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# Antimicrobial activity of selected herbal extracts against multi drug resistant oral pathogens isolated from leprosy patients

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**ABSTRACT**

Many systemic diseases reflect the poor oral health of an individual. Leprosy patients are functionally dependent and present enormous oral malformation which helps the colonization of pathogens. Oral health of leprosy patients were assessed based on the prevalence of the load of oral isolates from fifty volunteer patients. *Pseudomonas* sp. was found to be more prevalent in all patients which expressed a higher rate of co aggregation with other isolates (*Neisseria* sp., *Pseudomonas* sp., *Staphylococcus* sp., *Streptococcus* sp., *Candida albicans and C. tropicalis*). All bacterial and fungal isolates were subjected to antimicrobial sensitivity test using antibiotics and antifungal drugs recommended for oral infection (Amoxicillin, Clindamycin, Erythromycin, Penicillin G, Amphotericin B and Fluconazole) and aqueous extracts of five different herbs(*Berberis aristata, Cyminum cuminum, Piper nigrum, Syzygium aromaticum,* and *Zingiber officinale). Berberis aristata* exhibited highest antifungal activity and *Syzygium aromaticum* exerted highest anti bacterial activity. Hence we recommend the use of herbs for the preparation of choorna or oral rinse which can effectively control the colonization of multi drug resistant pathogens in the oral cavity.

**Key words:** Antibacterial, Antifungal activity, Herbal extracts, Oral isolates.

## INTRODUCTION

Leprosy is a chronic infectious granulomatous disease may manifest as indeterminate, tuberculoid and borderline.[1] Leprosy patients are categorized as pauci bacillary (five or fewer skin lesions) or multi bacillary (six or more lesions) for treatment purposes.[2] Borderline leprosy is characterised by numerous larger skin lesions with less well defined margins. Poor immune response and overwhelming bacterial load in lepromatous leprosy leads to systemic infection. If left untreated it may end in multi organ damage. Advance case may be present with the clinical condition of nasal cartilage damage with collapsed nose which are mainly influenced by the microbial population due to the lack of cell mediated immunity. [3]The oral cavity provides a permanent source of infectious agents. Historically, oral infections were thought to be localized to the oral cavity except in the case of some associated syndromes and untreated odontogenic abscesses.[4] Within human oral cavity, interactions of bacteria result in the formation of multispecies biofilms known as dental plaque which is comprised of more than 500 species of bacteria [3] there by overcoming many of the challenges associated with retention in the oral cavity such as high salivary flow and low nutrient conditions. Co aggregation and co adhesion are the two factors that contribute to biofilm formation by promoting mutualistic interactions between cells and provides more diverse attachment site for bacteria to adhere to the developing biofilm surfaces [5]. It has been renowned that oral infection may lead to a number of systemic diseases. Three mechanism or pathways linking oral infections to secondary systemic effects have been proposed (i) metastatic spread of infection from the oral cavity as a result of transient bacteraemia (ii) metastatic injury from the effects of circulating oral microbial toxins and (iii) metastatic inflammation caused by

immunological injury induced by oral microorganism. [6]

All of the variants of leprosy (indeterminate, borderline and lepromatous) may be associated with orofacial pathology and is directly proportional to the duration of the disease indicating the late manifestations and may act as the habitat for other pathogens.[7] Colonisation of the oral cavity by pathogenic bacterial species may be a precursor of various diseases in other organs. The present study was designed to explore the oral pathogens of leprosy patients with varying degrees of oral distortion and to evaluate their sensitivity to routine anti bacterial and anti fungal agents and selected herbal extracts.

## MATERIALS AND METHODS

**Selection of Study population:** Fifty volunteer leprosy patients who were the inmates of Government rehabilitation centre for Leprosy, Puduppatti, Madurai, Tamil Nadu, India were randomly selected. The study population involved 9 females and 41 males with varying degrees of deformity.

**Collection of sample:** A tongue depressor was placed on the extended tongue and with sterile cotton swabs, throat and palate were vigorously swabbed and immersed to tube containing sterile peptone broth and transported to the laboratory. Loopful of samples were streaked on to nutrient agar, chocolate agar, Mannitol salt agar, and Hi chrome Candida agar plates and incubated at room temperature. Isolated organisms were identified by staining techniques and biochemical testing.

