

ORIGINAL ARTICLE

Going Back to Where it all Started - An Overview of 5 Medicinal Plants

Rahul Deshpande¹, A. V. Khare², Anuj Shah³, Mayuri Mutha⁴, Nirmala R. Deshpande⁵

ABSTRACT

Introduction: Nature is an inexhaustible storehouse of riches. One of these is the therapeutic effect of various medicinal plants that have been described in traditional medicinal practice. Many traditional healing herbs and their parts have been shown to have medicinal value and can be used to prevent, alleviate or cure several human diseases. Consumption of herbal medicines is widespread and increasing in recent years and approximately 80% of the people in developing countries depend on traditional medicines for primary health care. The global market for the medicinal plants and herbal medicine is estimated to be worth US\$800 billion a year. India is one of the leading countries in Asia in terms of the wealth of traditional knowledge systems related to herbal medicine and employs a large number of plant species includes Ayurveda (2000 species), Siddha (1121 species), Unani (751 species) and Tibetan (337 species). The origin of Ayurveda can be traced back to Atharv veda, one of the four Vedas of vedic culture. The number of patients seeking alternate and herbal therapy is growing exponentially. Herbal medicines are the synthesis of therapeutic experiences of generations of practicing physicians of indigenous systems of medicine for over hundreds of years. Herbal medicines are now in great demand in the developing world for primary health care not because they are inexpensive but also for better cultural acceptability, better compatibility with the human body and minimal side effects.

Materials and Method: Extracts of *Morinda pubescens*, *Embelia basal*, *Juglans regia*, *Mimusops elengi* and *Cassia auriculata* were obtained and authenticated. Unstimulated saliva was accumulated, refrigerated and carried to the lab where the agar well diffusion method was used to prepare the microbial inhibition assay.

Results: All the herbal extracts discussed in the study showed variable antimicrobial properties, these properties are comparable with that of gold standard.

Conclusion: The present review of our original research attempts to describe the benefits of five such herbal extracts viz. '*Morinda pubescens*, *Embelia basal*, *Juglans regia*, *Mimusops elengi* and *Cassia auriculata* against the salivary microflora of children in the mixed dentition age group.

Keywords: Dentals Caries, Saliva, Ayurveda, Chlorhexidine, Amoxicillin

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INTRODUCTION

Dental caries has been one of the most commonly occurring diseases in children. According to the National Oral Health Survey in 2004 caries prevalence in India was 51.9%, 53.8%, 63.1% at ages 5, 12, 15 years respectively¹. The oral cavity consist of complex and highly diverse microflora containing colonies of bacteria like streptococcus, actinomycetes, staphylococcus etc. which varies in different individuals². So instead of concentrating on one group of bacteria we should think of whole salivary microflora.

Several synthetically prepared medicines such as ampicillin, chlorhexidine, sanguinarine, entridazole, phenolic antiseptics and quaternary ammonium-antiseptics, among others, have been very effective in preventing dental caries^{3,4}. However, various adverse effects such as tooth and restoration staining, increasing of calculus formation, diarrhoea, and disarrangements of the oral and intestinal flora has been associated with the use of these chemicals^{3,5}. Also there is a increase in prevalence of multidrug resistant strains of bacteria. These drawbacks justify the search for new effective and herbal antimicrobial compounds that could be employed in oral preventive measures with minimal or no adverse effects.

The Indian trans-Himalayas span over 186,000 km² above natural tree line zone and is known for its sparsely distributed vegetation and relatively low species diversity is a reservoir of a large number of medicinal and aromatic plants (MAPs) and designated as one of the global biodiversity hotspots, where ecological, phyto-geographical and evolutionary factors fa-

your high species diversity. More than 1000 plant species, 225 avian species and many rare and endangered mammalian fauna, including the snow leopard (Shiva, 1996) are found in this zone. Ladakh region of Jammu and Kashmir manifests the highest geographical area in the trans-Himalayan region of India, followed by Lahaul-Spiti in Himachal Pradesh, northern part of Sikkim and Uttaranchal. Though the high-altitude Himalayan zone is full of fragile habitats and shows decline in tree-species richness, it is rich in representative (native) and endemic biodiversity elements (Kala and Manjrekar, 1999). The western ghats is very rich in medicinal wealth. The forests and hills of this region is a treasure house of about 700 medicinal plants. Out of which some are used for traditional and folk medicinal practices. Many are exploited commercially for their active enzymes and their commercial values. Medicinal plants species of western ghats represent a variety of life form ranging from lichen, herbs, shrubs, climbers and trees which are annuals to perennials.

