

Minor Research Project (UGC)

“Study of Lemon Grass Extract as an Antimicrobial Agent on Oral Micro Flora”

Details

Sanctioning Authority: UGC -WRO, PUNE.

Sanctioned amount: Rs. 2,00,000=00

Period: March 2013-2015

F. No.47-515/12 (WRO)

PI: Dr. Bharati Bhadbhade

The above mentioned MRP was sanctioned in March, 2013, however the first installment of the funds (Rs. 1,47,500/-) was received in May 2013. Hence the work on the project was started in the month of May, 2013. The first report was submitted in the October, 2014, along with the utilization certificate for the above mentioned funds.

Introduction

Oral microbiology is the study of microorganisms of the oral cavity and their interactions with each other and with the host. These microorganisms present in the oral cavity are the major components of dental plaque. Dental plaque is the material that adheres to the teeth and consists of bacterial cells (mainly *Streptococcus mutans* and *S. sanguis* and anaerobes like *Porphyromonas gingivalis*, and microaerophilic organisms like *Campylobacter rectus*, *Fusobacterium nucleatum* etc., salivary polymers and bacterial extracellular products.

Dental plaque is a sticky, soft and colorless film of bacteria that constantly builds upon the surfaces of teeth and gums. Failure to remove dental plaque by regular tooth brushing, allows its build up in a thick layer. Dental plaque buildup can also become mineralized and form calculus. Dental plaque on teeth mainly consists of endogenous species like *Streptococcus mutans*. The bacteria other than *Streptococcus mutans* are *Streptococcus sanguis*, and *Lactobacillus acidophilus*. While bacteria most implicated in periodontal disease are *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis* and *Prevotella intermedia*. Other bacteria associated with periodontal disease are *Treponema denticola*, *Campylobacter rectus*, *Fusobacterium nucleatum*.

Since both gingivitis and periodontitis are plaque associated oral conditions, the removal of dental plaque should inhibit their occurrence and progression of the disease.

Plaque Control is essential to prevent both caries and periodontitis. Plaque controlled by mechanical means is not adequate and hence chemical means need to be used as an adjunct. Amongst the various chemical plaque control measures Antimicrobial agents form the mainstay of treatment since ages. Antimicrobials include antibiotics, naturally occurring & synthetic compounds. Prolonged use of antimicrobials leads to development of drug-resistant strains increase in calculus seen with gold standard Chlorhexidine.

Chemical control of plaque is considered to be adjunct to mechanical oral hygiene practices, the agents are most commonly used in the form of mouth rinse to prevent and control the plaque formation. To overcome the problems of dental caries & gingivitis many dentist prescribe Chlorhexidine mouthwash. The broad-spectrum antibacterial Chlorhexidine (CHX) is a very commonly used agent for the prevention or control of oral diseases. Studies have demonstrated that CHX is capable of arresting caries when applied to dentin.

However, CHX has some disadvantages, such as bacterial resistance, cytotoxic to human periodontal ligament cells, hinder protein synthesis & affect mitochondrial activity in this cell. Chlorhexidine could cause serious harm to the oral tissue. The side effects of different antibiotics and the global mouthwash- Chlorhexidine (CHX) led to a search for new antibacterial alternatives like plant extracts and essential oils substances that are safe for humans.

Hence, the search for alternative products continues and natural phytochemicals isolated from plants used as traditional medicines are considered as good alternatives. In this review, plant extracts or phytochemicals that inhibit the growth of oral pathogens, reduce the development of biofilms and dental plaque, influence the adhesion of bacteria to surfaces and reduce the symptoms of oral diseases will be discussed further. Clinical studies that have investigated the safety and efficacy of such plant-derived medicines will also be described.

Plants are a valuable source of natural products for maintaining human health. Medicinal plants are the only affordable and accessible source of primary health care for many, especially in the absence of access to modern medical facilities. Studies reveal that there are more

traditional medicine providers than the allopathic providers especially in the rural areas WHO (2002-2005). India has a rich & ancient tradition in use of medicinal plants as drugs. These naturally occurring phyto-chemicals are biodegradable with fewer side effects and hence would be very good source of a variety of drugs

Use of lemon grass extract has been extensively studied as an antibacterial agent in medicine, but its use is scarcely known and studied in dentistry.

Considering the various uses of lemongrass oil an attempt is being made to harness the properties, use of lemongrass oil as a mouth rinse was planned for its antiplaque property.

Cymbopogon citratus (lemon grass), commonly known as lemon grass is a native herb from India and is also cultivated in other tropical and subtropical countries. It is a tall perennial grass. It is consumed as an aromatic drink and used in traditional cuisine for its lemon flavor. Its leaves are used to make tea which can relieve stomach and intestinal problems. In many countries it is used as sleeping aid and an antidepressant agent. *Cymbopogon citratus* contains active ingredients like myrcene, an antibacterial agent and a pain reliever, citronellal, citronellol and geraniol.

MATERIALS AND METHODS

Chemicals Used - All other chemicals used were of AR grade. Commercial Lemon Grass Oil (LGO) was obtained from a local supplier. MS Agar was procured from Hi Media.

All the pH measurements were done using a Control Dynamics, model APX-175 EK digital pH meter.

Lemon Grass extraction was done by Soxhlet Extraction Method.

Collection of Samples.

- a. Samples were collected from **50 subjects**, 25 males and 25 females between the age group of 18 to 60 years.
- b. Samples were collected from different private dental clinics after a written informed consent was taken from the patient.

- c. c. Samples - collected in sterile wide-mouthed screw capped tubes containing 5ml of sterile nutrient medium.
- d. d. Samples - immediately carried to the Department of Microbiology, Abasaheb Garware College, Pune and processed within 2 hrs. of collection.

