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RESEARCH ARTICLE

EVALUATION OF ANTIBIOTIC POTENTIAL OF MEDICINAL PLANT EXTRACTS AGAINST ORAL BACTERIA

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ABSTRACT

A survey conducted by IMRB International for Colgate Palmolive India found that only 2.5% Indians visit a dentist at least once a year compared with 48% which is the global average. 67% of Indians have never gone for a dental check-up. Out of those that do finally seek treatment for their dental problems, 1 in 3 have to get tooth extractions as generally it is too late. Tooth decay (dental caries) is a disease of multifactorial etiology. The essential factors include: the appropriate number and species of bacteria, the type, quantity and frequency of consumption of fermentable carbohydrates, and susceptible tooth surfaces. The aim of this study was to determine the oral bacteria counts and their sensitivity to plant extracts. Swab samples were collected from male persons without smoking and pan or nut products chewing habit. From the bacterial isolates obtained *Streptococci* spp. were identified by using HiStrep™ identification kit and tested for antibiotic sensitivity (Ampicillin, Tetracycline, BenzylPencillin, Amoxycillin, and Erythromycin). Resistant species were tested against aqueous plant extracts of *Punica grantum*, *Phyllanthus emblica*, *Terminalia catappa*, *Azadirachta indica*, *Mentha arvensis*, *Acacia nilotica*. Plant extract concentrations were tested against *Streptococci* spp. The total bacteria counts enumerated from the swab samples were 50.8×10^8. 10 isolates of *Streptococci* spp. were found multidrug-resistant. MIC, MBC and statistical analysis (mean, SD) were analyzed. From our study we could prove that the multi drug resistant isolates oral bacteria were sensitive to the test plant extracts. Based on our findings the plant extracts possess good taste and aroma is better than antibiotics.

INTRODUCTION

Health hygiene is important to keep oneself fit and away from illnesses. Hygiene covers routine activities taught among the young ones in early days of life. Cleaning teeth, hair, bathing, nail cutting are principal habits to be cultivated. Use of sticks from plants like – neem, babool and dried tobacco leaves powder with salt; common salt (sodium chloride) crystals were age old methods to clean teeth in rural communities. Poor oral health, untreated oral diseases (dental caries, gingival inflammation, periodontal, tooth loss) and conditions have a significant impact on quality of life including the ability to eat and drink, swallow, maintain proper nutrition, smile and communicate. India exposes discrepancies in oral health, with lower income groups having higher disease rates, limited or no access to care. The ratio between dentists versus population in rural areas is as low with less than 2% dentists being available for 72% of rural population. Statistics present the gloomy reality, that 95% of the population in India suffers from gum disease, only 50% use a toothbrush and just 2% of the population visit the dentist (National Oral Health Programme).

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Dental plaque is typically the precursor to tooth decay and contains more than 600 different microorganisms, contributing to the oral cavity overall dynamic environment that frequently undergoes rapid changes in pH, nutrient availability, and oxygen tension. Dental plaque adheres to the teeth and consists of bacterial cells while plaque is the biofilm on the surfaces of the teeth. Twenty-five species of oral *Streptococci* live in the oral cavity. Each species has developed specific specialized properties for colonizing different oral sites and constantly changing conditions to fight competing bacteria and to withstand external challenges. Imbalances in the microbial biota can initiate oral diseases. Under special conditions, commensal streptococci can switch to opportunistic pathogens, initiating disease and damaging the host. Oral *Streptococci* (*S. mutans, S. mitis, S. sanguis, S. salivarius, S. sobrinus*), *Actinomyces* sp., *Lactobacillus* sp. have both harmless and harmful bacteria (Pathak *et al*., 2012).

The World Health Organization has reported that more than 80% of global population in developing countries depends on herbal medicine for heath care needs. Plants with medicinal significance have been familiar in traditional folklore from pre-historic times and today proved as source of components that could be new antibacterial, antiviral, anticancer and treat multiple aliments without contraindications. These components

were investigated against agents causing oral diseases (plaque/caries) because the increased resistance is reported towards available therapeutic compounds. These agents are proved as cost effective and better potency than synthetic drugs. The genus *Acacia* (Family: Mimosaceae), common to use as goat feed in most of semi-arid regions, *Acacia nilotica, A. erubescens, A. sieberiana, A. erioloba, A. karro,* and *A. aroma* were investigated for nutritional effects of leaf meals. *A. nilotica* is locally named as babul, young stem is used in teeth cleaning. The powdered bark of this plant is known for treatment of acute diarrhea by folk and anti-viral against Hepatitis C virus (HCV) (Abdulrazak *et al*., 2000, Ramana *et al*., 2000, Arias *et al*., 2004, Halimani *et al*., 2005, Jansen *et al*., 2007, Mlambo *et al*., 2008, Banso, 2009, Rehman *et al*., 2011).

