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Short Communication

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 Minium 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.

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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 (Salviniaceae) 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 prokarytotic *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 promotory (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^{o}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^{o}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^{o}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*

Isolates	Zone of inhibition in cm			
	A. pinnata	***A. rubra***	**Streptomycin**	**DMSO**
Sm-01	3.4	3.1	3.9	0.0
Sm-02	2.9	2.7	3.7	0.0
Sm-03	3.1	2.8	4.1	0.0
Sm-04	2.8	2.6	3.4	0.0
Sm-05	3.3	2.9	4.0	0.0
Sm-06	2.6	2.3	3.5	0.0

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

Isolates	MIC (mg/ml)	
	A. pinnata	***A. rubra***
Sm-01	0.312	0.625
Sm-02	1.250	1.250
Sm-03	0.625	1.250
Sm-04	0.625	1.250
Sm-05	0.312	0.625
Sm-06	1.250	2.500

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 (Aneja *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 methanoli 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.

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