic bacteria against antibiotics [53-56]. Flowers of plants have shown to be potential candidates for development of agents active against dental caries. Tsai *et al*. [54] showed the inhibitory effect of methanol extract of flowers of *Lonicera japonica, Jasminum sambac, Chrysanthemum morifolium, Lavandula angustifolia, Osmanthus fragrans* and *Rosa damascene* against cariogenic bacteria *S. mutans, S. sanguinis* and *S. sobrinus*. Erturk *et al*. [57] showed the efficacy of flower extracts of four species of *Rhododendron* against *S. mutans*. In the present study, the flower extract of *L. speciosa* displayed marked inhibition of *S. mutans* recovered from caries teeth.

Candida albicans and *Cryptococcus neoformans* are the most common etiological agents of opportunistic fungal infections accounting majority of nosocomial fungal infections causing disease predominantly in immunocompromised and HIV patients. These organisms are the frequent cause of fatal mycotic infections among patients with AIDS. The clinical manifestation of Candidiasis and cryptococcal meningoencephalitis is usually incurable in immunocompromised patients despite antifungal therapy. Both *C. albicans* and *C. neoformans* have shown to be resistant to commonly used antifungal agents such as Azoles, Amphotericin B etc [58-60]. Fluconazole is currently and widely used antifungal antibiotic for therapy as it can be given orally, lacks major side effects, penetrates the central nervous system, and has broad efficacy against most pathogenic yeasts. It perturbs the biosynthesis of ergosterol by blocking an alpha-14-demethylation step in the biosynthetic pathway. However, fluconazole-resistant fungal pathogens are becoming increasingly common [58]. This alarming situation triggered an immense interest in scientific community to search alternatives for the prevention and control of infectious organisms. Flowers have shown to one of the natural sources of antifungal agents. In our study, the extract of *L. speciosa* flower exhibited inhibition of *C. neoformans* and *C. albicans*. Jawlaa *et al*. [61] showed the inhibition of *C. albicans* by ethanol and ethanol:water (1:1) extract of flowers of *Musa paradisiaca*. In another study, Abubacker *et al*. [62] showed a dose dependent inhibition of *C. albicans* by flower extract of *Cassia alata*. Similarly, flower extracts of *Inula viscosa* [63], *Peltophorum pterocarpum* DC [21], *Lippia nodiflora* [51], *Cassia fistula* [64] have been reported to possess antifungal activities. In the present study, the extract of *L. speciosa* caused inhibition of *C. neoformans* to higher extent than *C. albicans*. Similar observation was made in the study of Karamoko *et al*. [65] in which the flower extracts of *Thonningiasanguinea* were more potent against *C. neoformans* than *C. albicans*.

A free radical is an atom or molecule having an unpaired electron in an outer shell. The production of reactive oxygen species (ROS) during metabolism is a normal and necessary process that provides several important physiological functions. However, when an imbalance between ROS production and antioxidant defences occurs, it leads to oxidative stress which is implicated in over hundred human disease conditions. This free radical induced oxidative stress can be prevented by the intake of sufficient amount of antioxidants. It has been reported that antioxidant capacity of plants is because of phenolics, flavonoids and anthocyanins. These phytochemical act on ROS and prevent damage to DNA, proteins and membrane lipids and therefore are significant from the point of health [14,66-69]. Flowers are known to possess marked antioxidant activity due in part to the presence of polyphenolic compounds including flavonoids. In this study, the radical scavenging activity of different concentrations of flower extract of *L. speciosa* was evaluated by ABTS radical scavenging assay. This assay has been extensively used to estimate free radical scavenging nature of antioxidants. On interaction with ABTS,

antioxidants either transfer electrons or hydrogen atoms to ABTS and thereby neutralizing the free radical character [70]. The extract of *L. speciosa* has shown a dose dependent scavenging of radicals. At extract concentration 5µg/ml and higher, the scavenging activity was >50%. The radical scavenging effect observed in this study was higher than that of the ABTS radical scavenging activity of methanol extract of *Crocus sativus* flowers [71]. Vidyalakshmi *et al*. [72] showed the ABTS radical scavenging activity of white and pink flowers of *Mussaenda glabra*. Park *et al*. [73] determined the antioxidant activities of butanol and hexane extracts of white *Rosa rugosa* flowers. The hexane fraction had excellent scavenging potential as it contained significant amount of polyphenols and volatile components. In another study, Ozsoy *et al*. [74] showed superior ABTS radical scavenging activity of flower extract when compared with that of leaf extract of *Rosa horrida*. The high radical scavenging activity of flower may be attributed to the presence of polyphenol compounds in the flower extract.

CONCLUSION

An appreciable quantity of various mineral elements such as potassium, calcium, magnesium, iron etc. was found to be present in the flower of *L. speciosa*. The flower can be used as a source of mineral elements needed for health. The flower extract displayed a marked antimicrobial and radical scavenging activity which might be attributed to the presence of phytochemicals. Further, isolation of bioactive components from flower extract and their bioactivity determination is under progress.

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