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Comparative evaluation of antibacterial properties of different extracts of *Mimusops elengi* (bakul) & *Juglans regia* (walnut) against salivary microflora.

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ABSTRACT

Dental caries is a chemico-parasitic process where the oral micro-organisms play a pivotal role. For prophylactic purposes, we must target processes involved in formation of mixed bacterial communities that have the potential to cause initiation of dental caries, without perturbing the balance of the normal flora. Herbal extracts used in traditional medicinal systems are known antibacterial agents. In this study, the bark extracts of *Mimusops elengi*(Bakul) *and Juglans regia*(Walnut),each in aqueous and acetone solvents were evaluated and compared for antibacterial activity against salivary microflora. The salivary samples were collected from children of 6-12 years of age with moderate caries (DMFT=3-4). Antibacterial assay was carried out using paper disc diffusion method. The antibacterial potential of both the plants was confirmed. The acetone extract of *J. regia* showed highest zones of inhibition which was found to be statistically significant ($p>0.032$), thus indicating its use as a potent antibacterial agent. Comparatively, the aqueous and acetone extracts of *M. elengi* did not show any significant zones of inhibition. Thus, this in vitro study supports its folklore application as a preventive remedy for microbial diseases of hard & soft tissues in the oral cavity.

Keywords: Salivary microflora, *Mimusops elengi*(Bakul), *Juglans regia*(Walnut), antibacterial, primordial prevention.

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INTRODUCTION

Oral health is an important aspect of the overall health of an individual. The diseases produced by a number of micro-organisms are manifested in the oral cavity. Dental caries, prevalence as high as 60-80% in children, is major health problem in India [1]. Due to the growing evidence of relation between oral health and whole body health dental practitioners may seek to respond to their patient's oral hygiene needs with newer products [2,3]. These research based products come with naturally occurring active ingredients, that achieve the desired antibacterial and anti inflammatory effect [3]. Moreover, chemicals like chlorhexidine and amine fluorides have undesirable side-effects such as staining of teeth and restorations, increase in calculus deposition and imbalance of the oral and intestinal flora, thus leading to vomiting and diarrhea. These drawbacks justify the search for new effective anticariogenic compounds that could be employed in caries prevention [4,5].

In this sense, efforts have been made to evaluate & compare the antimicrobial properties of bark extracts of *Mimusops elengi* and *Juglans regia* with 2% chlorhexidine gluconate against human salivary microflora. Furthermore, the effectiveness of acetone and aqueous extract of each of the herbs at various concentrations was also investigated.

MATERIALS AND METHODS

Source of data

Patients who enrolled at the Dr. Deshpande's Oral Health Clinic at Pune, were included in the study after obtaining their informed consent.

Inclusion criteria

Patients of 6 – 12 years old in mixed dentition age group with moderate caries (DMFT=3-4) (modified WHO criteria 2003) having good general health [6].

Exclusion criteria

Patients with history of antibiotic and oral drug therapy, chemical anti-plaque agents prior to six months of study initiation, physically and mentally handicapped patients were excluded from the study.

Plant extracts

Plant materials used in this study were procured from the local market of Pune, Maharashtra, India, and the bark extracts were obtained using acetone and distilled water as solvents. The plant materials of *M.elengi* & *J.regia* were authenticated at Agharkar Research Institute, Pune, India with authentication number AHMA S/B – 065 & 14319, respectively.

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Microbial Flora

The saliva samples (5 samples of each of the two groups) from the patients were collected using sterile cotton tipped swabs placed in the floor of the mouth. It was then placed in a sterile container with saline (2 ml) and was used to inoculate on the agar plates.

Anti-Microbial assay

The paper disc diffusion method (Kirby-Bauer method) was employed [7,8]. Samples of acetone and aqueous extracts (30 mg) of each plant were dissolved in respective solvents (1 ml). Sterile 5mm diameter filter paper discs were impregnated with these extracts of different concentrations ranging from 100 µg to 600 µg per disc.

