INTRODUCTION

Dental caries is one of the most infectious diseases in human being and is still a main public health concern in many nations [1]. Mutans streptococci (MS), Streptococcus mutans, and Streptococcus sobrinus are the most common bacteria isolated from human dental caries and it’s considered the major etiologic agents of caries disease. Many studies have reported that S. sobrinus is less prevalent than S. mutans in dental caries [1,2]. However, the prevalence of S. sobrinus is more likely connected with a high dental caries [3].

Plants have been one of the essential sources of medicines from the start of human development. There is a developing interest in plant based medicines, health items, pharmaceuticals, nutrient supplements, beautifying agents, and so forth. As per the WHO review 80% populations living in the third world countries depend solely on conventional medication for their essential human needs [4].

The plant Annona squamosa and Annona reticulata belong to genus Annona and family Annonaceae. A. squamosa commonly known as Custard apple in English and Sharifa in Hindi is cultivated all over India and other tropical countries [5]. Annona reticulata is likewise known as Ramphal, Bullock’s heart and Custard apple [6]. Different parts of A. reticulata like leaves, bark, seed, and root are therapeutically helpful and they indicate numerous remedial activities as anticancer, central nervous system depressant, pain relieving, anti-hyperglycemic, anti-inflammatory, antiproliferative, wound healing, and antiulcer activity [7,8].

Various parts of A. squamosa are used in conventional remedies for the treatment of several disorders and useful for heart ailments, diabetes, hyperthyroidism, and tumor [9]. A. squamosa is traditionally used for the treatment of epilepsy, diarrhea, worm infestation, constipation, hemorrhage, dysuria, fever, thirst, ulcers, and also as an abortion agent [10-12].

The leaves of A. squamosa is reported to contain glycoside, alkaloids, saponins, flavonoids, tannins, carbohydrates, proteins, phenolic compounds, phytosterols, amino acids [13,14]. The volatile constituents of A. squamosa bark contains annonaine, and alkaloid which is found to have many benefits [15]. An ethanol extract of A. squamosa and bark is reported to have anticancer activity [16,17]. Methanolic extract of A. squamosa bark possesses antimicrobial activity against gram-positive and gram-negative bacteria [18]. Methanolic leaves extract of A. reticulata showed significant activity against Bacillus subtilis, Staphylococcus aureus, and Vibrio algolyticus. In comparison to the extracts of A. reticulata, A. squamosa had strong antibacterial activity [19].

In an earlier study, there have been observed increases in antibiotic resistant strains of S. mutans, which have led to the emergence of new strains with a multidrug-resistant [20]. Plant products have been tested in an attempt to prevent dental caries [21,22]. However, most studies on dental caries had been performed using the strains of MS derived from Westerns countries. It is unclear if the plant products previously used would have a similar effect on the MS of Indian population. In order to test the anti-cariogenic effect of plant extracts, it would be important to evaluate the antibacterial activity against clinical strains of the MS isolated from the dental plaque obtained from Indian population.

To best of our knowledge, there is no antibacterial activity study of A. squamosa and A. reticulata against MS species been reported. Hence,
the objective of the study was to test the antibacterial effectiveness of *A. squamosa* and *A. reticulata* (leaves and bark) against MS species the causative agents of dental caries.

**METHODS**

**Collection of plant**

Leaves and bark of *A. squamosa* and *A. reticulata* were collected in the month of February from UAS university, GKVK Bangalore and authenticated by Dr. Vasundhara M. Professor of the Horticulture Department, UAS university, GKVK Bangalore, India. With an authentication number as A no. 29 and A no. 30, respectively. The leaves of both the species were separately rinsed with distilled water. Leaves and bark were kept separately in hot air oven at 50°C for complete drying. The leaves and the bark were powdered by using an electrical blender.

**Extract preparation**

A total of 40 g of powder was extracted with 600 ml of 100% v/v methanol using a soxhlet apparatus, the soxhlet was run for 30 hrs at 20°C, methanolic extract then concentrated by rotary vacuum.

**Bacterial isolates**

The Ethical Approval of this study was taken from the Institutional Ethics Committee of PMNM Dental College, Bagalkot, Karnataka, India. Clinical isolates were isolated from dental caries subjects. Reference cultures used were *S. mutans* ATCC 25175, *S. mutans* MTCC 497, and *S. sobrinus* ATCC 33478. Dental plaques were collected from the patients and placed in sterile phosphate buffered saline (PBS) (HiMedia, India). The samples were diluted by 100-fold in 1× PBS and streaked on mitis salivarius agar (HiMedia, India) supplemented with 15% sucrose and 0.2 units of bacitracin (HiMedia, India) (MSB agar) the plates then incubated anaerobically at 37°C for 48 hrs. Out of 65 clinical samples, four isolates were selected randomly for the present study.

**Genomic DNA isolation**

CTAB method [23] was used for bacterial genomic DNA isolation. DNA concentration was determined by measuring the OD at 260 and 280 nm using an UV spectrophotometer (Sartorius stedim biotech, Germany).

