Anticaries Activity of Azolla pinnata and Azolla rubra

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Abstract

The present study was carried out to investigate anticaries activity of two Azolla species viz., A. pinnata and A. rubra. Inhibitory efficacy of methanolic extract of both Azolla species was tested against six oral isolates of Streptococcus mutans by Agar well diffusion and Minimum inhibitory concentration (MIC) determination. The S. mutans isolates were shown to be susceptible to extracts. Among Azolla species, A. pinnata displayed high inhibitory effect against oral isolates when compared to A. rubra as evidenced by wider inhibition zones and low MIC values. These Azolla species can be used to treat dental caries.

INTRODUCTION

Dental caries is one of the most important infections of the oral cavity affecting people of all age groups and remains a major problem worldwide. Among cariogenic flora, mutans streptococci in particular Streptococcus mutans is a primary cause of dental caries. It is acidogenic and aciduric and has the ability to adhere to tooth surfaces and forms biofilm. If left untreated, dental caries gradually leads to tooth loss with a variety of health problems. Hence, prevention of dental caries is preferable than treatment. Conventional methods used for prevention and treatment of dental caries include the use of antibiotics and mouth rinses. However, these strategies have some drawbacks such as side effects, development of resistance, high cost etc. Hence, search for alternatives is of much interest. Plants have been used for the prevention and control of dental caries and a number of researchers have shown the efficacy of plants against microflora causing dental caries [Fani et al., 2007; Ambrosio et al., 2008; Gupta et al., 2012; Chaiya et al., 2013; Junaid et al., 2013; Vivek et al., 2013; Vivek et al., 2014].

Azolla (Salviniacaeae) is a small aquatic pteridophyte with agronomic importance worldwide. It grows faster and produces maximum biomass in short time. It is an example for symbiotic interaction between eukaryotic Azolla and prokaryotic Anabena. Anabena lives as an endosymbiont in the leaf cavities of Azolla and is associated with all stages of fern’s development. Azolla supplies carbon sources to Anabena and in return it gets its nitrogen requirements. Because of its ability to fix nitrogen at high rates and low cost, Azolla is used as biofertilizer especially in paddy fields. Besides, Azolla is used as green manure, animal feed, human food and medicine, water purifier, hydrogen fuel, biogas producer, weed and insect controller, and reduces ammonia volatilization after chemical nitrogen application. Azolla improves the water quality by removing excess quantity of nitrates and phosphorus [Ray et al., 1979; Pabby et al., 2003; Chris et al., 2011 and Sadeghi et al., 2013]. It is experimentally shown that Azolla species exhibit plant growth promitory [Bindhu et al., 2013], hepatoprotective [Kumar et al., 2013], antioxidant [Nayak et al., 2014], bioremediation [Zazouli et al., 2014], and antimicrobial activity [Nayak et al., 2014]. The present study was conducted to determine anticaries activity of methanol extract two Azolla species viz., A. pinnata and A. rubra.

MATERIALS AND METHODS

Collection and Extraction of Plant Materials

The Azolla species viz., A. pinnata and A. rubra were obtained from UAS, GKVK, Bangalore. The whole plant materials were dried under shade and powdered in a blender. 10g of powdered A. pinnata and A. rubra was added to 100ml of methanol (HiMedia, Mumbai) in separate conical flasks and left at room temperature for two days with occasional stirring. The solvent extracts were filtered using Whatman No. 1 filter paper and the solvent was evaporated to obtain concentrated extract (Vivek et al., 2014).

Anticaries activity of A. pinnata and A. rubra

The efficacy of extracts to inhibit cariogenic bacteria was tested by Agar well diffusion method against 6 oral isolates of S. mutans (Sm). The bacterial isolates were inoculated into sterile Brain heart infusion broth (HiMedia, Mumbai) tubes and incubated at 37°C overnight. The broth cultures were aseptically swabbed on sterile Brain heart infusion agar (HiMedia, Mumbai) followed by punching wells of 6mm diameter in the inoculated plates.
100μl of extract (20mg/ml of 25% dimethyl sulfoxide [DMSO; HiMedia, Mumbai]), standard (Streptomycin, 1mg/ml of sterile distilled water) and DMSO (25%, in sterile distilled water) were transferred into respectively labelled wells. The plates were incubated aerobically at 37°C for 24 hours. The zone of inhibition formed around each well was measured using a ruler (Vivek et al., 2014).

### Minimal Inhibitory Concentration (MIC)

The MIC of Azolla extracts was determined by dilution method. The extract dilutions (ranging from 20 to 0.0mg/ml) were tested against each clinical isolate. Two-fold dilutions of Azolla extracts were prepared in sterile Brain heart infusion broth tubes. Broth tubes with different concentrations of extracts were inoculated with test bacteria and incubated at 37°C for 24 hours. The MIC was determined by observing the visible growth of the isolates after incubation. The extract dilution revealing no visible growth was considered as the MIC (Kosanic and Rankovic, 2010).

