

Evaluation of Antimicrobial Activity of Six Medicinal Plants Against Dental Pathogens

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Abstract: All these selected medicinal plants have secondary metabolites which inhibit the microbial growth. In the present study six medicinal plants namely *Terminalia chebula*, *Mimusops elengi*, *Achyranthes aspera*, *Acacia catechu*, *A. arabica* and *Glycyrrhiza glabra* extracts were prepared in four different solvents such as petroleum ether, acetone, methanol and water. Each extract was tested for their antibacterial activity against five dental infection microorganisms such as *Staphylococcus aureus*, *Streptococcus mutans*, *S. salivarius*, *S. sanguis*, *Lactobacillus acidophilus* and *Candida albicans* by well diffusion method. All the plants showed significant activity against all pathogens, but the methanolic extract of *T. chebula* showed maximum zone of inhibition against *S. aureus* (27 mm) and *Candida albicans* (26 mm) and the minimum zone of inhibition were determined in petroleum ether extract of *M. elengi* and *A. aspera* against *S. mutans*, *S. aureus* and *Candida albicans* (9 mm). Methanolic and aqueous extracts showed greater activity as compare petroleum ether and acetone extracts because more phytoconstituents were leached in it. Phytochemical analysis of these medicinal plants showed the presence of many biologically active constituents of plant which might have exerted synergistic antimicrobial effect. [Report and Opinion 2010;2(6):37-42]. (ISSN:1553-9873).

Key Words: Dental infections, Antibacterial activity, phytoconstituents.

Introduction:

Many bacteria and fungi produce diseases which are manifested in or about the oral cavity. Some of these diseases or lesions are of a specific nature and are produced by a specific contribution to the problem of caries etiology (William et al., 1994). The highest caries susceptibility is in the age group of 20-40 years, also the females are more susceptible to dental caries as compared to males (Nigam and Srivastawa, 1990). Dental decay is a chemical parasitic process consisting of two stages, the decalcification of enamel or its total destruction and the decalcification of dentine (dissolution of the softened residue (Marsh, 1992). The cariogenic Streptococci is critical to the development of pathogenic plaque. A large number of Streptococcus, Actinomyces and Lactobacillus species are involved in root caries and periodontal diseases (Schiipbach et al., 1995, Slots and Rams, 1992). Antibiotic resistance has increased substantially in the recent years and is posing an ever increasing therapeutic problem (Guillemot, 1999, Jones, 1988, Austin et al. 1999) one of the methods to reduce the resistance to antibiotics is by using antibiotic resistance inhibitors from plants (Kim et al. 1995). Plants are known to produce a variety of compounds to protect themselves against a variety of pathogens. It

is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant pathogens. (Ahmad and Beg, 2001). Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years and in many parts of the world. In rural areas of the developing countries, they continue to be used as the primary source of medicine (Chitme et al., 2003). About 80% of the people in developing countries use traditional medicines for their health care (Kim, 2005).

Material and Methods

Plant material: The plants were collected from the foot hills of Shivalik range of Himalayas in Harwar and were further confirmed in the Department of Botany and microbiology Gurukul Kangri University, Harwar and identified at Botanical Survey of India, Dehradun, Uttarakhand.

Preparation of plant extracts: The method of Alade and Irobi (1993) was adopted for the preparation of plant extracts with little modification. 100 gm of the powdered plant materials were loaded in soxlet

assembly and extracted in four different solvents *i. e.* petroleum ether, acetone, methanol and aqueous for 72 hours by successive method. At the end of each extract, it was passed through Whatman filter paper No 40. The filtrate obtained was concentrated by using vacuum evaporator at 30°C.

Culture media: Muller Hinton agar media no 173 (Hi media Pvt. Ltd., Mumbai, India) was used for conducting antibacterial tests.

Inoculums: The microorganisms were inoculated into nutrient broth (Hi media Pvt. Ltd., Mumbai, India) and incubated at 37°C for 24 h. and the suspension was checked to provide approximately 10⁵CFU/ml.