Herbal medicine is still the mainstay of about 75 - 80% of the world population, mainly in the developing countries, for primary health care.⁶ This is primarily because of the general belief that herbal drugs are without any side effects besides being cheap and locally available.⁷ According to the World Health Organization (WHO), the use of herbal remedies throughout the world exceeds that of the conventional drugs by two to three times (Evans, 1994).⁸ The use of plants for healing purposes predates human history and forms the origin of much modern medicine.

This paper is a review of our original research on the herbal extracts of five medicinal plants - '*Morinda pubescens*, *Embelia basal*, *Juglans regia*, *Mimusops elengi* and *Cassia auriculata* and their antimicrobial property at different concentrations compared against 0.2% chlorhexidine.

MATERIAL METHOD

Plant material: Extracts of *Morinda pubescens* (AHMA-21220)*, *Embelia basal* (AHMA F- 084)*, *Juglans regia* (AHMA S/B - 14319)*, *Mimusops elengi* (AHMA S/B - 065)* and *Cassia auriculata* (BSI/WC/Tech/2009/95)**.

*authentication no., authenticated at Agharkar Research Institute, Pune, India. ** authenticated at Botanical Survey of India, Pune (Maharashtra)

Criteria for selection of patients: (Standard method of saliva collection)

In the studies conducted, patients of 6-12 years of age, in mixed dentition period with DMFT four or above four were included, where the patients had no history of antibiotic therapy or use of chemical anti-plaque agents prior to six months of study initiation.

Method of saliva collection and storage: (Standard method of saliva collection and storage in dental institutes)

The subjects were asked to rinse with water; saliva (unstimulated) was allowed to accumulate in the floor of the mouth for approximately two minutes and by asking the subject to spit in funnel, saliva (3ml) was collected in vial. 10 samples were collected in the early morning time, by following the above mentioned method. These salivary samples were diluted (3:1) in a sterile vial containing 1ml of normal saline and were used to inoculate on the agar plates. The samples were refrigerated within 30 minutes and frozen within 4 hours. (If collection is being carried out in the field, it may not be practical to freeze the samples until the end of the day, such samples should be kept cold until they are returned to the lab).

Antimicrobial Assay: (Standard method of Antimicrobial Assay used in biotech institutes)

The agar well diffusion method was used to prepare the microbial inhibition assay. Sterile well of 8.0mm diameter were impregnated with the extract of different concentrations ranging from 250µg to 4000µg per well. Adequate amount of Muller Hinton Agar were dispensed into sterile plates and was allowed to solidify under aseptic conditions. The test samples of saliva (0.1ml) were inoculated with a sterile spreader on the surface of solid Muller Hinton Agar medium in plates.

After the media was solidified; a well was made in the plates with the help of a cup-borer (8.0mm). The well was filled with different concentrations of the extract (250µg to 4000µg/ well) and plates were incubated at $37 \pm 0.1^\circ\text{C}$ for 24 hours. After incubation, the plates were observed for zones of growth of inhibition and the diameters of these zones were measured in millimeters by using bacterial inhibition zone reading scale. All the tests were performed under sterile conditions. Chlorhexidine was used as positive control. The lowest dose required to attain maximum inhibition of a mixed oral micro flora was recorded.

RESULTS

STUDY 1: Comparative evaluation of antimicrobial activity of active

Components of '*cassia auriculata*'; 0.2% chlorhexidine in different concentration of human salivary microflora.⁹

1.1:- Mean Value of Zones of Inhibition (in mm) of Active Compound (Emodine) of '*Cassia auriculata*'

Concentrations	Mean value of average zone of inhibition (mm)
5mg	0.0000
10mg	1.4000
15mg	3.7000
20mg	4.4000
30mg	5.2000
50mg	7.0000
80mg	10.2000

1.2:- Mean Value of Zones of Inhibition (in mm) of Standard Antimicrobial Agent

Antimicrobial agent	Mean value of zone of inhibition (mm)
0.2 % Chlorhexidine	20.0000

This study proves that the antimicrobial activity of cassia auriculata at higher concentration is comparable with 0.2% chlorhexidine.