**16S rDNA sequencing**

16S rDNA sequencing was done to identify the species of the isolates, the PCR was performed using universal primer 16S FP (AGA GGT TGA TCC TGG CTC AG), 16S RP (AAG GAG GTG ATC CAG CCG CA), and the following conditions: Initial denaturation 94°C for 2 minutes, denaturation 94°C for 50 seconds, annealing 48°C for 30 seconds, extension 72°C for 1 minutes 30 seconds, and final extension 72°C for 6 minutes. The sequences were submitted to National Centre for Biotechnology Information (NCBI) database to obtain GenBank accession numbers.

**Disc diffusion method**

5 mg, 10 mg, 15 mg, 20 mg, 25 mg, 30 mg, 35 mg, 40 mg, 45 mg, 50 mg and 100 mg of the extract were dissolved. The sterile discs (HiMedia, India) were aseptically soaked overnight in different concentrations (5-50 mg/ml), while leaf extracts of *A. squamosa* did not show any inhibition even at 100 mg/ml as shown in Fig. 1. *A. reticulata* (leaves and bark) did not possess antibacterial activity against both MS species even with higher concentration, such as 100 mg/ml as shown in Fig. 2.

Ten different concentrations of the *A. squamosa* bark were tested against MS species as shown in Table 1, Fig. 3 and Fig. 4.

**DISCUSSION**

*A. squamosa* is a multipurpose tree with edible fruits and is a source of the medicinal and industrial products. The antibacterial activities of the leaves and bark extract of *A. squamosa* and *A. reticulata* has been evaluated against MS (*S. mutans and S. sobrinus*).

The result indicates that 5-50 mg/ml concentration of the bark extract of *A. squamosa* showed a significant antibacterial activity against *S. mutans* and *S. sobrinus*. However, in this study, 100 mg/ml concentration of methanolic leaves extract of *A. squamosa* and *A. reticulata* did not show any inhibition zone to any isolates. No antibacterial activity of *A. reticulata* bark against *S. mutans* and *S. sobrinus* been observed either.

**Table 1: Antibacterial activity of different concentration of *A. squamosa* bark against MS species, the zone of inhibition measured by millimeter in diameter mm**

<table>
<thead>
<tr>
<th>Bacterial sample number</th>
<th><em>A. squamosa</em> bark (mg/ml)</th>
<th>Amp 10 μg</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td><em>S. mutans</em> MTCC 497</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td><em>S. mutans</em> ATCC 25175</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td><em>S. mutans</em> KP975192</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td><em>S. mutans</em> KP975193</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td><em>S. sobrinus</em> ATCC 33478</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td><em>S. sobrinus</em> KP975179</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td><em>S. sobrinus</em> KP975203</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Amp: Ampicillin antibiotic
There were differences in the susceptibility between the reference strains and the clinical isolates of MS. In addition, the antibacterial effectiveness of the bark-extract from *A. squamosa* differed among the clinical isolates, this is a similar finding with Lim et al. 2003 [24]. Our study revealed, *S. sobrinus* is more susceptible to the extract than *S. mutans* as shown in Table 1. Not much difference showed between the species in regards of different concentration. In contrast to an old study, the methanolic extract of *A. squamosa* and *A. reticulata* leaf showed high inhibition zone against *Bacillus subtilis*, *Staphylococcus epidermidis*, *S. aureus*, and *V. alginolyticus* [19]. While in this study no activity been shown against *S. mutans* and *S. sobrinus*. 

Fig. 2: Antibacterial susceptibility testing of *A. reticulata* (leaves and bark) against (a) *S. mutans* MTCC 497, (b) *S. mutans* KP975192 and (c) *S. sobrinus* ATCC 33478

Fig. 3: Antibacterial susceptibility testing of *A. squamosa* bark against (a) *S. mutans* ATCC 25175, (b) *S. mutans* MTCC 497, (c) *S. sobrinus* ATCC 33478, (d) *S. mutans* KP975193, (e) *S. sobrinus* ATCC 33478, (f) *S. mutans* MTCC 497. AMP: ampicillin 10 unit

Fig. 4: Distribution of antibacterial activity values into mutans Streptococci species. S.m: *S. mutans*, S.s: *S. sobrinus*
CONCLUSION

This study reveals the presence of antibacterial activity of *A. squamosa* bark against *S. mutans* and *S. sobrinus*. These results suggest that *A. squamosa* bark could be employed as a potent antibacterial agent for preventing dental caries. These extracts might be added in toothpaste or mouthwash to prevent its decay. The results suggest that traditional remedies may lead to treatment of dental caries.

ACKNOWLEDGMENTS

The authors like to thank Dr. Vasundhara M. and Mrs. Ashwini Jayaram, Horticulture Department, UAS university, GKVK Bangalore, India, for their help.

REFERENCES