Azadirachta indica A. Juss. (Family: Meliaceae), neem is popular in traditional medicine system – Ayurveda, for treatment of liver disorders, diabetes, hypertension. This plant is grown in many Asian countries and tropical regions and young stem is used for teeth cleaning. Leaves and seeds are traditionally used in cure of human ailments and pest control (Azadirachtin: insect antifeedant, growth regulatory). Laboratory studies on this plant reveals that extracts of leaves possess significant antihyperglycemic, antiserotonin, anti-inflammatory, hypotensive, hypolipidemic, and anti-fertility activities (Sidhu *et al*., 2003, Chattopadhyay, 2003, Thakurta *et al*., 2007, Shori and Baba, 2013).

The genus *Mentha* (family: Lamiaceae) consists of 25 species grown commonly in temperate region of the world. *Mentha arvensis, M. piperita, M. longifolia* and *M. spicata* locally named as menthol mint, peppermint, wild mint and spear mint. These are cultivated in Asia, Europe, America and Australia for production of essential oils and in cuisines. In folk medicine, the leaves are used for relief of sore throat, mouth or throat irritation, digestive tract diseases. It is also used in treatments for headaches, migraines, neuralgia, myalgia, nausea, bronchitis, flatulence, anorexia, ulcerative colitis, sprains, nasal decongestants and liver complaints. Among the pharmacological effects of *Mentha* are antipruritic, anti-inflammatory, anti-spasmodic, anti-catharrhal, emmenagogue, carminative, antiseptic, antimicrobial, radical-scavenging, antioxidant, cytotoxic and stimulant properties (Iscan *et al*., 2002, Proestos *et al*., 2005, Singh *et al*., 2005, Hussain *et al*., 2010).

Punica granatum L. (Pomegranate) is a fruit bearing deciduous shrub or small tree of 5-8 m height. This plant is native of India, Iran and Asia, Africa, Middle East, Mediterranean region has long history as medicinally valuable and has been extensively used by traditional herbal practitioners and folk of many cultures. Fresh aril of fruits are edible and used for preparation of juice, canned beverages, jelly, jam, paste, flavoring and colorant products. The known components from fruit are citric acid, ascorbic acid, phenolics - anthocyanins including (glucosides, delphinidin, cyanidin, pelargonidin), catechins, ferulic acids, quercetins, gallagic acid, ellagic acid, punicalin, punicalagin, pedunculagin and flavanols. Fruit juice is a provider of anti-viral, anti-fungal, anti-fungal, antioxidant, anti-cancer, anti-diarrhea, anti-worm, anti-gastric ulcer and anti-atherosclerotic effects (Gil *et al*., 2000, Singh *et al*., 2002, Elfalleh *et al*., 2009, Mousavinejad *et al*., 2009, Opara *et al*., 2009, Tehranifar *et al*., 2010).

Phyllanthus emblica L. (Family: Euphorbiaceae) Indian goose berry, is one among the most often used herb and widely available in tropical sub tropical regions. Fruit of this plant is reputed to possess good dietary source with highest content of vitamin C, super oxide dismutase compared with any other natural substances and amino acids, mineral content, tannins, lignans, flavonoids and alkaloids. Different traditional systems of medicine i.e. Ayurveda, Greco-Arab, Chinese uses all parts especially fruit of this plant. Extracts have been showed pharmacological properties like anti-diarrhea, anti-dysenteric, analgesic, anti-inflammatory, anti-cancer, antimicrobial, antioxidant, anti-ulcerogenic, anti-pyretic, hypolipidemic, hepatoprotective and chemoprotective (Mayachiew and Devahastin, 2008, Liu *et al*., 2008a,b, Mehmood *et al*., 2011).