### RESULTS

The result of inhibitory effect of extract of *A. pinnata* and *A. rubra* against the clinical isolates of *S. mutans* is shown in Table 1. The *S. mutans* isolates were susceptible to the extract of both Azolla species. The extract of *A. pinnata* was more effective in inhibiting the test bacteria (zone of inhibition ranging 2.6 to 3.4cm) than that of *A. rubra* (zone of inhibition ranging 2.3 to 3.1cm). Inhibition caused by reference antibiotic was higher than that of extracts of Azolla species. DMSO did not cause inhibition of any bacteria. In MIC determination also, similar kind of inhibition of oral isolates by Azolla extracts was observed. Extract of *A. pinnata* inhibited oral isolates at low concentration when compared to *A. rubra*. MIC ranged between 0.312 to 1.25 and 0.625 to 2.5mg/ml in case of *A. pinnata* and *A. rubra* respectively (Table 2).

### Table 1: Anticaries activity of extract of *A. pinnata* and *A. rubra*

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Zone of inhibition in cm</th>
<th><em>A. pinnata</em></th>
<th><em>A. rubra</em></th>
<th>Streptomycin</th>
<th>DMSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sm-01</td>
<td>3.4</td>
<td>3.1</td>
<td>3.9</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Sm-02</td>
<td>2.9</td>
<td>2.7</td>
<td>3.7</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Sm-03</td>
<td>3.1</td>
<td>2.8</td>
<td>4.1</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Sm-04</td>
<td>2.8</td>
<td>2.6</td>
<td>3.4</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Sm-05</td>
<td>3.3</td>
<td>2.9</td>
<td>4.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Sm-06</td>
<td>2.6</td>
<td>2.3</td>
<td>3.5</td>
<td>0.0</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2: MIC of extract of *A. pinnata* and *A. rubra*

<table>
<thead>
<tr>
<th>Isolates</th>
<th>MIC (mg/ml)</th>
<th><em>A. pinnata</em></th>
<th><em>A. rubra</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sm-01</td>
<td>0.312</td>
<td>0.625</td>
<td></td>
</tr>
<tr>
<td>Sm-02</td>
<td>1.250</td>
<td>1.250</td>
<td></td>
</tr>
<tr>
<td>Sm-03</td>
<td>0.625</td>
<td>1.250</td>
<td></td>
</tr>
<tr>
<td>Sm-04</td>
<td>0.625</td>
<td>1.250</td>
<td></td>
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<td>Sm-05</td>
<td>0.312</td>
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<td>Sm-06</td>
<td>1.250</td>
<td>2.500</td>
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</tbody>
</table>

### DISCUSSION

Dental caries can be effectively controlled by mechanical removal of dental plaque by tooth brushing and flossing. However, the majority of the human population (particularly aged people) may not follow this mechanical plaque removal sufficiently. In such cases, the use of antimicrobial mouth rinses may be preferred to limit plaque-related oral infections. However, these chemicals show undesirable side effects such as tooth staining, taste alteration and development of hypersensitivity reactions. Antibiotics are routinely used to prevent oral infections. These antibiotics also suffer from problems such as side effects and risk of development of resistance against antibiotics in cariogenic flora (Anjea et al., 2010; Fani and Kohanteb, 2012; Chaiya et al., 2013). Plants are routinely used for prevention and control of dental caries and periodontal infections. These are safer and do not cause side effects that are observed in case of the antibiotics and other synthetic chemicals. Researchers have shown the potential of plants against cariogenic bacteria and have come out with promising results (Wolinsky et al., 1996; Prashant et al., 2007; Fani et al., 2007; Gupta et al., 2012; Chaiya et al., 2013, Junaid et al., 2013; Vivek et al., 2014; Kekuda et al., 2014).

In this study, methanolic extract of *A. pinnata* and *A. rubra* were screened for their inhibitory activity of *S. mutans* isolates. Both species of Azolla were effective in inhibiting the clinical isolates of *S. mutans*. Marked inhibitory activity was observed in case of *A. pinnata* when compared to *A. rubra* as indicated by wider zones of inhibition and low MIC. It has been shown that extract of some Azolla species possess antimicrobial activities. In a study, Angalao et al. (2012) found antimicrobial activity of *A. filiculoides* against fungi. However, bacteria were not inhibited by extract. The study of Gerard (2013) showed that the methanolic extract of *A. microphylla* exhibit inhibitory activity against several strains of *Xanthomonas*. More recently, Nayak et al. (2014) observed marked antibacterial activity of methanolic extract of *A. caroliniana* against multidrug resistant pathogenic bacteria such as *S. aureus*, *P. mirabilis*, *Enterococcus sp.*, *E. aerogenes*, *E. coli* and *P. aeruginosa*.

### CONCLUSION

A marked anticaries activity of *A. pinnata* and *A. rubra* was observed in this study. These Azolla species can be used to control dental caries. Further studies on purification of active components from Azolla extracts and determination of their inhibitory activity against cariogenic bacteria are under progress.

### ACKNOWLEDGEMENTS

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### REFERENCES

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