

Study To Evaluate Antimicrobial Efficacy Of Rubia Cordifolia Extract Against Cariogenic Organisms

Research Article

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Abstract

Introduction: Streptococcus mutans, Lactobacillus acidophilus and Actinomyces viscosus have been identified as cariogenic organisms. Various mechanical and chemical aids have been designed to control the levels of these organisms. In view of instances of development of resistance to currently used chemicals or antibiotics and side effects, search for alternatives has led to herbal compounds being used as antimicrobials. Rubia cordifolia also known as Indian madder is a commonly used herb in Ayurveda for the treatment of various medical conditions. It is known for its antioxidant, anti-inflammatory and antimicrobial activity. The aim of this study is to check the anti cariogenic activity of Rubia cordifolia against Streptococcus mutans, Lactobacillus acidophilus and Actinomyces viscosus.

Methodology: Rubia cordifolia root powder was taken and methanol extract was prepared using Soxhlet apparatus. The bacterial strains of Streptococcus mutans, Lactobacillus acidophilus and Actinomyces viscosus were grown on Mueller Hinton Agar. The minimum inhibitory concentration and minimum bactericidal concentration was determined for the above mentioned organisms.

Results: Rubia cordifolia extract was found to be effective against cariogenic organisms with a mean MIC of 12.5 mg/ml and MBC of 25 mg/ml.

Conclusion: Rubia cordifolia extract can be used as an anticaries agent in mouth washes and dentifrices due to its effectiveness against cariogenic organisms.

Keywords: Dental Caries; Streptococcus Mutans; Lactobacillus Acidophilus; Actinomyces Viscosus; Rubia Cordifolia; Anticaries.

Introduction

Dental caries is the most common chronic dental disease which is of multifactorial origin [1, 2]. Micro organisms form one of the main causative factors for this condition [3, 4]. Streptococcus mutans is a primary dweller in the oral cavity and is believed to be a primary colonizer in the initiation of caries [5, 6]. Once the caries process is initiated, Lactobacillus acidophilus takes part

in progressing the caries activity [7, 8]. The role of Actinomyces viscosus, an acidogenic species, has also been proved in the development of senile caries that occurs commonly on the root surfaces [9, 10].

Despite various attempts in caries control, a skewed distribution in caries development and progression has been noted all across the globe [11]. This has resulted in development of novel ideas in caries prevention and control strategies in the form of various

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mechanical and chemical aids. These chemically available anticaries agents carry the side effects of teeth staining and antimicrobial resistance [12].

Herbal mouth rinses are devoid of alcohol and sugar which are the two most common components of chemical mouthwash [13]. These components undergo further breakdown by oral microbiota to release by products that cause halitosis. This brings herbal mouth wash a step forward towards maintaining better oral hygiene.

Manjistha (*Rubia cordifolia*) often known as Indian madder is commonly found through out the hilly subtropical to sub temperate regions of India. It is a medicinal plant commonly used in Ayurveda and is known for its anti inflammatory and antiseptic actions [14]. Studies have shown the action of *Rubia cordifolia* extract against a wide range of organisms.

Previously our team has a rich experience in working on various research projects across multiple disciplines [15-29]. Now the growing trend in this area motivated us to pursue this project. Our aim is to evaluate the efficacy of *Rubia cordifolia* extract against cariogenic organisms.

Materials and Methods

Preparation Of Plant Extract

Rubia cordifolia root powder was purchased from Herbal Care and Cure Centre, Chennai. The *Rubia cordifolia* powder was soaked in methanol (1:30) and heated to 50°C for 24 hours on a rotary shaker, the solution was then filtered thrice through filter paper (Whatman No.1). The methanolic extract was prepared using Soxhlet apparatus and the supernatant was evaporated in a rotary evaporator. The dry crude extract obtained was kept in an air tight bottle at 20°C until use.

