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# Comparing the Effectiveness of Aqueous and Alcoholic Rubia Cordifolia Extracts against *Streptococcus mutans* and *Lactobacillus Acidophilus* with Chlorhexidine: An in Vitro Study

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**Abstract**--Introduction: Because of the majorly reported undesirable effects of excessive antibiotic usage like drug hypersensitivity and drug virulence, herbal extracts serve as alternatives with potent therapeutic effects and lesser side effects. The aim of the current study was to evaluate the effectiveness of aqueous and alcoholic Rubia cordifolia extracts against *S.mutans* and *L.acidophilus*. Materials and Methods: The antibacterial activity of aqueous and alcoholic extracts of R.cordifolia was tested on *S.mutans* and *L.acidophilus* and compared with the gold standard Chlorhexidine. Aqueous extract and alcoholic extracts of R.cordifolia was formulated. The antibacterial activity of R.cordifolia was determined by agar well diffusion method. Results: The aqueous extract of R.cordifolia showed a higher zone of inhibition for *L.acidophilus* at 100 µl measuring 21mm. The alcoholic extract of R.cordifolia showed a higher zone of inhibition for *S. mutans*

which was the maximum at 100 µl measuring 21mm. The zone of inhibition of Aqueous and Alcoholic extracts of *R.cordifolia* was much superior to chlorhexidine against *L.acidophilus* and *S.mutans* respectively. Conclusion: The aqueous and alcoholic *Rubia cordifolia* extracts were effective against *S.mutans* and *L.acidophilus*. Aqueous extract of *R.cordifolia* is superior in inhibiting *L.acidophilus* than chlorhexidine. Alcoholic extracts of *R.cordifolia* is superior in inhibiting *S.mutans* than chlorhexidine.

**Keywords**---alcoholic extract, aqueous extract, chlorhexidine, *r.cordifolia*, *streptococcus mutans*.

## Introduction

Ayurvedic herbs are at a great interest across the world as they are free of adverse reactions or side effects(1).According to Cragg's investigation, approximately 62% of drugs that are available commercially have natural plant formulations. However, the emergence of serious side effects of antibiotics like drug resistance, increased virulence and drug hypersensitivity have paved way for the alternative ayurvedic herbs for their widespread use(2) and these are the majorly reported undesirable effects of excessive antibiotic usage(3).

Dental caries is appraised as a disease of modern living. It is a microbial disease of the oral cavity that is irreversible(4).*Streptococcus mutans* and *Lactobacillus* are the primary cariogenic microorganisms causing dental caries(5).In 1924,*Streptococcus mutans* was identified by Clarke. And till date, these microorganisms one amongst the pathological bacteria in the oral cavity because of their characteristic abilities to survive in low pH and to produce extracellular polysaccharides and acids(6).However, these microorganisms are investigated comprehensively for the prevention of dental caries(7).

*Rubia cordifolia*, also known as Indian Madder or Common Madder, is a flowering plant species in the coffee family. The phytochemicals of *Rubia* plants have considerable potent bioactivities(8).The roots of *Rubia cordifolia* have its genesis in Ayurveda medicine. The root of the plant is commonly called as Manjistha. It is also known by a number of vernacular names like Aruna, Bhandi, Majathi, Manjathi, Manjith. The roots of this plant are predominantly used in the treatment of abnormal uterine bleeding, internal and external haemorrhage, bronchitis, rheumatism, stones in the kidney, to lower the blood pressure etc. It comprises of an organic compound called Mollugin, that is known to possess anti inflammatory property(9).

Antimicrobial compounds from herbal sources have a good therapeutic potential. Various plant and fruit extracts have been evaluated recently to assess their activity against oral pathogens. *Punica granatum*(pomegranate) has been signified as an effective herbal extract in Iranian medicine(10).Lotus leaves have been used for the treatment of mouth inflammation and halitosis according to ancient Chinese medicine. Traditional practices support the use of *Piper betle* extracts to

maintain oral hygiene(11).Coffee has been identified to possess significant antibacterial properties against cariogenic bacteria(12).

Despite abundant literature on general health benefits of *R.cordifolia* exists, its effect on cariogenic microbes is limited and none of the studies have compared them with chlorhexidine. Hence, the aim of this in vitro study was to compare the efficacy of aqueous and alcoholic *R.cordifolia* extracts against *S.mutans* and *L.acidophilus* in comparison to chlorhexidine.