Terminalia catappa L. (Family: Combretaceae), tropical almond, widely distributed tree grown for shade and ornamental purpose. It is a medium sized tree with leaves clustered at the ends of the branches; leaf size is 25 cm long and purple-red after full ripening turn yellow during senescence. Flowers are white and fruit is drupe with 5 cm long, ovoid, red in color with almond in seed. The leaf and bark extracts were reported for anti-cancer, antioxidant, anti-HIV reverse transcriptase, hepatoprotective, anti-inflammatory, anti-diabetic and aphrodisiac. Fruit contains glucoside, punicalin, punicalagin (anti-HIV), terflavin - A, B, tergallagin, tercatain, chebulagic acid, geramin, granatin B, corilagin (topoisomerase I, II and xanthine oxidase inhibitor), ellagic acid (anti-HIV, xanthine oxidase inhibitor, aldose reductase inhibitor, anti-asthmatic), gallic acid, eugenic acid (anticataract) and pentosans. Dried raw nuts are highly relished by children and roasted or boiled nuts are used as snacks at tea time, steamed nuts are sprinkled over cereal or yogurt for breakfast in India, Jamaica, Nigeria and Malaysia (Lopez-Hernandez *et al*., 2001, Nagappa *et al*., 2003, Ko *et al*., 2003, Kloucek *et al*., 2005, Laskan and Abbas, 2010).

In this study, we have selected six medicinal plants: *Punica grantum* L., *Phyllanthus emblica* L., *Terminalia catappa* L., *Azadirachta indica* A., *Mentha arvensis* L., *Acacia nilotica* L. extracts against antibiotic resistant ***Streptococci*** isolates for minimum inhibitory concentrations and minimum bactericidal concentrations for screening of novel antibacterial agents.

MATERIALS AND METHODS

Chemicals and Growth media

Nutrient agar, blood agar, *Mutans-Sanguis* agar, Muller-Hinton agar, HiStrep™ identification kit, Ampicillin, Tetracycline, Benzyl penicillin, Amoxycillin, and Erythromycin paper discs (Hi-Media Laboratories Pvt. Ltd. Mumbai, India).

Test Bacterial strains

Swab samples were collected from male persons without smoking and pan or nut products chewing habit. Swabs were streaked on the sterile nutrient agar and blood agar plates and incubated at 37°C for 24 h. typical colonies after sufficient growth on agar plates were subcultured on nutrient agar slants.

From the bacterial isolates obtained *Streptococci* spp. were identified by using HiStrep™ identification kit, Mutans-Sanguis agar plates and other biochemical tests such as starch hydrolysis, gelatin liquefaction and urease hydrolysis were performed to identify using Bergey's manual of systematic bacteriology.

Antibiotic sensitivity assay

Ampicillin, Tetracycline, Benzyl penicillin, Amoxycillin, and Erythromycin paper discs were used to test sensitivity clinical isolates. Muller-Hinton agar plates were prepared and 0.1 ml of 24 hr culture was spreaded uniformly with sterile glass spreader. Antibiotic discs were introduced in the centre of each plate and kept in refrigerator for diffusion for 30 min and then incubated at 37°C for 24 h. experiment was in triplicates.

Collection of plant material

Six different plants i.e. *Punica grantum* L., *Phyllanthus emblica* L., *Terminalia catappa* L., *Azadirachta indica* A., *Mentha arvensis* L., *Acacia nilotica* L. were selected for testing against the antibiotic resistant clinical isolates. Fruits of *P. grantum*, *P. emblica* and *T. catappa*, young stem of *A. indica*, and *A. nilotica*, leaves of *M. arvensis* were used for antibacterial activity. The plant material was air dried and processed for aqueous extracts by using Soxhlet apparatus. The extracts were filtered with muslin cloth. Filtrate was then concentrated using reflux method at 100°C for 6 h in Soxhlet apparatus to 1/4th of the original volume and used.

Antibacterial assay

5%, 10%, 15%, 20%, 25% concentrations of the plant extracts were prepared with sterile distilled water. The assay was performed using agar well diffusion method. Plates were prepared and 0.1 ml of culture was added and spread with sterile glass spreader. A well was made in the centre of plate with sterile (0.65 cm) cork borer (steel). 100µl of each concentration was introduced into the well in separate plate. The plates were kept in refrigerator for diffusion for 30 min and then incubated at 37°C for 24 h. experiment was in triplicates.

Minimum Inhibitory concentration

MIC was determined for each plant extract showing activity against clinical isolates in disc diffusion assay. Micro broth was followed for MIC value determination.

Minimum Bactericidal concentration

MBC was determined by subculturing 50µl from each well showing no apparent growth. Least concentration of extract showing no visible growth on subculturing was taken as MBC.