The salivary flora were inoculated on nutrient broth and incubated for 24 hours at 37 ± 0.1 °C. Adequate amount of Mueller-Hinton Agar were dispensed into sterile plates and allowed to solidify under aseptic conditions [7,8]. The test samples of saliva (0.1 ml) were inoculated with a sterile spreader on the surface of solid medium in plates. The agar plates inoculated with these test samples were incubated for one hour before placing the extract impregnated paper discs on the plates. Following this, the sterile discs impregnated with acetone & aqueous extracts were placed on agar plates. The bacterial plates were incubated at 37 ± 0.1 °C for 48 hours. After incubation, all the plates were observed for zones of inhibition and the diameter of these zones was measured in millimeters. The minimum inhibitory concentration (MIC) was taken as the lowest concentration that will prevent the growth of the salivary micoflora [8].

STATISTICAL ANALYSIS

Data were expressed as mean standard deviation. All results were statistically analyzed and compared using Kruskal-Wallis test:

Table 1: Mean values of J. regia and M. elengi

Group	Type of extract	N	Mean rank
J. regia	aqueous	5	11.10
	acetone	5	15.00
M. elengi	aqueous	5	6.90
	acetone	5	9.00
Total		**20**	

Table 2: Statistical analysis

	Value
Chi-square test	5.363
Df	3
P value	0.147
NS	

Df- degree of freedom
NS- not significant

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Table no. 3: Comparative mean values of J. regia and M. elengi

Concentration of the extract	N	Mean rank
100 µg	4	6.25
150 µg	4	9.50
200 µg	4	8.25
250 µg	4	10.00
Control	4	18.50
Total	**20**	

Table no. 4: Statistical analysis of comparative mean values of J. regia and M. elengi

	Value
Chi-square test	10.586
Df	4
P value	0.032

Significant

RESULTS & DISCUSSION

About 80,000 species of plants are utilized for treating various diseases in different systems of Indian medicine. Since 1990's there has been a growing shift in interest towards plants as significant sources for new pharmaceuticals. This shift is mainly due to the current widespread belief that 'Green Medicine' is safe & more dependable than the costly synthetic drugs, which have adverse side effects.

As per the World Health Organization (WHO) report, 80% of the world population presently uses herbal medicine for some aspect of primary health care [9]. Since the last decade, the rise in the failure of chemotherapeutics and antibiotic resistance exhibited by oral pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity [10,11].

In the present study, two plants viz., *Mimusops elengi*(bakul) and *Juglans regia*(walnut) were selected based upon their traditional medicinal uses in the treatment of oral diseases. This paper reports the antibacterial activity and the effectivity of different extracts of the above mentioned plants against salivary microflora.

The results of antibacterial assay using the paper disc diffusion method of sample no.2 can be observed in Figure no. 1.

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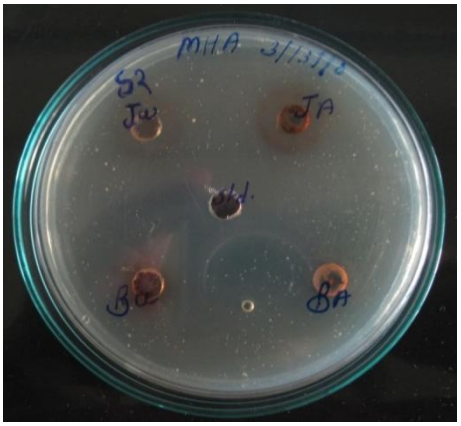

Jw- aqueous extract of J. regia
Ja- acetone extract of J. regia
Bw- aqueous extract of M. elengi
Ba- acetone extract of J. regia
Std.- chlorhexidine as standard

Figure no. 1: Antibacterial assay of aqueous and acetone extracts of J. regia & M. elengi with chlorhexidine as standard in sample no.2,showing maximum zones of inhibition with acetone extract of J. regia.