## **Materials and Methods**

The present in-vitro study was conducted at the Department of Nanomedicine, Saveetha Dental College and Hospitals, Chennai. Bactericidal activity was determined by agar well diffusion method.

### *Preparation of Rubia cordifolia*

Total of 100gms fresh root pieces of manjishta were purchased from online retailer, Chennai city, of which 2gms of manjistha roots were crushed and homogenized in sterile mortar and pestle under aseptic conditions. Chlorhexidine was used as a control because it is commonly used as a standard to compare the effects of new products.

### *Preparation of Rubia cordifolia ethanolic extract*

Rubia ethanolic extract was prepared by adding 1g of Rubia to 50ml of ethanol in a conical flask. The extract was then filtered through Whatman No.1 filter paper and was stored in an air tight container for further use.

### *Preparation of Rubia cordifolia aqueous extract*

The dry powder of Rubia was weighed about 2g. The powder was then added to 100 ml of distilled water in a conical flask. The extract was then filtered through Whatman No.1 filter paper and was stored in an air tight container for further use

### *Microbial Assay*

Agar well diffusion method was used to evaluate the antibacterial potential of the extracts. Petri dishes contained Mueller Hinton agar for both *S.mutans* and *Lactobacillus* species. These suspensions were then smeared on agar plates using swab technique. With the aid of a sterile 5mm metal borer, 4 equally spaced wells were bored in the agar plates aseptically.

The agar plates were loaded with 25 $\mu$ l, 50  $\mu$ l, 100  $\mu$ l of Aqueous extract of *R.cordifolia* with an antibiotic in the respective prepared wells for *Streptococcus mutans* and *Lactobacillus acidophilus*. After 24 hours of incubation, the agar plates were observed for zone of inhibition. It was measured as the maximum width from the edge of the well to the periphery of the inhibition zone with the help of vernier calliper. Maximum zone of inhibition would determine the inhibition of bacterial growth on an agar plate. Similarly the experiment was

repeated for Alcoholic extract of *R.cordifolia* for *Streptococcus mutans* and *Lactobacillus acidophilus*.

### Results

GROUP	ORGANISM	25µl	50µl	100µl
R.cordifolia (Aqueous extract)	<i>S.mutans</i>	12mm	13mm	15mm
	<i>L.acidophilus</i>	10mm	16mm	21mm
R.cordifolia (Alcoholic extract)	<i>S.mutans</i>	13mm	16mm	21mm
	<i>L.acidophilus</i>	10mm	11mm	16mm
Chlorhexidine	<i>S.mutans</i>	19mm	21mm	19mm
	<i>L.acidophilus</i>	10mm	12mm	16mm

Table 1: Zone of inhibition of Aqueous and Alcoholic extracts of *R.cordifolia* compared with Chlorhexidine against *S.mutans* and *L.acidophilus*

Table 1 shows the antimicrobial activity of Aqueous extracts of *R.cordifolia* against *S.mutans* and *L.acidophilus* at 48hrs.

From the above results, it is evident that Aqueous extract of *R.cordifolia* is superior in inhibiting *L.acidophilus* than chlorhexidine. Alcoholic extracts of *R.cordifolia* is superior in inhibiting *S.mutans* than chlorhexidine.

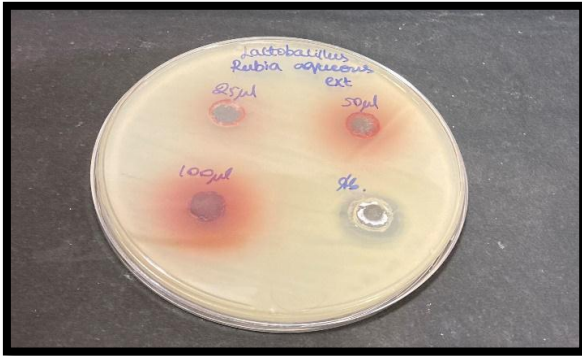
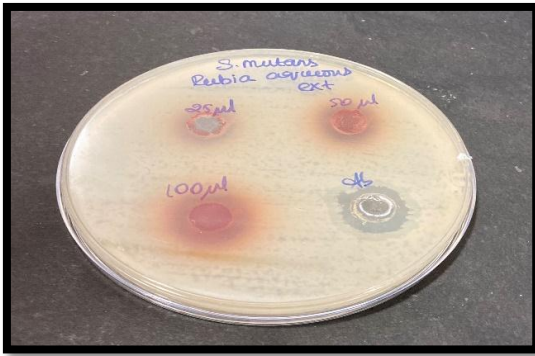


