ISSN: 2226-7522(Print) and 2305-3327 (Online)
Science, Technology and Arts Research Journal
Jan-March 2012, 1(1): 53-56
www.starjournal.org

Short Communication

Anticariogenic Activity of *Lagerstroemia speciosa* (L.)

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Abstract

Dental caries is the common infectious diseases of the oral cavity and is caused mainly by oral streptococci. The present study was carried out to investigate the anticariogenic activity of methanol extract of Lagerstroemia speciosa (L.) (Lythraceae) leaves. The inhibitory efficacy of methanol extract was tested against 12 oral isolates of Streptococcus mutans by Agar well diffusion method. The broth cultures of bacteria were swabbed uniformly on sterile Brain heart infusion agar plates and wells of 6mm were punched in the inoculated plates. Standard antibiotic and different concentrations of extract were transferred into labeled wells. Zone of inhibition was measured after incubation. The extract caused a concentration dependent inhibition of cariogenic isolates. Inhibition caused by standard antibiotic was higher than the methanol extract. Preliminary phytochemical analysis showed the presence of saponins, glycosides, tannins and terpenoids. The result of the present study reveals that methanol extract showed significant inhibitory activity against cariogenic isolates. The inhibitory efficacy of extract against cariogenic isolates could be due to the presence of these metabolites. In suitable form, the leaves could be used to treat dental caries.

Article Information

Article History:

Received : 12-01-2012

Revised : 19-02-2012

Accepted : 21-02-2012

Keywords:

Lagerstroemia speciosa

Anticariogenic activity

Streptococcus mutans

Agar well diffusion

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INTRODUCTION

Lagerstroemia speciosa (L.) synonym *Lagerstroemia flos-reginae* Retz. belongs to the family Lythraceae. It is distributed in Tropical Himalaya, and Assam, Western and Eastern Ghats, up to 1000m. It is known as Pride of India, Queen's Flowers and Queen Crape Myrtle in English. 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. Mature leaves and fruits, in fresh condition, exhibit hypoglycaemic activity experimentally. The leaf extract, when administered as powder and as tannin-free extract, showed hypoglycaemic activity in mice. The plant contains triterpenoids, colocolic acid and maslinic acid. Colocolic acid is known to possess hypoglycaemic activity. Leaves contain lageracetal and sitosterol. Ellagitannins have been isolated from fruits and leaves (Khare, 2007). Extensive literature review carried on the *L. speciosa* revealed that no work on anticariogenic activity is being carried. Hence, the present study was carried out to investigate anticariogenic activity of methanol extract of *L. speciosa* leaves against oral isolates of *Streptococcus mutans*.

MATERIALS AND METHODS

Collection & Identification of Plant Material

The plant material was collected during January 2012 from the college campus and authenticated by Prof. Rudrappa D, Taxonomist, Dept. of Botany, SRNMN College of Applied Sciences, Shivamogga-01, Karnataka. Voucher specimen was deposited in the department herbaria for future reference.

Extraction

The leaves were washed thoroughly to remove extraneous matter on surface, shade dried, powdered mechanically and subjected for extraction. A known quantity of powdered leaf material (100gm) was subjected to soxhlation and exhaustively extracted with methanol (HiMedia, Mumbai) for about 48 hours. The extract was filtered and concentrated in vacuum under reduced pressure and dried in the desiccator (Kekuda *et al*., 2012).

Phytochemical Analysis of Methanol Extract

Methanolic extract was subjected to preliminary phytochemical screening to screen secondary metabolites namely alkaloids, saponins, flavonoids, glycosides, tannins and Terpenoids (George *et al*., 2010).

Test for Tannins: About 0.5 g of the extract was stirred with 10 ml of distilled water and filtered. 5% ferric chloride reagent was added to the filtrate. A Blue-black precipitate indicates the presence of tannin.

Test for Saponins: 0.5 g of the extract was dissolved with 5 ml of distilled water and filtered. Persistent frothing observed when the filtrate was shaken vigorously indicates the presence of saponins.

Test for Terpenoids: 0.5 g of extract was dissolved with 5 ml of chloroform and filtered. 10 drops of acetic anhydride was added to the filtrate followed by two drops of concentrated acid. Presence of pink colour at the interphase was an indication of the presence of terpenoids.

Test for flavonoids: Few pieces of magnesium metal were added to 5 ml of the extract and concentrated hydrochloric acid was carefully added. The formation of orange or crimson colour was taken as evidence of the presence of flavonoids.

Test for Glycosides (Salkowski test): 0.5 g of the extract was dissolved in 2 ml of chloroform. concentrated sulphuric acid was carefully added to form a lower layer. A reddish-brown coloration at the interphase indicates the presence of a steroidal ring of glycoside.

Test for Alkaloids: 5 ml of 1% aqueous hydrochloric acid was added to 5 g of the extract and warmed in a steam bath while stirring. It was filtered and the filtrate was used to test for alkaloid. i) 1 ml of the filtrate was treated with a few drops of Dragendorff's reagent. Formation of a reddish -brown turbid dispersion or precipitate indicates the presence of alkaloid. ii) 1 ml of the filtrate was treated with a few drops of Mayer's reagent. Formation of creamy turbid dispersion indicates the presence of alkaloid.

Anticariogenic Activity of Methanol Extract

The anticariogenic efficacy of methanol extract was tested against 12 oral isolates of *S. mutans* recovered from dental plaque and saliva samples of dental caries patients by Agar-well-diffusion method (Swathi *et al*., 2011). The *S. mutans* isolates were maintained on sterile Brain heart infusion agar (HiMedia, Mumbai) slants. Briefly, 24 hours old broth cultures of *S. mutans* isolates were swabbed uniformly on solidified sterile Brain heart infusion agar plates using sterile cotton swab. Then, wells of 6mm diameter were punched in the inoculated plates with the help of sterile cork borer. The methanol extract (50, 25 and 10mg/ml of 10% DMSO), Standard (Chloramphenicol, 1mg/ml) and Control (10% DMSO) were filled separately into respectively labeled wells. The inoculated plates were incubated at 37°C for 24 hours in upright position and the zone of inhibition formed around the well was measured with a ruler. The experiment was carried in triplicates to get average reading.