**Antibiogram assay:** Antibiotic and anti fungal discs were obtained from Hi media laboratories, Mumbai. To sterile Muller-Hinton agar plates, 18 hour cultures were streaked on which sterile antibacterial and antifungal discs (Amoxicillin, Clindamycin, Erythromycin, Penicillin G Amphotericin-B, Ketaconazole) were placed and incubated at 37$^{o}$C for 24 hours in an inverted position.

**Preparation of herbal extracts**: Fresh herbs *Berberis aristata, Syzygium aromaticum, Cyminum cuminum, Zingiber officinale* and *Piper nigrum* were obtained from the local market and cleaned, descaled when necessary and washed in sterile distilled water. About 100g of each washed herbs were crushed with mortar and pestle and aqueous extracts were prepared by cold maceration method. The extracts were sieved through a fine mesh cloth followed by Whatman No.1 filter paper under aseptic condition. This extract was considered as the 100% concentration of the extract. The concentrations viz., 75%, 50%, 25% and 10% were made by diluting the concentrated extract with appropriate volumes of sterile distilled water[8].

**Antibacterial sensitivity testing using filter paper method:** Filter paper discs of 7 mm diameter were prepared and sterilized. Using an ethanol dipped and flamed forceps, these discs were aseptically placed over nutrient agar plates seeded with the respective test microorganisms. One hundred micro litres of the various herbal extracts (100%, 75%, 50%, 25% and 10%) were aseptically transferred to these discs and incubated at 37ºC for 24 hours. Inhibition zones with diameter less than 12 mm were considered as having no antimicrobial activity. Diameters between 12 and 16 mm were considered moderately active and those with zones >16mm were considered highly active.[8]

## RESULTS AND DISCUSSION

Various clinical symptoms observed in the oral cavities of leprosy patients were bleeding in gums (9%), cracks in lips (17%), dental caries (15%), inflammation in throat (15%), mal alignment of teeth (9%), oral lesions (12%), tongue coating and fissures (7%) and oral wounds (16%). These clinical symptoms may be formed due to the lepromatous stage of leprosy and were reported to have direct role in the pathogenesis of oral micro flora.

Inpatients had a notably higher detection rate for *Pseudomonas* sp. (100%) and *Staphylococcus* sp. (94%) and had a lower detection rate for *Candida tropicalis* (4%) and *Candida albicans* (18 %). Detection rates for *Neisseria* sp. and *Streptococcus* sp. were found to be 66% and 64% respectively. In healthy individuals, *Streptococci* are dominant species that may grow better than opportunistic bacterial species including *P. aeruginosa* and Methicillin resistant *Staphylococcus aureus* in the oral cavity. But the detection rate of *Pseudomonas* sp. and *Staphylococcus* sp. were higher in leprosy patients and this coincides with the findings of Costerton *et al.*[9-10] Microbial communities within the human oral cavity are energetic association of bacterial and fungal species that forms biofilms in the oral cavity. In the present study, isolates were found to be co aggregates of two to five organisms. *Pseudomonas* sp. was found to have co-infection with all isolates and was found to be more capable of forming biofilm than other bacterial species. This result correlates the findings of Tada *et al.* [10]
In the current study, the bacterial isolates were partially sensitive to antibiotics, Amoxicillin (44%)

and Penicillin (39.5%), Clindamycin (42.6%) and Erythromycin (43.7%). Multidrug resistance is often noted among immunocompromised patients and those who undergo multi drug therapy. Compared with normal sterile sites, higher rate of resistance was noted in the oral cavity and respiratory tract. [11] Both fungal species were found to be sensitive to Amphotericin B and Fluconazole.

Among the five herbal extracts tested, *Berberis aristata* exerted highest antifungal activity against *Candida albicans* followed by *Candida tropicalis* in a dose dependent manner and the results supported the findings of Wagh and Vidhale. [12] No isolate showed sensitivity to 50% concentrations of *Berberis aristata* except *Candida albicans*. A dose dependent moderate antifungal activity was observed at 100% and 75% concentrations of aqueous extracts of *Syzygium aromaticum.* The anti fungal activity of tested herbs against oral isolates can be concluded as *Berberis aristata* > *Syzygium aromaticum* > *Cyminum cuminum* > *Zingiber officinale* > *Piper nigrum*. At 100% concentration, *Syzygium aromaticum* extract revealed a highest anti bacterial activity against *Pseudomonas* sp. and a moderate activity against *Neisseria* sp. whereas *Streptococcus* sp. was resistant to *S. aromaticum.* The anti bacterial activity of the tested herbs against oral isolates can be concluded as *Syzygium aromaticum*> *Zingiber officinale*> *Piper nigrum*> *Cyminum cuminum* > *Berberis aristata* (Table1).

