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Antibacterial Activity of Methanolic Fruit Extract of *Randia dumetorum* Lamk against Oral Pathogens

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Abstract : Dental caries and periodontal diseases have been documented as the most common health problems universally. Most of the chemicals and synthetic drugs currently in use have marked side effects. Hence, there has been an ideal shift from the use of modern drugs to the age-old herbs. *Randia dumetorum Lamk* is one such important plant with various established medicinal properties. The aim of the present study was to evaluate the preliminary antibacterial activity of methanolic extract of *Randia dumetorum Lamk. (Xeromphis spinosa* Thumb.)against common oral pathogens such as *Streptococcus mutans, Streptococcus sobrinus* and *Lactobacillus acidophilus.* Methanolic extract of the dried fruits of the plant was prepared. Different concentrations of the dried fruit extracts*(R. dumetorum)* were transferred to the nutrient agar plates, which had been previously inoculated with the test microroganisms. The plates were incubated at 37°C for 24 h in an incubator and the zones of inhibition were measured using well diffusion method. The extract showed potential antibacterial properties comparable with that of the standard chlorhexidine against the organisms tested. The methanolic extract of *R. dumetorum* displayed a concentration related antibacterial activity. The results showed that the inhibition of the bacterial growth was more pronounced on *Lactobacillus acidophilus* as compared to the other tested organisms.

Key words : *Randia dumetorum Lamk,* antibacterial activity, *Streptococcus mutans, Lactobacillus acidophilus.*

Introduction:

Dental caries and periodontal diseases have been documented as the most common health problems universally[1].The oral cavity is colonized by numerous bacterial species, but only a selected number of these species participate in the dental decay (caries) or periodontal disease. Dental decay is due to the irreversible solubilization of tooth mineral by acid produced by certain bacteria that adhere to the tooth surface in bacterial communities known as dental plaque. *Streptococcus mutans* and *Streptococcus sobrinus* are the main causes of the dental decay. Various *lactobacilli* are associated with progression of the lesion[2].

Many chemicals and synthetic drugs have proven to be effective in the prevention of these diseases, but they also have marked side effects. In recent times, there has been a marked shift toward herbal cures because of the pronounced cumulative and irreversible reactions of modern drugs [3,4.]

Randia dumetorum lamk is a species of plant in the Rubiaceae family, commonly known as Mainphal, Mindhal. It is found in waste places & jungles all over India, extending northwest to the Bias river& ascending to outer Himalaya to 4000 ft[5].

Ripe fruitsof the plant contains glycosides, randioside A, mollisidialtriterpenoid glycosides and randianin, six saponins dumetoronins A to F [6,7]. The fruit is reported to have various pharmacological actions such as aphrodisiac, emetic, purgative, carminative, antipyretic, cures abscess, ulcers, inflammations, wounds, tumors, skin diseases and have antibacterial activity. The pulp of fruit is believed to have anthelmintic properties, and also used as an abortificient in folklore remedy.The bark is astringent and is given in cases of diarrhoea and dysentery [8].

Various studies report that the Methanolic extract of *Randia dumetorum Lamk* has shown antibacterial activity against *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus, Bacillus subtilis, Escherichia coli* and *Salmonella typhi*[9]. Very few studies have been conducted on the antibacterial effect of *Randia dumetorum Lamk* extracts against oral pathogens. Therefore, the aim of this study is to evaluate the *in vitro* antibacterial activity of *R.dumetorum* against some selected oral pathogens.

Materials and Methods

Plant collection and authentication

Randia dumetorum seeds were obtained from local market and authenticated by Head of Botany department, Malla Reddy college of Pharmacy, Maisammaguda, Telangana. A voucher specimen has been deposited in the museum of department of Pharmacognosy, M.R.C.P Telangana. Voucher specimen (PH- 709) was also deposited in the herbarium of Pharmacy Department of M.R.C.P Telangana**.**

Plant preparation and extraction

The fruits were dried in sunlight and reduced to a coarse powder. Then the powder was subjected to soxhlet extraction with methanol for 72 hours at a temperature of 50-60ºC. The extract was concentrated and the solvent was completely removed. They were freeze dried and stored in the vacuum dessicator until further use. Preliminary phytochemical screening were carried out to identify the chemical constituents [10].