RESULTS

The yield of extract was 5.6%. Preliminary phytochemical analysis revealed the presence of tannins, saponins, glycosides and terpenoids. The anticariogenic activity of methanol extract was tested against 12 oral isolates of *S. mutans* recovered from dental plaque and saliva samples of dental caries patients. Results were recorded as presence or absence of zones of inhibition around the well. The result of inhibitory activity of extract is shown in table 1. The inhibitory activity of extract was found to be

Table 1. Anticariogenic activity of methanol extract of *Lagerstroemia speciosa* (L.) leaves.

Bacterial isolates	Zone of inhibition in cm			
	Methanol extract (mg/ml)			Standard (mg/ml)
	50.0	25.0	10.0	1.0
1	2.5	2.0	1.3	2.8
2	2.0	1.6	1.4	3.5
3	1.8	1.3	1.1	2.6
4	1.0	0.8	0.0	2.4
5	2.3	1.9	1.4	3.1
6	2.6	2.1	1.9	3.3
7	2.0	1.5	1.0	2.8
8	2.3	1.8	1.5	3.6
9	1.0	0.8	0.0	2.3
10	1.6	1.3	0.8	2.6
11	1.9	1.5	1.2	2.9
12	1.4	1.1	0.8	2.5

concentration dependent i.e., inhibitory efficacy of extract was found to increase with increase of extract concentration. The zone of inhibition ranged from 1.0 to 2.6, 0.8 to 2.1 and 0.0 to 1.9 at extract concentrations 50, 25 and 10mg/ml respectively. Inhibition caused by standard antibiotic was higher than that of methanol extract. DMSO did not cause any inhibition of bacterial isolates.

DISCUSSION

Dental caries is the common infectious diseases of the oral cavity and approximately 200 to 300 bacterial species colonize human dental plaques. Only a finite number of bacteria have been associated with either dental caries or periodontal disease. Among them, *Streptococci* are the major group when compared to other genera. The study of the genus *Streptococci* is of clinical significance because of their pathogenic potential particularly in oral science as there is concern about the group called *viridans streptococci*. These streptococci form a significant part of the normal flora of the human oral cavity and are associated with several disease conditions including dental caries, infective endocarditis and septicaemia, as well as purulent infections of oral and other sites. The species most frequently isolated from the oral cavity are *Streptococcus salivarius*, *Streptococcus sanguis*, *Streptococus mitis*, and *mutans Streptococci* (Loesche, 1986; Whiley and Beighton, 1998; Thurnheer *et al*., 2001).

Several studies have been carried on the inhibitory role of natural compounds/extracts against mutans streptococci. Yanagida *et al.* (2000) investigated the inhibitory effects of apple polyphenols on the synthesis of water-insoluble glucans by glucosyltransferases of streptococci of the mutans group and on the sucrose-dependent adherence of the bacterial cells. Lim *et al.* (2003) reported that the leaf-extract from *Camellia sinensis* had an antimicrobial effect on mutans streptococci. Chung *et al.* (2006) isolated macelignan from the methanol extract of *Myristica fragrans* and showed its potent anticariogenic activity against *S. mutans* and other oral pathogens. The inhibitory activity of methanol extract of *Rheum undulatum* root against *S. mutans* and *S. sorbinus* was investigated by Song *et al.* (2006). The dichloromethane fraction showed the most active antibacterial activity. The fraction significantly inhibited the caries-inducing factors of these bacteria. The activity of the fraction was related to the presence of anthraquinones, cardiac glycosides, coumarines, sterols/terpenes, and phenolics. Esmaeelin *et al.* (2007) investigated anticariogenic effect of ethanol and chloroform extracts of *Alcea longipedicellata* against *S. mutans*, *S. salivarious*, *S. sorbinus* and *S. sanguis*. Both the extract were found to be bacteriostatic while malvidin-3,5-diglucoside, isolated from ethanol extract of flowers was found to be the principal constituent for antibacterial activity. In a study, Zheng *et al.* (2010) observed inhibitory efficacy of methanol extract of *Aceriphyllum rossii*

Engler root and its components aceriphyllic acid A and 3-oxoolean-12-en-27-oic acid against all cariogenic bacteria tested. Aceriphyllic acid A was found to possess faster bacteriostatic activity and the inhibitory action was shown to be membrane disruption leading to killing of bacteria. In a previous study, Venugopal *et al*. (2011) showed anticariogenic activity of leaf extract of *Scleropyrum pentandrum* against oral isolates of *S. mutans*. The leaf extract has shown inhibition of oral bacteria in a dose dependent manner. Swathi *et al*. (2011) found dose dependent inhibition of oral isolates of S. mutans by methanol extract of *Croton gibsonianus* Nimm. Grah leaves. In our study, dose dependent inhibition of cariogenic isolates was observed.

CONCLUSION

In this study, we reported anticariogenic activity of leaf extract of *L. speciosa*. The leaf extract was found to possess marked anticariogenic activity and the inhibitory efficacy could be related to the presence of secondary metabolites present in the extract. In suitable form, the plant could be used to prevent and treat dental caries.

Acknowledgements

Authors express sincere thanks to Principal, SRNMN College of Applied Sciences, Shivamogga-01, Karnataka for the facilities provided. Authors also thank NES, Shivamogga-01, Karnataka for the moral support.

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