The literature review gives an account on a high anti bacterial activity of *Berberis aristata* against some human pathogenic bacteria which is contrary to the present result.[13] Lower antibacterial activity of *Cyminum cuminum* was already reported by Iacobellis *et al.*[14] Piper nigrum exerted a moderate antibacterial activity and a least anti fungal activity. Similar results were demonstrated by Karsha and Lakshmi [15], where good anti bacterial activity of *P. nigrum* was noted against both gram negative and gram positive bacteria.

## CONCLUSION

Pathogenic bacterial species in the oral cavity paves way for the development of systemic diseases in functionally dependent people such as leprosy people and hence the oral care in the above mentioned group form a crucial part to avoid colonization by pathogenic bacteria. Aqueous extracts of raw herbs themselves can act as a good herbal oral rinse. Various bioactive components present in the herbs confer their antimicrobial activity and simple powder or choorna preparations ensure their activity as they do not undergo any chemical or heat treatment. They are cost effective and harmless when compared to antibiotics. Herbal drugs are more advisable in patients who have been under long term antibiotic therapy as they develop resistance towards most of the antibiotics. To overcome the strategy of multi drug resistance against antibiotics and to protect the leprosy individuals from clinical pathological consequences of these organisms, present study recommends herbal therapy as an alternative choice of treatment.

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Table 1: Antimicrobial potential of *Berberis aristata DC, Cuminum cyminum Linn, Piper nigrum Linn, Syzygium aromaticum and Zingiber officinale* Rosc against oral isolates

| Herbs | Conc(%) | *C.albicans* | *C.tropicalis* | *Neisseria* | *Psuedomaonas* | *Staphylococcus* | *Streptococcus* |
|---|---|---|---|---|---|---|---|
| *Berberis aristata DC* | 100 | 20.1±0.15 | 14±0.10 | 08±.09 | 10±0.11 | 11±0.12 | 11±0.13 |
| | 75 | 16.2±0.20 | 12±0.20 | 08±0.20 | 08±0.20 | 10±0.21 | 09±0.11 |
| | 50 | 15.26±0.10 | - | - | - | - | - |
| | 25 | 15.2±0.30 | - | - | - | - | - |
| *Cuminum cyminum* Linn. | 100 | 12.5±0.10 | 12.1±0.10 | 11±0.10 | 14.3±0.15 | 8.6±0.11 | 10±0.12 |
| | 75 | 12±0.10 | 10±0.11 | - | 12.3±0.12 | 11.3±0.15 | - |
| | 50 | 09.75±0.30 | 09.5±0.31 | - | 12.3±0.10 | 8.6±0.11 | - |
| | 25 | - | - | - | 11±0.10 | - | - |
| *Piper nigrum* Linn. | 100 | - | 11.5±0.21 | 12.5±0.09 | - | 12.5±0.05 | 12.7±0.10 |
| | 75 | - | 8±0.90 | 10.5±0.05 | - | 12.1±0.15 | 12±0.10 |
| | 50 | - | - | - | - | 10.5±0.10 | 10±0.15 |
| | 25 | - | - | - | - | - | - |
| *Syzygium aromaticum* | 100 | 14.6±0.16 | 13.4±0.05 | 13.4±0.09 | 16.2±0.10 | 13.2±0.15 | 11±0.12 |
| | 75 | 13.6±0.11 | 12.4±0.11 | 12.4±0.10 | 15±0.10 | 12.4±0.11 | 9.5±0.12 |
| | 50 | 11.6±0.09 | 10±0.12 | 10±0.10 | 13.8±0.11 | 09±0.10 | 10±0.13 |
| | 25 | 09.5±0.10 | - | - | 12±0.12 | 10±0.10 | - |
| *Zingiber officinale* Rosc. | 100 | 16.3±0.15 | 14.6±0.15 | 14.6±0.15 | 12±0.15 | 14.8±0.15 | 13±0.12 |
| | 75 | 11.5±0.09 | - | - | - | 12.6±0.09 | 12±0.13 |
| | 50 | - | - | - | - | - | - |
| | 25 | - | - | - | - | - | - |
| Distilled water | | - | - | - | - | - | - |

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