Bacterial Strains

Screening for antibacterial activity in the tested supernatants against several bacterial species was then performed. The strains used were *Streptococcus mutans*, *Lactobacillus acidophilus* and *Actinomyces viscosus*. The bacterial strains were grown in Muel-

ler- Hinton Agar (MHA) plates that were maintained at 37°C. The bacteria were grown in nutrient broth at 37°C and maintained on nutrient agar slants at 4°C (Figure 1). The organism was checked for its purity regularly on the basis of its culture characteristics, gram staining and biochemical tests.

Determination Of Minimum Inhibitory Concentration (MIC)

The MIC of the plant extract was carried out against *Streptococcus mutans*, *Lactobacillus acidophilus* and *Actinomyces viscosus* using broth dilution method (Figure 5). The cultures were incubated and then serially diluted to reach the density of 2×10^4 cells per ml. Cell counting was done using a hemocytometer. Two millilitres of Muller Hinton broth was dispensed in tubes and 100µl of cell culture was inoculated in it. Then 100µl of different concentrations of plant extract was added to each tube (Figure 2). Growth control was run parallel with every experiment. All experimental tubes were incubated in anaerobic jars for 48 hours. After completion of the incubation period, the optical density was measured at 600nm. MIC was defined as the minimum concentration of the extract that caused 20% inhibition in growth of test microorganism. Each experiment was carried out in a triplicate set. The lowest concentration prior to color change was considered as the Minimum Inhibitory Concentration (MIC).

$$\text{Percentage Inhibition} = \frac{\text{OD in control} - \text{OD in test}}{\text{OD in control}} \times 100$$

MBC value was determined by sub-culturing the test dilution (which showed no visible turbidity) on to freshly prepared nutrient agar media. The plates were incubated further for 18-24 hours at 37°C. The highest dilution that showed no single bacterial colony on the nutrient agar plates was considered as MBC.

Determination Of Minimum Bactericidal Concentration (MBC)

The antimicrobial activity of the methanol extract was evaluated by the agar well diffusion method using Mueller Hinton Agar (Figure 6). The microorganism was then either inoculated (0.25ml) in to molten petri dishes, or spread (0.1ml) on the surface of plates by spreading technique. Wells of uniform diameter (6mm) were

Figure 1. Nutrient agar slants.



Figure 2. Broth dilution method to determine MIC of *Rubia cordifolia* extract.



then made on solidified agar. About 0.1ml of the plant extract at the designated concentration (6.25, 12.5, 25, 50 and 100mg/ml) and the negative control (solvent without plant extract) were placed separately in each well. Plates were then left at room temperature for 1 hour to allow diffusion of the solution into MHA and incubated at 37°C overnight. Finally, the zones of inhibition were measured from the base of the plates and the experiments were performed in duplicate and repeated independently three times (Figure 3).

Statistical Analysis

For statistical analysis of data, multiple comparisons were performed using one way analysis of variance (ANOVA) followed

by the LSD test for post hoc analysis. Statistical significance was accepted at a level of $p < 0.05$. Data was analysed using SPSS (version 11).

Results and Discussion

MIC and MBC of the extract were evaluated at different concentrations (Tables 1 and 2). The mean MIC of the extract was determined as 12.5mg/ml and MBC as 25mg/ml (Table 3).

The results show that the mean MIC of the *Rubia cordifolia* root extract to be 12.5mg/ml and the MBC to be 25mg/ml against the cariogenic organisms. These findings are statistically significant ($P < 0.05$) as compared to the negative control that was distilled

Figure 3. Agar well diffusion method- NC- Negative control; A- *Rubia cordifolia* extract 6.25%; B- *Rubia cordifolia* extract 12.5%; C- *Rubia cordifolia* extract 25%; D- *Rubia cordifolia* extract 50%; E- *Rubia cordifolia* extract 100%.

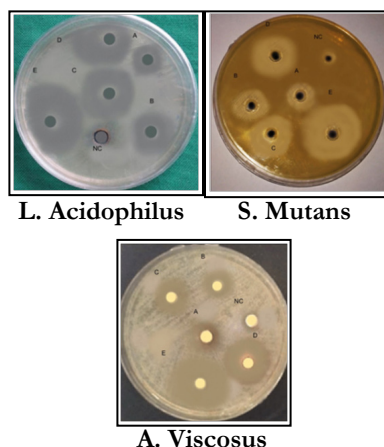


Table 1. Readings of MIC of *Rubia cordifolia* extract at different concentrations using broth dilution method.

