



## Research Article

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### ANTICARIES AND $\alpha$ -AMYLASE INHIBITORY ACTIVITY OF *JASMINUM ARBORESCENS* ROXB. (OLEACEAE) LEAVES EXTRACT

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Received on: 08/10/13 Revised on: 21/11/13 Accepted on: 17/12/13

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DOI: 10.7897/2277-4343.04622

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#### ABSTRACT

The aim of the present study was to determine anti caries and  $\alpha$ -amylase inhibitory activity of leaf extract of *Jasminum arborescens* Roxb. (Oleaceae). Anti caries activity was determined by Agar well diffusion assay against seven clinical isolates of *Streptococcus mutans* (Sm-01 to Sm-07) recovered from dental caries subjects. Enzyme inhibitory activity was tested against  $\alpha$ -amylase by spectrophotometric method using starch as substrate. The extract exhibited dose dependent inhibition against cariogenic isolates. Among seven isolates, isolate Sm-04 and Sm-06 were inhibited to higher and least extent respectively. The extract was found to cause inhibition of  $\alpha$ -amylase activity in a dose dependent manner and its IC<sub>50</sub> value was found to be 17.45 mg/ml. The inhibitory activity could be attributed to the presence of secondary metabolites. The plant may be a potential source for development of agents which are active against dental caries pathogens and for diabetes mellitus.

**Keywords:** *Jasminum arborescens*, Dental caries, *Streptococcus mutans*, Diabetes mellitus,  $\alpha$ -amylase

#### INTRODUCTION

*Jasminum arborescens* Roxb. (Oleaceae) is distributed in Sub-Himalayan tract, Bengal, Central and South India. The plant is commonly called Tree Jasmine in English. It is known as Nava-mallikaa in Ayurveda and Nagamalli in Siddha. The leaves are astringent and stomachic. Juice of leaves, with pepper, garlic and other stimulants, is used as an emetic in obstruction of the bronchial tubes due to viscid phlegm<sup>1</sup>. *Jasminum arborescens* is used in household remedies of Keshavraipatan tehsil with 112 villages, situated in eastern part of Bundi district of Rajasthan, India. The leaves are chewed for treatment of mouth sores or rashes and drops of leaf extract are put in the ear to treat hearing problems<sup>2</sup>. Bhagath *et al.*<sup>3</sup> screened antibacterial potential of various solvent extracts of leaves of *Jasminum arborescens*. Marked antibacterial activity was observed in case of aqueous extract followed by ethanol, chloroform and petroleum ether extract. In another study, Bhagath *et al.*<sup>4</sup> screened the antioxidant and anthelmintic activity of ethanol, chloroform and petroleum ether extracts of leaves. It was found that the extracts caused a marked dose dependent antioxidant activity. The time taken for paralysis and death of worms was found lesser in case of ethanol extract followed by chloroform and petroleum ether extract. Nandini *et al.*<sup>5</sup> observed marked dose dependent larvicidal effect of leaves. 2<sup>nd</sup> instar larvae were more sensitive to leaf extract than 3<sup>rd</sup> instar larvae of *Aedes aegypti*. The present study was carried out to investigate anticaries and  $\alpha$ -amylase inhibitory activity of methanol extract of *Jasminum arborescens* leaves.

#### MATERIALS AND METHODS

##### Collection and Identification of Plant Material

The leaves of *J. arborescens* were collected in December 2009 at a place called Haniya, Hosanagara (Taluk), Shivamogga (District), Karnataka, India. Plants were cleaned off adhering soil/dust in field by shaking properly and using soft brush. Plants were placed in paper bags and brought to the laboratory. Remaining dust particles were removed by quick rinsing using clean water. The plant was authenticated by Dr. Vinayaka KS and voucher specimen (KB/Chem/001-09) was deposited.

##### Extraction

Leaves were separated from the plant, shade dried and powdered mechanically. About 50 g of powdered material was subjected to soxhlet extraction and exhaustively extracted with ethanol (Hi Media, Mumbai, India) for 48 hours. The extract was filtered and concentrated in vacuum under reduced pressure using rotary flash evaporator and dried in the dessicator<sup>6</sup>.