Table 2: Samples Collected for the isolation of Bacteria causing Dental Diseases

Sample. No.	Source of samples	No. of Samples	No. of Isolates
1.	Supra Gingival	09	09
2.	Dental plaque & caries	41	50

Isolation of Organisms:

The clinical samples were homogenized by a vortex mixer and 100µl of sample was streaked on sterile Mitis Salivarius (MS) agar plate and sterile Nutrient Agar (NA) plate respectively. The plates were incubated under aerobic and anaerobic conditions at 37⁰C for 24 - 48 hours.

All the isolates were purified using sterile MS agar plate and NA agar plates

All the purified isolates were maintained on sterile MS agar and NA agar slants.

Screening of bacterial isolates for further studies

The samples obtained were of dental caries & plaque and supragingival origin, and the most common organisms naturally associated with these, belong to genus *Streptococci*.

Hence on the basis of characteristic colony Morphology, Gram Character, distinctive cell shape, selective biochemicals, growth on MS Agar + 1% K-tellurite, out of the 59 isolates obtained, 10 isolates were selected for further identification and studies.

Lemon Grass extraction was done by Soxhlet Extraction Method.

Organic Extraction of Lemongrass:

In this type of extraction procedure, the successive extraction with solvents of increasing polarity from a non polar (Hexane) to a more polar solvent (Methanol) to ensure that a wide polarity range of compound could be extracted.

The antimicrobial action of these extracts was determined using agar well diffusion assay along with positive control (0.2% CHX) as well as negative control (DMSO).

RESULTS:

Isolation of Cultures from Dental Samples. Several Cultures were isolated from the dental samples procured from dental clinics, as shown in the following table.

Sr. No.	Source of samples	No. of Samples	
1	Supra Gingival	09	09
2	Dental plaque	41	50

The fifty nine have been isolated from the samples as above

The samples obtained were of dental caries & plaque and supragingival origin. On the basis of characteristic colony morphology, Gram Character, distinctive cell shape, selective biochemicals, growth on MS Agar + 1% K-tellurite, out of the 59 isolates obtained, 10 isolates were selected for further identification and studies.

Identification of the isolates

The selected isolates were identified on basis of morphological, cultural & physiological characteristics according to Bergy's Manual of Systematic Bacteriology Vol. I and II.

All the 10 selected isolates were found to belong to Genus *Streptococcus*.

Further species level identification is in progress.

The testing of the antimicrobial action of the lemon grass extract is in progress.

The antimicrobial actions of these extracts against the isolates is being determined, using agar well diffusion assay along with positive control (0.2% CHX) as well as negative control (DMSO). The following results were obtained.

figure: 9 Antibacterial effect of different extracts on S-1 and S-2.

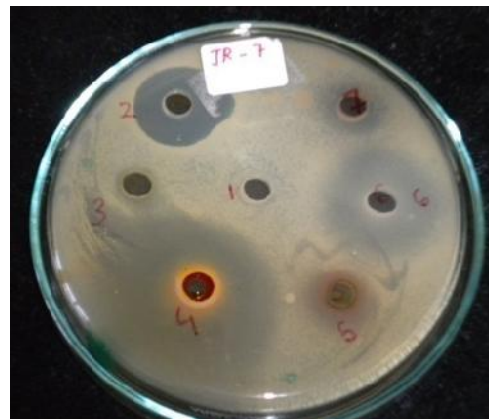
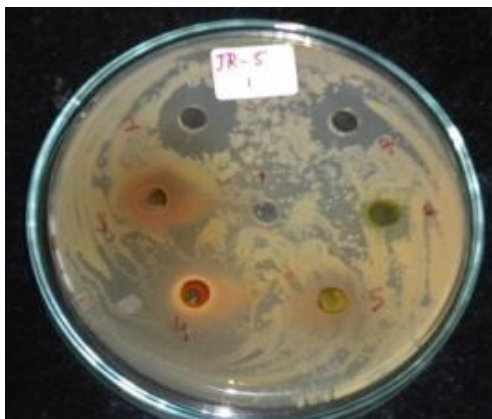
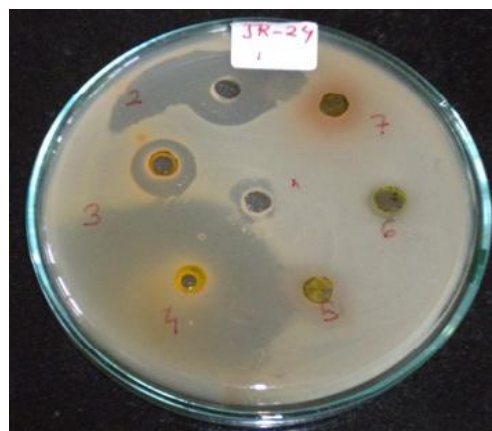
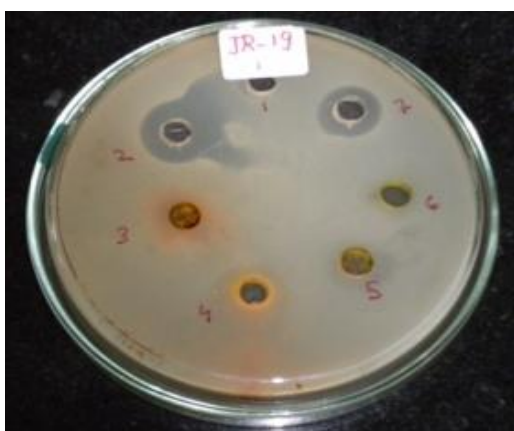


figure: 10 Antibacterial effect of different extracts on S-3 and S-4.



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