Microorganism:

Pure cultures of *S.mutans, Streptococcus sobrinus,* and *Lactobacillus acidophilus* were obtained from MTCC, Institute of Microbial Technology, Chandigarh.

Preparation of inoculums

The suspension of all organisms were prepared by inoculating a single colony of the strain in 20 ml of sterile nutrient broth and incubated at 37ºC for 24 hours. The suspension is adjusted such that it contained approximately 1 x 10^6 cells/ml. It was obtained by calculating the cells by Neubers chamber. Nutrient agar (HiMedia) was prepared for the study.

Controls for the test

The controls were needed to confirm all the necessary nutritional conditions were suitable for the growth of microorganism and for absence of inhibitory substance in the medium. The positive controls were observed by streaking the organism on agar plates for observing morphology of colonies. Any contamination during the assay was ruled out by keeping the negative control. This was checked by adding the sterile saline and observing for growth as a contamination. The results indicated that the medium was free from contamination.

Culture medium

Readymade dehydrated medium supplied by Hi Media was used for testing the antimicrobial activity of plant extracts. The dehydrated medium was prepared by incorporating 13grams of NA in 100 ml of distilled water and heated to boiling to dissolve the medium completely. The medium was distributed into clean glass tubes and plugged with cotton and sterilized by autoclaving at 15 lb/sq. inch pressure at 121^0 C for 20 min.

Antimicrobial Agent

0.2% chlorhexidine at 10 µl was used as the reference standard was procured as gift sample from Hindustan Antibiotics Ltd., Pune.

Determination of MIC (minimum inhibitory concentration)

Broth dilution method was followed for the determination of minimum inhibitory concentration of the extract. Fresh amount of the nutrient broth was prepared and sterilized by autoclaving.20 ml of the sterilized nutrient broth was transferred to the test tubes. Measured amount of the extract was added in to the test tubes containing nutrient broth in such as way that the final concentration per ml was 0 (control), 5,15,25,50 and 100mg.Loopful of test microorganism was incorporated into the test tubes and incubated at 37^0C for 24hrs. After completion of the incubation period, the tubes were checked for the growth. Growth in the liquid cultures were seen in the form of turbidity. Tubes showing growth was denoted by '+' and '-' for absence of growth.

Determination of zone of inhibition by cup plate method [10]

The antimicrobial activity of the methanolic extract was determined by well diffusion method.20ml of sterile nutrient agar medium was transferred into the sterile petri-dishes and allowed to solidify. The petri dishes were incubated at 37°C for 24 hours to check for the sterility. The medium was seeded with the organisms by pour plate method using sterile top agar layer (4 ml) containing 1 ml of the culture. Wells were made on the medium using sterile borer. Dried methanolic extract of fruits of *Randia dumetorum* was dissolved in Dimethyl sulfoxide (DMSO) to obtained different concentration (50, 100 and 150 mg/ml) and sterilized by filtration through a Whatman filter paper no. 1, and 0.1 ml of the different concentrations of extract were added to the respective bores. 0.1ml of 0.2% chlorhexidine at a concentration of (0.5 mg/ml, 1mg/ml) was taken as standard reference. The plates were incubated overnight at 37°C with appropriate positive and negative controls. The petri-dishes were kept in refrigerator at 4°C for ½ hour for diffusion. After diffusion the petri-dishes were incubated at 37°C for 24 hours and zone of inhibition were observed and measured. Dimethyl sulfoxide was used as the control.

Result and Discussion

Table 1. Determination of MIC of methanolic fruit extract of *R. dumetorum* against different bacteria

Name of the bacteria	**Growth in nutrient broth containing different concentration of extract in mg/ml**					
	0	**5**	**15**	**25**	**50**	**100**
Streptococcus mutans	+	+	+	-	-	-
Streptococcus sobrinus	+	+	+	–	–	–
Lactobacillus acidophilus	+	+	–	–	–	–