Microorganisms used : 6 pathogenic microorganisms such as *Staphylococcus aureus*, *Streptococcus mutans*, *S. salivarius*, *S. sanguis*, *Lactobacillus acidophilus* and *Candida albicans* were isolated from infected patients in Aggarwal dental clinic and the isolates were identified according to published guidelines (Burnetti *et.al.*, 1994).

Antimicrobial assay:

The cup- plate method was used to evaluate the antibacterial activity (24). This method depends upon the diffusion of the tested material to such an extent that growth of added microorganisms is prevented entirely in a zone around the hole containing a solution of tested material. One hundred microlitres of diluted inoculums of 10⁵CFU/ml of 24hours old cultures of test organisms were mixed in Muller Hinton agar medium and shaken. Then media was poured (25-30ml) in sterilized Petri dishes (20 × 90 mm). Wells of 6 mm diameter were punched into the agar medium and filled with 45µl of synthesized complexes (100mg/ml). All the solvents served as negative control. Antibiotic (ampicilline concentration 100mg/ml.) was simultaneously used as positive control. Each sample was assayed in triplicate and the mean values were observed. The plates were then

incubated at 37°C for 24 h. The antimicrobial activity was interpreted from the size of the diameter of zone of inhibition measured in mm, it was observed as the clear zones surrounding the hole evaluated by measuring the inhibition zone diameter.

Results

The botanical name family, common name, Vedic name, part used, chemical phytoconstituents and the traditional uses of all the six plants are given in table 1. Each of the six plants were prepared in extracts of four different forms, namely petroleum ether, acetone, methanol and aqueous extracts and tested against dental infection pathogens. Table 2 represents the summary of the antibacterial activities of the various extracts with respect to each of the test organism at concentration of 200mg/ ml. Antibacterial activity was found in all the 24 plant extracts of different plant parts. *Terminalia chebula* (fruits), *Glycyrrhiza glabra* (roots) and *Acacia arabica* (bark and fruits) exhibited significant antibacterial activity with maximum zone of inhibition in methanolic extract. The antibacterial activity of active extracts were observed in the increasing order - *.aspera* < *M.elengi* < *A.catechu* < *A.arabica* < *G.glabra* < *T.chebula*. The maximum zone of inhibition against *S.aureus* i.e. 27mm was observed in methanolic extract of *T.chebula* and the minimum zone of inhibition in petroleum ether extract of *A.aspera* against *S.aureus* i.e. 9mm. Similarly methanolic extracts of *T.chebula* showed maximum antimicrobial activity against *S.mutans* (23mm), *L.acidophilus* (24mm) *S.salivarius* and *C. albicans* (26mm) and, while *G.glabra* is effective against *S.sanguis* (20mm). The phytochemical analysis of these six plants showed the presence of many biologically active plant constituents as depicted in Table 3. Glycosides, flavonoids, alkaloids and saponins were detected in *A.aspera*, *A.arabica* and *Mimusops elengi*. Tannins, were detected in all extracts, saponins were not found in *T.chebula* *A.catechu* and *G.glabra*.

Table 1. The ethnobotanical and phytochemical data of six Indian Vedic Medicinal plants.