##### Anticaries Activity of Ethanol Extract

The anticaries efficacy of leaf extract was tested against seven isolates of *S. mutans* (Sm-01 to Sm-07) recovered previously from dental caries subjects. Agar-well-diffusion method was employed. Briefly, 24 hours old Brain heart infusion broth (Hi Media, Mumbai, India) cultures of *S. mutans* isolates were swabbed uniformly on solidified sterile Brain heart infusion agar (Hi Media, Mumbai, India) plates. Then, wells of 6 mm diameter were punched in the inoculated plates with the help of sterile cork borer and the extract (10 and 20 mg/ml of 10 % DMSO), standard (Chloramphenicol, 1 mg/ml) and dimethyl sulfoxide ([DMSO], 10 % in sterile water) were

added into respectively labeled wells. The inoculated plates were incubated at 37°C for 24 hours in upright position and the zone of inhibition was measured<sup>6</sup>.

**α-amylase Inhibitory Activity of Ethanol Extract**

The inhibitory activity of different concentrations of leaf extract (0-50 mg/ml) was determined against fungal α-amylase (Diastase (Fungal) 3240, Lobachemie Laboratory reagents and fine chemicals, Mumbai, India) by following the method Karthik *et al.*<sup>7</sup> with minor modifications. The enzyme (0.5 %) was prepared in phosphate buffer (pH 6.8). Briefly, 500 µl of different concentrations of leaf extract and 500 µl of 0.1M phosphate buffer (pH 6.8) containing α-amylase were incubated at 25°C for 10 minutes. After pre incubation, 500 µl of a 1 % starch solution in 0.1M phosphate buffer (pH 6.8) was added to each tube and further incubated at 25°C for 10 minutes. The reaction was stopped by addition of 1 ml of dinitrosalicylic acid reagent. The same was performed for control where extract was replaced with buffer. The test tubes were placed in a boiling water bath for 10 minutes and cooled. To each tube, 10 ml of distilled water was added and the absorbance was measured spectrophotometrically at 540 nm. The percentage (%) inhibition of enzyme activity was calculated using formula:

$$\text{Inhibition of } \alpha\text{-amylase activity (\%)} = \frac{[A_{540}\text{Control} - A_{540}\text{Extract} / A_{540}\text{Control}] \times 100.}$$

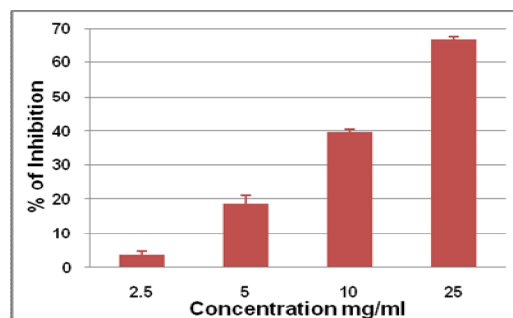
**RESULTS**

Table 1 shows the result of anti caries activity of leaf extract. The extract was found to exhibit dose dependent inhibition of cariogenic isolates. Isolate Sm-04 and Sm-06 were inhibited by the extract to higher and least extent respectively. Standard antibiotic caused higher inhibition of test bacteria when compared to leaf extract. DMSO did not cause inhibition of test bacteria.

**Table 1: Anticaries activity of extract of *Jasminum arborescens* leaf**

Test bacteria	Zone of inhibition in cm (Mean ± SD)			
	Leaf extract		Standard	DMSO
	10 mg/ml	20 mg/ml		
Sm-01	1.3 ± 0.0	1.6 ± 0.1	3.2 ± 0.2	0.0 ± 0.0
Sm-02	1.5 ± 0.2	1.9 ± 0.0	3.6 ± 0.0	0.0 ± 0.0
Sm-03	1.1 ± 0.2	1.3 ± 0.2	3.1 ± 0.0	0.0 ± 0.0
Sm-04	1.6 ± 0.1	2.1 ± 0.0	3.2 ± 0.1	0.0 ± 0.0
Sm-05	1.2 ± 0.0	1.5 ± 0.1	3.4 ± 0.2	0.0 ± 0.0
Sm-06	0.8 ± 0.1	1.0 ± 0.0	2.9 ± 0.0	0.0 ± 0.0
Sm-07	1.3 ± 0.2	1.5 ± 0.2	3.1 ± 0.2	0.0 ± 0.0

The enzyme inhibitory activity of the leaf extract was tested against Diastase (α-amylase). The extract was found to display inhibitory activity against α-amylase and the effect was dose dependent. The highest inhibition of α-amylase (66.68 %) was observed at extract concentration 25 mg/ml (Figure 1). The IC<sub>50</sub> value found to be 17.45 mg/ml.