STUDY 2: Screening of antimicrobial activity of herabal extract of active compound of *Cassia auriculata*, Chlorhexidine and Amoxicillin against salivary microflora of mixed dentition age group¹⁰

2.1:- Mean Value of Zones of Inhibition (in mm) of Active Compound (Emodine) of ‘Cassia auriculata’

Concentrations	Mean value of average zone of inhibition (mm)
5mg	0.0000
10mg	1.4000
15mg	3.7000
20mg	4.4000
30mg	5.2000
50mg	7.0000
80mg	10.2000

2.2:- Mean Value of Zones of Inhibition (in mm) of Standard Antimicrobial Agent

Antimicrobial agent	Mean value of zone of inhibition (mm)
0.2 % Chlorhexidine	20.0000
Amoxicillin 125mg	40.4000
Amoxicillin 250mg	48.4000

This study proves that the antimicrobial activity of *Cassia auriculata* at higher concentration is comparable with 0.2% chlorhexidine and Amoxicillin 125mg and Amoxicillin 250mg.

STUDY 3: Screening of Antimicrobial Activity of Active Compound of *Embelia basal*, Chlorhexidine and Amoxicillin against Salivary Microflora of Mixed Dentition Age Group¹¹

3.1:- Diameter of Zone of Inhibition

Conc. (Mg)	Diameter of zone of inhibition(mm)									
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
5mg	2	10	7	10	10	6	6	5	5	6
10mg	5	12	17	12	12	10	10	7	7	7
20mg	6	13	20	15	15	11	11	11	11	8
40mg	8	15	18	17	17	12	12	12	12	9
80mg	10	18	18	19	19	12	15	15	14	10

3.2:- Mean Value of Zones of Inhibition (in mm) of Standard Antimicrobial Agent in salivary samples

Antimicrobial agent	Mean value of zone of inhibition (mm)
0.2 % Chlorhexidine	20.0000
Amoxicillin 125mg	40.4000
Amoxicillin 250mg	48.4000

This study concludes that the antimicrobial activity of *Embelia basal* at higher concentration is comparable with 0.2% chlorhexidine. But to assert antimicrobial activity of active compound of *Embelia basal* with chlorhexidine, amoxicillin 125mg and amoxicillin 250mg, we need to take further higher concentration, because mean of zone of inhibition of chlorhexidine, amoxicillin 125mg and amoxicillin 250mg are 20mm, 40.40mm and 48.40mm respectively and mean of zone of inhibition at 80 µg is 15mm.

STUDY 4: Screening of antimicrobial activity of herabal extract of ‘*Morinda pubescence*’, chlorhexidine and amoxicillin against salivary microflora of mixed dentition age group¹².

4.1:- Mean Values of Zones of Inhibition (in mm) of ‘Morinda pubescence

Concentrations(ug)	Mean value of average zone of inhibition(mm)
50	4
100	5.2
200	7.2
400	9.2
800	14

4.2:- Mean Value of Zones of Inhibition (in mm) of Standard Antimicrobial Agent

Antimicrobial agent	Mean value of zone of inhibition (mm)
0.2 % Chlorhexidine	20.0000
Amoxicillin 125mg	40.4000
Amoxicillin 250mg	48.4000

This study compared and evaluated the antimicrobial activity of ‘*Morinda Pubescence*’ with 0.2% chlorhexidine and Amoxicillin 125mg and Amoxicillin 250mg. The zone of inhibition were measured by excluding the diameter of well. The mean value of average zone of inhibition of ‘*Morinda Pubescence*’ with 0.2% chlorhexidine and S-flo in ten salivary samples was taken for comparison. These zones of inhibition were directly proportional to the concentration.

STUDY 5: Comparative evaluation of antibacterial properties of different extracts of *Mimusops elengi* (bakul) and *Juglans regia* (walnut) against salivary microflora¹³

Efforts have been made to compare and value the antimicrobial properties of bark extracts of *Mimusops elengi* and *Juglans regia* with 2% chlorhexidine gluconate against human salivary microflora.

5.1:- 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	

5.2:- Comparative mean values of *J.regia* and *M.elengi*

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

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.

This study also compared the effectiveness of acetone extract of *M. elengi* and *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 and acetone extract of *J. regia*, it was observed at 150µg and 100µg with 4.25 and 8.75mm zone of inhibition, respectively.