Figure 1: Zone of inhibition of Aqueous extract against *S. mutans* and *L. acidophilus*



Figure 2: Zone of inhibition of chlorhexidine against *S. mutans* and *L. acidophilus*

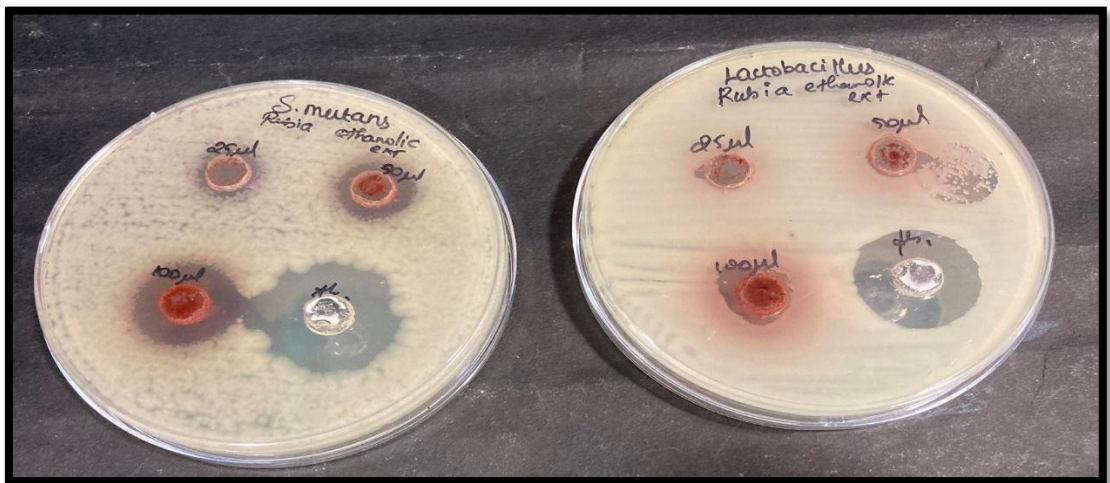


Figure 3: Zone of inhibition of Alcoholic extracts against *S. mutans* and *L. acidophilus*

## Discussion

In spite of its use in medicinal field, *R.cordifolia* is less explored in the field of dentistry. Therefore, the aim of the study was to evaluate the antimicrobial effect of Aqueous and Alcoholic extracts against caries causing microorganisms *S.mutans* and *L.acidophilus* in comparison to chlorhexidine.

Chlorhexidine was considered as positive control in the study. This is one of the promising agents with an effective antimicrobial activity against cariogenic microorganisms. Various studies have signified that CHX induces precipitation of nucleic acids and cytoplasmic proteins(13).It operates by blocking phosphoenolpyruvate-phosphotransferase activity ,that is fundamental for sugar transport, thereby curbing the acid production in oral streptococci(14).

The results of the current study was found to be similar to the previous investigations conducted by Basu et al where it is stated that methanol and chloroform extracts from *R.cordifolia* were very effective against gram positive bacteria than gram negative strains(15).

The present study demonstrated that the zone of inhibition to both aqueous and alcoholic extracts of *Rubia cordifolia* was effective when compared to chlorhexidine against the cariogenic microorganisms *L.acidophilus* and *S.mutans* respectively. Aqueous extracts has a higher zone of inhibition for *L.acidophilus* than *S.mutans*. This might be because of increased dissolving capacity in alcohol, bioavailability, polarity of antimicrobial compounds.

## Conclusion

The zone of inhibition of Aqueous and Alcoholic extracts of *R.cordifolia* was much effective than chlorhexidine against *L.acidophilus* and *S.mutans* and hence can be used as an alternative to commercial agents provided it is subjected to various chemical, biological methods and or enzymatic methods.

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