**Figure 1: α-amylase inhibitory activity of extract of *Jasminum arborescens* leaf**

**DISCUSSION**

Dental caries is one of the common and most important infections of the oral cavity which affects people of all age groups worldwide. Among cariogenic flora, mutans streptococci, in particular *Streptococcus mutans* is the primary cause of dental caries. The prevention of dental caries can be achieved by mechanical removal of dental plaque by tooth brushing and flossing. The majority of the human population (in particular aged people) may not follow this mechanical plaque removal sufficiently. In such cases, the use of antimicrobial mouth rinses such as chlorhexidine and others may be preferred to reduce plaque-related oral infections. These chemicals are shown to exert some undesirable side effects such as tooth staining, taste alteration and development of hypersensitivity reactions. Antibiotics are routinely used to prevent oral infections. Like mouth rinses, these antibiotics also possess side effects in addition to development of resistance by cariogenic flora<sup>8-10</sup>. Plants and the formulations containing them have been routinely used worldwide for prevention and control of oral infections. These agents are safer and are free from side effects which are observed in case of the antibiotics and mouth rinses. Researchers have revealed the potential of a number of plants against cariogenic bacteria<sup>9-20</sup>. In the present study, the leaf extract of *Jasminum arborescens* was shown to possess antibacterial activity against clinical isolates of *S. mutans* recovered from dental caries subjects.

Millions of people all over the world suffer from Diabetes mellitus, a common endocrine disorder. This disorder is characterized by hyperglycaemia which occurs due to absolute or relative lack of insulin secretion. The Long term complications include retinopathy, nephropathy, neuropathy, microangiopathy and increased risk of cardiovascular diseases. There two forms of diabetes are Type 1 (insulin-dependent) and Type 2 (non insulin-dependent) among which Type 2 is the major form of diabetes which results from defects in insulin secretion or rather insulin resistance. One of the strategies employed to manage type 2 diabetes is the use of drugs which inhibit the enzymes responsible for carbohydrate metabolism. Drugs such as Acarbose, Miglitol and Voglibose slow down the absorption of carbohydrates through the inhibition of enzymes responsible for their digestion *viz.*, pancreatic α-amylases and disaccharidases (glucosidases). These drugs are used to treat type II diabetes as they reduce post-prandial hyperglycemia.

However, the use of these drugs may cause side effects such as abdominal discomfort, flatulence, meteorism and diarrhea<sup>21-24</sup>. The use of natural products as alternate approaches for existing medications for the treatment of diabetes mellitus is growing worldwide. Antidiabetics of plant origin are commonly used in traditional systems of medicine worldwide with no apparent side effects. Plants have the ability to lower postprandial blood glucose level and can be used as treatment of choice for diabetes. The most important phytoconstituents are polyphenolic compounds which show inhibitory activity against  $\alpha$ -amylase<sup>22,24-27</sup>. In the present study, the leaf extract of *Jasminum arborescens* showed dose dependent inhibition of  $\alpha$ -amylase activity. It is shown that extracts/fractions of sorghum<sup>28</sup>, foxtail millet<sup>28</sup>, proso millet<sup>28</sup>, *Phaseolus vulgaris*<sup>21</sup>, *Rubus chamaemorus* and *Sorbus aucuparia*<sup>29</sup>, *Corchorus olitorius*<sup>30</sup>, *Salvia virgata*<sup>27</sup>, Green tea<sup>24</sup>, *Pithecellobium dulce*<sup>23</sup> possess  $\alpha$ -amylase inhibitory activity.

## CONCLUSION

Leaf extract of *Jasminum arborescens* exhibited dose dependent inhibition of cariogenic bacterial isolates and  $\alpha$ -amylase activity. The preliminary phytochemical analysis of leaf extract showed the presence of saponins, tannins, flavonoids, glycosides, sterols and terpenoids<sup>4</sup>. The observed inhibitory activity could be attributed to the presence of secondary metabolites mainly phenolic compounds. The plant can be a potential candidate for the development of anti caries and  $\alpha$ -amylase inhibitory agents. Further studies need to be conducted to isolate active principles and to determine anti caries and  $\alpha$ -amylase inhibitory activity of purified components.

## ACKNOWLEDGEMENTS

The authors thank Principal, Sahyadri Science College (Autonomous), Shivamogga, Karnataka, India for providing facilities to conduct work and moral support.

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**Cite this article as:**

Bhagath K, Prashith Kekuda T.R, Mallikarjun N, Raghavendra H.L. Anticaries and  $\alpha$ -amylase inhibitory activity of *Jasminum arborescens* Roxb. (Oleaceae) leaves extract. Int. J. Res. Ayurveda Pharm. 2013;4(6):885-888 <http://dx.doi.org/10.7897/2277-4343.04622>

Source of support: Nil, Conflict of interest: None Declared