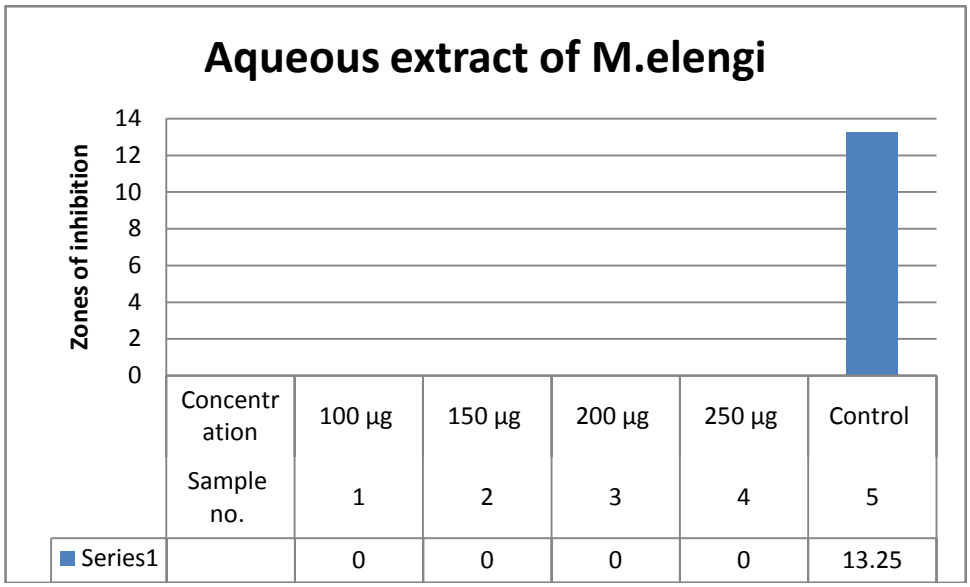

Figure no. 2: Effect of Aqueous Extract of M. elengi on micro-organisms, showing no zones of inhibition except the control group(13.25mm).

The results of the antibacterial assay of *M. elengi* are represented in Figure no. 2, which showed no zone of inhibition when the aqueous extract was used. It could be concluded that the aqueous extracts of *M. elengi* had no antibacterial activity on the salivary microflora .

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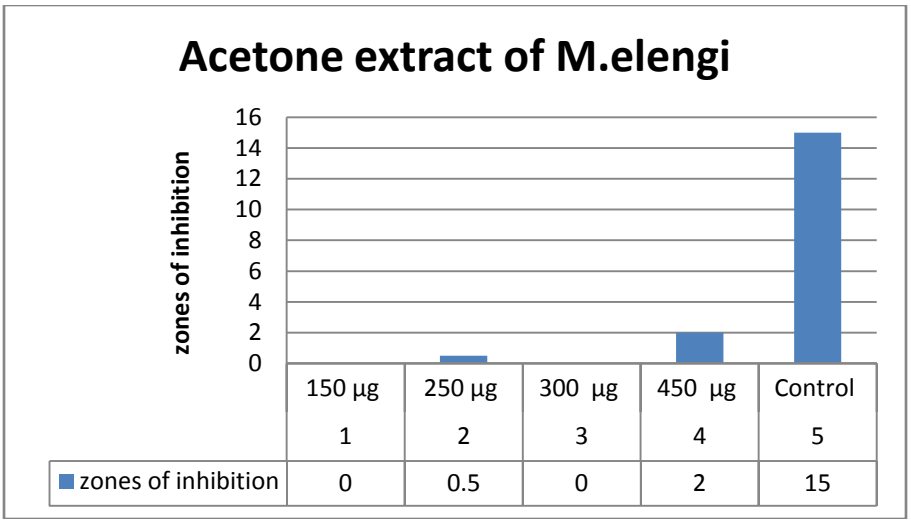

Figure no. 3: Effect of Acetone Extract of M. elengi on micro-organisms,showing maximum zone of inhibition(2mm) at 450µg.

On the contrary, acetone extract of *M. elengi* showed high zones of inhibition and hence, more antimicrobial activity. The acetone extract of *M. elengi* at concentrations of 250µg & 450µg showed 0.5mm & 2mm of zones of inhibition, respectively (Figure no. 3).