'0' – Control (without extract); '+' – Growth; '-' – No growth

Table 2. Antibacterial activity of Chlorhexidine and fruits methanolic extract

Micro organism	**Zone of inhibition in mm**				
	Extract Conc. mg/ml			**Conc. Chlorhexidine mg/ml**	
	50	**100**	**150**	**0.5**	**1**
Streptococcus mutans	12 ± 0.42	16 ± 0.53	18 ± 0.17	20 ± 0.31	26 ± 0.54
Streptococcus sobrinus	14 ± 0.51	15 ± 0.18	16 ± 0.57	22 ± 0.42	24 ± 0.72
Lactobacillus acidophilus	20 ± 0.73	21 ± 0.41	23 ± 0.61	21 ± 0.15	25 ± 0.68

Plant based medications have been used from many ages. Our ancestors had been known to use herbal drugs to treat and alleviate illnesses that affect the human body. Medicinal plants are today being once again preferred because of the various reasons such as their easy availability, negligible side effects, low cost of treatment, and their effectiveness. The present study proved that *R. dumetorum* possesses a significant antibacterial activity against *S.mutans, Streptococcus sobrinus, and Lactobacillus acidophilus*, which are the causative organisms playing a major role in the pathogenesis of dental caries.

The antibacterial activity of the extract was confirmed by observing zone of inhibition around the well containing test solution after specified incubation period. Absence of bacterial growth in the negative control plate confirmed the sterility of the medium. The remaining plates were examined for the presence or absence of growth. The positive control without *R. dumetorum*extract was checked to ensure that each test strain was capable of exhibiting adequate growth in the medium. The negative control was checked for the absence of growth there by indicating the sterility of the medium. In reading the end points, a faint haze of growth of a single colonywas evident for antimicrobial activity. A dense film of growth or more than one colony was considered as evidence that the plant extract failed to inhibit the growth.

The results of the MIC as well as well diffusion are summarized in Table. 1 and 2. The largest zone of inhibition of methanol extracts was found to be 20 mm against *Lactobacillus*. Second largest zone was observed against *Streptococcus sobrinus* i.e. 14 mm at 50mg/ml. Chlorhexidinegluconate was used as the standard drug to check the efficacy of the test compound. At 0.5 mg/ml concentration, it showed a zone of inhibition of 21 mm and 22 mm against *Lactobacillus acidophilus* and *Streptococcus sobrinus respectively.*Though the plant extract was found to be effective at a higher concentration and volume than chlorhexidinegluconate, it showed a marked antibacterial activity and should be considered to replace the synthetic drugs and chemicals because of their irreversible side effects.

Any antimicrobial agent is considered effective, given the size of inhibition zone produced by it measures 2 mm or more. In the present study, the minimum zone of inhibitions obtained were 12 mm and 14 mm for methanolic extracts of *R. dumetorum*, respectively. It has proved to have potent antibacterial property.

The result showed that the methanolic fruit extract of *R. dumetorum* displayed concentration dependent antibacterial activities. It indicated that *R. dumetorum* exhibited antibacterial activity towards all three oral pathogens. The highest antibacterial activity was found towards Lactobacillus acidophilus, while it was less active against *S.mutans*. The compounds responsible for this antimicrobial property were not investigated. However preliminary phytochemical analysis of the methanolic extract revealed the presence of phytosterol, polyphenol, saponins, flavonoids and carbohydrates[11]. The antimicrobial potency of the plant may be attributed to the single or combined effect of the above mentioned chemical groups. The methanolic fruit extract of *R. dumetorum* had impressive antibacterial activity and could lead to the discovery of new antibacterial agents. This becomes more relevant as the current drugs in use are fast loosing effectiveness due to their irreversible side effects.

Conclusion

Antibacterial agents against oral microorganisms, especially those contributing to sub- and supra-gingival biofilm formation, play an important role in the prevention of dental caries and periodontal disease. Since some chemical synthetic drugs including chlorhexidine can cause brown staining of the teeth, tongue, transient impairment of taste perception toxic effects on connective tissues, dryness and soreness of oral cavity, allergic reactions in patients, and oral desquamation in children, herbs with medicinal properties can serve as a useful and effective source of treatment for various dental diseases. Hence, they can be utilized along with conventional medicine that can assure us of greater health in the future. In the present study methanolic extract of *Randia dumetorum Lamk* showed good activity against the oral pathogens. Further studies must be conducted for the separation of the active components of the extract and to assess its safety levels.

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