DISCUSSION

From these studies it is inferred that the lowest dose of the herbal extract required to attain a maximum inhibition of oral microflora was recorded, and their efficacy was found to be comparable with these agents at higher concentrations. The significant zones of inhibition indicates that an active molecule must be present in these plants and further studies need to be carried out in order to confirm and isolate the active ingredients.

Thus these studies have confirmed the antimicrobial potentials of the plant, supporting its application as a preventive remedy for various microbial diseases of hard tissues in the oral cavity.

The use of plants for healing purposes predates human history and forms the origin of much modern medicine. Many conventional drugs originated from plant sources: a century ago, most of the few effective drugs were plant based. Examples include aspirin (willow bark), digoxin (from foxglove), quinine (from cinchona bark), and morphine (from the opium

poppy) (Vickers and Zollman, 1999).¹⁴ The wide spread use of herbal medicine is not restricted to developing countries, as it has been estimated that 70% of all medical doctors in France and German regularly prescribe herbal medicine (Murray and Pizzorno, 2000).¹⁵

The utility of herbal medicines is also seen in dentistry due to the lack of effective medicines for some dental problems. The herbs aloe vera, bloodroot, Calendula extract are useful for treating periodontal problems, while for fighting tooth-aches clove oil, garlic, and propolis, are helpful. Calendula, lavender oil, and echinacea are being used to reduce and heal inflamed gingival tissues and treat opportunistic yeast that causes thrush.¹⁶

Ayurveda, one of the paramount traditional forms of medical practice in India, has produced many useful leads in developing medications for chronic diseases. Almost 25 centuries ago, Hippocrates proclaimed, 'Let food be thy medicine and medicine be thy food.'¹⁷ Amalgamating the strengths of the knowledge base of traditional systems such as ayurveda with the dramatic power of combinatorial sciences can provide new functional leads to reduce time, money and toxicity—the three main hurdles in drug development.

From the studies we performed, it was evident that the antimicrobial Activity of these plants may be compared with other 'synthetic' antimicrobial agents. A clinician can exploit these antimicrobial agents as a prophylactic measure in Dental Caries, Intracanal Medicaments and in prevention of Periodontal Disease. Primary prevention among children and adolescents is of particular importance in India, due to high population numbers and wide economic, social, and health disparities amongst its population¹⁸. Oral and dental health awareness has improved tremendously over the last century but the prevalence of dental caries in children remains a significant clinical hurdle¹⁹. There is a need to focus on the prevention of Dental Caries as the proverb goes 'Prevention is always better than cure'. These studies compare the antimicrobial properties obtained from a plant source and which is easily available to the common man.

Thus these extracts can be formulated in the form of a mouth wash, or as an intracanal medicament where an antimicrobial agent is required.

CONCLUSION

The requirement of plant based therapeutics is increasing in both developing and developed countries due to growing recognition as they are natural products, non narcotic, easily biodegradable producing minimum environmental hazards, having no adverse side effects and easily available at affordable prices. Various studies by Deshpande R.R et. al. have confirmed the antimicrobial potentials of the plants *Morinda pubescens*, *Embelia basal*, *Juglans regia*, *Mimusops elengi* and *Cassia auriculata*, thus supporting its application as a

preventive remedy for various microbial diseases of hard tissues in the oral cavity. However bona fide use of these plants as a preventive and therapeutic agent requires much more fundamental study regarding higher concentrations and samples as well. These studies can act as a stencil and prove to be a head start for a newer generation of herbal substitutes for the synthetic agents used in dentistry.

The demand on plant based therapeutics is increasing in both developing and developed countries due to growing recognition that they are natural products, non narcotic, easily biodegradable producing minimum environmental hazards, having no adverse side effects and easily available at affordable prices. Various studies by Deshpande R.R et. al. have confirmed the antimicrobial potentials of the plants *Morindapubescens*, *Embelia basal*, *Juglansregia*, *Mimusopselengi* and *Cassia auriculata*, thus supporting its application as a preventive remedy for various microbial diseases of hard tissues in the oral cavity. However authentic use of these plants as a preventive and therapeutic agent requires much more fundamental study regarding higher concentrations and samples as well. These studies can prove to be a head start for a newer generation of herbal substitutes for the synthetic agents used in dentistry.

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