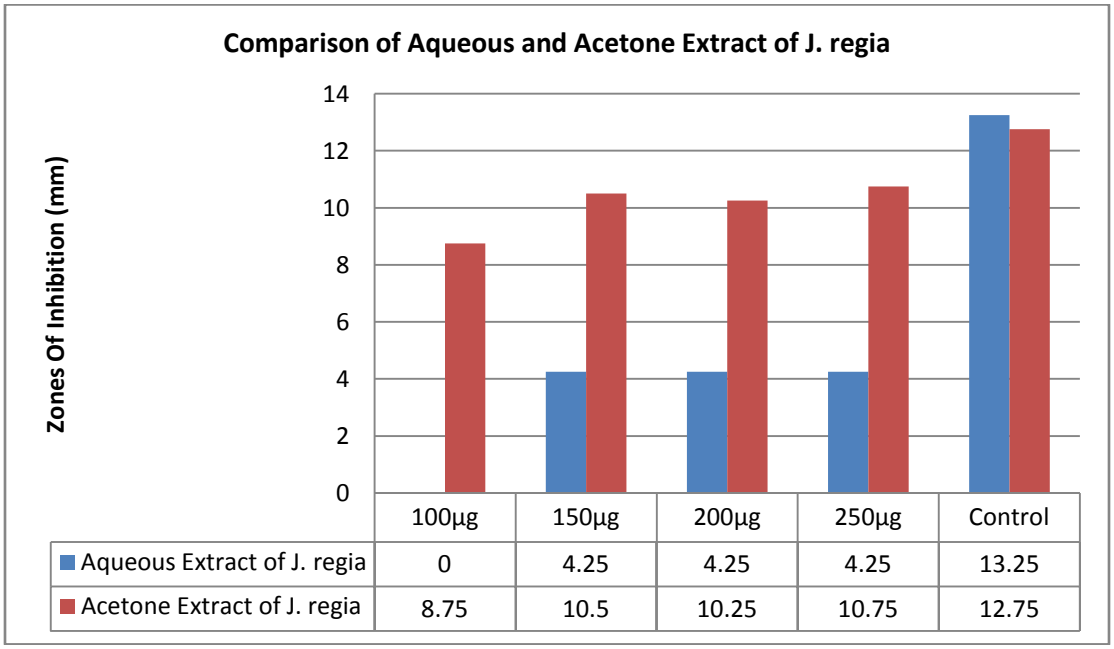

Figure no. 4: Comparison of Effect of Aqueous and Acetone Extracts of J. regia on micro-organisms,showing maximum zones of inhibition with acetone extract(10.75mm) at 250 µg.

On comparison, among the two extracts, the aqueous extract of the bark of the plant *J. regia* showed zones of inhibition against all the tested samples. The acetone extract showed the highest percentage of inhibition of micro-organisms and thus was found to be more

effective as anti-microbial medicine. Figure no. 4 shows the graphical representation of these comparative results.

In the present study, the results confirmed the antimicrobial potential of the plants and indicated that the extracts can be used in the prevention of infectious diseases caused by salivary microflora. The bark extracts showed significant antibacterial activities. The solvents used in the extraction procedure were found to have pronounced effect on the solubility of the antibacterial compounds.

Acetone extract was found to be more effective among the two extracts, because more organic compounds were leached in this solvent. Although water is reported by the traditional healers and herbalists to be the most commonly used solvent for extracting the active compounds due to its easy availability [12].

This study also compared the effectiveness of acetone extract of *M. Elengi* & *J. regia* at different concentrations on salivary micro-organisms using disc diffusion method.The minimum inhibitory concentration (MIC) was taken as the lowest concentration that will prevent the growth of the salivary microflora. This minimum concentration was found to be 250µg with 0.5mm zone of inhibition for acetone extract of *M. elengi*, while the aqueous extract of *M elengi* showed no zone of inhibition at various tested concentrations. For the aqueous & acetone extract of *J. regia*, it was observed at 150µg & 100µg with 4.25 & 8.75mm zone of inhibition, respectively.

CONCLUSION

The data obtained from this study shows that the acetone extract of *Juglans Regia* was a more effective antibacterial agent against salivary microflora as compared to aqueous extracts of *Juglans Regia* and, aqueous and acetone extracts of *Mimusops elengi*.

For *Juglans regia* to be an effective antibacterial agent it must be used in a solvent which is soluble in water and saliva. Thus, aqueous extract of *Juglans regia* can be effectively used in various chemotherapeutic agents to inhibit the growth of predominant oral bacteria and cariogenic pathogens.

The antibacterial properties of *J. regia*, may be due to the presence of phenolic compounds, terpenoids, alkaloids, flavonoids and steroids [13].

The resistance in many dental pathogens to currently used antibiotic drugs is ever increasing. The ingredients derived from *Juglans regia* & *Mimusops elengi* plants used in this study are herbal, they are ecofriendly and do not produce any side effects as well as are effective and economical, as compared to the synthetic drugs. The results of the present work, and for its inexpensive cost, show that the use of *J. regia* can help developing countries limit oral infections.

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