RESULTS AND DISCUSSION

The total bacteria counts enumerated from the swab samples were 50.8×10^{8}. Two different types of organisms *S. mutans*, *S. sanguis* were identified based on morphological and biochemical characters as per Bergey's manual of systematic bacteriology. 10 isolates of *Streptococci* spp. were found multidrug-resistant. The resistant bacterial isolates were tested against the plant extracts showed sensitive at different concentrations and the highest titer of all the extracts was found efficient (Fig: 1-6).

Fig 1: Antibacterial assay of *P. grantum*

Fig 2: Antibacterial assay of *P. emblica*

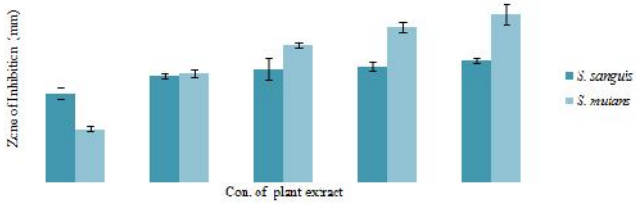

Fig 3: Antibacterial assay of *T. catappa*

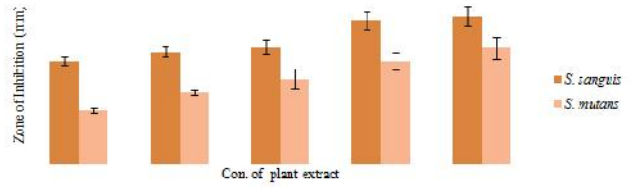

Fig 4: Antibacterial assay of *A. indica*

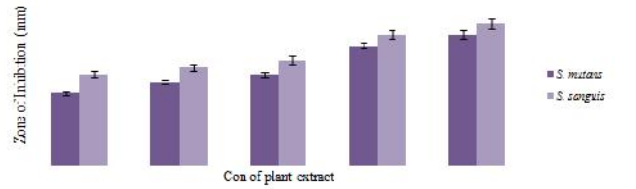

Fig 5: Antibacterial assay of *M. arvensis*

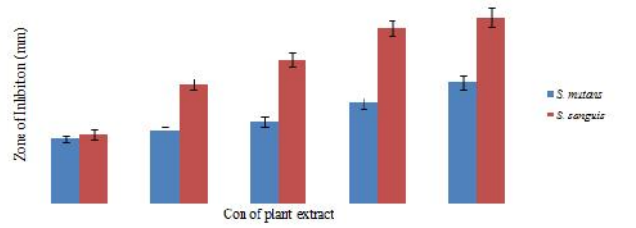

Fig 6: Antibacterial assay of *A. nilotica*

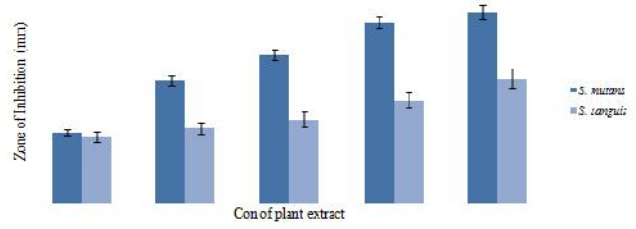

Table 1: Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of plant extracts

Plant extracts	Test organisms			
	S. mutans		*S. sanguis*	
	MIC	MBC	MIC	MBC
Punica grantum	15	19	14	18
Phyllanthus emblica	18	21	14	20
Terminalia catappa	14	19	15	22
Azadirachta indica	10	15	10	12
Mentha arvensis	20	20	14	17
Acacia nilotica	18	22	18	20

MIC and MBC of the test species were different for the plant extracts tested *A. indica* was more efficient, *P. granatum*, *P. emblica* and *M. arvensis* were inhibitory at the same concentration for *S. sanguis* (Table 1) hence, these extracts could be used in single or combination in tooth paste or tooth powder and mouth wash that could be effective.

Our study was against the antibiotic resistant isolates that were resistant against antibiotics of common practice to treat dental problems by physicians. Plant extracts tested could be safer than those antibiotics and most of them inexpensive, used in daily walk of life by common man. Some plants that we have tested are already used by most of rural people in teeth cleaning practice and also available in tooth powders and tooth pastes by multinational companies like Vicco laboratories, Colgate-Palmolive, Hindustan Unilever and Dabur. From our results such herbal formulations are required to increase and made familiar to create the environment without antibiotic resistant forms of bacteria.

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