Plant extract	Concentration (mg/ml)	S. Mutans		A. Viscosus		L. Bacillus	
		Mean OD ± SD	% of inhibition	Mean OD ± SD	% of inhibition	Mean OD ± SD	% of inhibition
Rubia cordifolia	6.25	0.416±0.03	19.6	0.421±0.03	19.33	0.421±0.03	16.7
	12.5	0.378±0.02	27.13	0.398±0.02	31.99	0.396±0.02	21.7
	25	0.214±0.01	58.68	0.234±0.01	52.99	0.228±0.01	54.9
	50	0.16±0.01	69.1	0.161±0.01	67.6	0.179±0.05	64.6
	100	0.094±0.01	81.8	0.102±0.02	79.4	0.098±0.08	80.6
Negative control		0.518±0.06		0.497±0.05		0.506±0.04	

Table 2. Readings for MBC of *Rubia cordifolia* extract at various concentrations using agar well diffusion method.

Plant extract	Concentration (mg/ml)	S. Mutans (mm)	A. Viscosus (mm)	L. Bacillus (mm)
Rubia cordifolia	6.25	9.3±0.56	5.6±0.41	8.1±0.79
	12.5	11.6±1.0	7.3±0.51	10.1±0.92
	25	14.5±1.1	10.4±0.96	13.1±1.1
	50	16.1±1.0	13.5±1.1	15.9±1.2
	100	19.3±1.7	16.8±1.2	17.8±1.4
Negative control		Nil	Nil	Nil

Table 3. Mean MIC and MBC (mg/ml) results.

	S. Mutans	A. Viscosus	L. Bacillus
MIC (mg/ml)	12.5	12.5	12.5
MBC (mg/ml)	25	25	25

water.

Our institution is passionate about high quality evidence based research and has excelled in various fields [19, 30-40].

Rubia cordifolia root extract is commonly used in Ayurveda [41]. It has a wide range of pharmacological activity ranging from anti-oxidation, neuroprotection, anti inflammatory, antitumor and immunomodulation [42-44]. The roots of this herb contain various compounds like anthraquinones (munjistin, purpurin, alizarin), naphthoquinones (mollugin, furomollugin), cyclic hexapeptides (rubicordin, RA I, II) and triterpenoids (oleanolic acid, rubiarbanol A,B) [43, 45]. Alizarin present in the extract gives red color to it. The antibacterial action of this extract is mainly due to the components anthraquinones and flavonoids. The mechanism of action of these compounds is by altering the cell membrane fluidity and disruption of the cell wall, inhibiting DNA and RNA synthesis by binding to the phosphate groups of DNA and inhibition of metabolic processes by acting on the various bacterial cell processes and bacterial enzymes [46-48].

Various studies have been conducted to check its antimicrobial action. Aldehyde acetate, dihydromollugin and ribamallin have shown significant antibacterial activity against Klebsiella pneumonia [49]. Ethanolic extract has inhibited beta lactamase producing E.coli [50]. As stated by Basu et al, the aqueous extract is active against Bacillus subtilis and Staphylococcus aureus compared with streptomycin and penicillin G. Rubia cordifolia root extract has also been shown to have antifungal activity.

Since Rubia cordifolia extract is red in color, the study was extended by evaluating the MIC and MBC values with the distillate of the extract which was colorless. This showed marked increase in MIC and MBC values indicating that the red colored dye possessed antimicrobial activity.

Conclusion

In view of the developing resistance against most of the commonly used antimicrobials for preventing caries, research is on to look for herbal alternatives that are easily available, cost effective and prevent the development of resistance among cariogenic organisms. Many studies have been carried out to check the antimicrobial activity of numerous herbs. In this study Rubia cordifolia root extract was used and it showed inhibitory activity against anticariogenic organisms. This paves the way for further research in the use of this extract as anticaries mouthwash there by controlling and minimizing the incidence of caries development.

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