Botanical Name Family	Common & Vedic name	Part used	Known phytoconstituents	Traditional Use
<i>Terminalia chebula</i> (Combretaceae)	Harar Haritiki	Fruit	Chebolic, tannic acid Anthroquinone, Chebuligicacid, Coritagit, (Harborneand Baxter. 1995)	Laxative, ulcers teeth, piles.
<i>Mimusops elengi</i> (Sapotaceae)	Moulshri Bakul	Bark Fruit	Saponin, pentacyclic triterpens mimusopgenonre steroidal glycosides (Shaha <i>et.al.</i> 2001)	Diarrhoea, dysentery, astringent, Gum diseases,
<i>Achyranthes aspera</i> (Amaranthaceae)	Chirchita Apamarga	Whole Plant	Achyranthin,ecdysterone,stigmasterol,sitostero l Triterpenoides, rhamnose glycosides17entatriacontanol (Mishra <i>et al.</i> , 1993)	Cough asthma, bronchitis snake bite, anemia, dropsy, leprosy, teeth (Gokhale <i>et al.</i> , 2002)
<i>Acacia catechu</i> (Mimosaceae)	Khair Khadhira	Stem	Quercetin, poriferasterol, B-Dglucoside, tannicacid, catechuicacid, orcatechin flavone glycoside(5,7,3,4, tetrahydroxy 3- methoxyflavene (Sharma <i>et.al.</i> 1997 and Yadava, 2002	Ulcers, urinary discharges, strengthens teeth, diarrhea, and tonsils (Nadakarni, 1976)
<i>A.arabica</i> (Mimosaceae)	Babool Baboola	Bark, Fruits	Galactoaraban, Arabic acid	Astringent, cough, eucoderma, gum diseases, gonorrhea, diarrhea, Mouth and teeth, skin disease.
<i>G.glabra</i> (Leguminaceae)	Mulahathi	Roots, Rhizomes.	Phenolics, Glycosides, Cinnamic acid, Isobatin, Liqirstinasparagive, liquirtin Coumarin andtannin (Bhardwaj <i>et.al.</i> 19...)	Rheumatoid, arthritis, Tonsil, Expectorant, antitussive, cough, anti-inflammatory, laxative.

Table-2. The antimicrobial activity of plant extracts

PATHOGENS	A. ASPARA				A. ARABICA				A. CATECHU				T. CHEBULA				M. ELENGI				G. GLABRA			
	Pt. Ether	Acetone	Methanol	Water	Pt. Ether	Acetone	Methanol	Water	Pt. Ether	Acetone	Methanol	Water	Pt. Ether	Acetone	Methanol	Water	Pt. Ether	Acetone	Methanol	Water	Pt. Ether	Acetone	Methanol	Water
<i>Staphylococcus Aureus</i>	9	10	12	11	19	20	22	21	12	14	14	13	25	26	27	26	14	15	16	16	20	22	23	24
<i>Streptococcus Mutans</i>	10	12	12	11	15	16	17	17	15	15	16	17	22	22	23	22	9	12	16	14	19	18	20	21
<i>S.sanguis</i>	10	12	13	11	15	16	17	14	15	15	16	14	17	18	19	17	10	11	13	12	17	18	20	19
<i>S.salivarius</i>	10	11	12	10	16	17	17	16	13	12	15	14	22	23	26	25	10	13	15	14	18	18	19	18
<i>Lactobacillus acidophilus</i>	12	13	16	14	18	19	21	20	18	19	20	19	20	22	24	22	12	13	15	14	17	18	19	18
<i>Candida albicans</i>	0	10	14	12	15	17	18	16	9	11	13	14	12	18	26	22	9	11	14	12	9	12	13	13

* Solvents did not show any zone of Inhibition

** Inhibitions zone in mm.

Table-3. The phytochemical screening of methanolic extracts.

S. No	Plants	Phytoconstituents					
		Glycosides	Alkaloids	Flavonoids	Phenols	Tannins	Saponin
1.	<i>Terminalia chebula</i>	+	+	+	+	+	-
2.	<i>Acacia Arabica</i>	+	+	+	+	+	+
3.	<i>Acacia catechu</i>	+	+	-	+	+	-
4.	<i>Glycyrrhiza glabra</i>	+	+	+	+	+	-
5.	<i>Mimusops elengi</i>	+	+	-	+	+	+
6.	<i>Achyranthes aspera</i>	+	+	+	+	+	+

+ = Present

- = Absent

Discussion:

The diseases produced by a number of microorganisms are manifested in or about the oral cavity. Some of these diseases are of a specific nature and are produced by a specific microorganism while others are clinically specific and may be caused by any of a broad group of microorganisms. This microbial specificity or non specificity is characteristic of All pathogens isolated during present disease wherever they may occur in the body. Dental caries is caused by infection with streptococcal, staphylococcal, Lactobacillus and candida species. The streptococcal and staphylococcal organisms of the beta hemolytic type that elaborate an erythrogenic toxin in the early stages of characteristic of disease wherever they may occur in the body. *S. mutans* is the main causative agent of dental caries. *Streptococcus mutans*, fermented sugars (Yamada et al., 1980) and produced acids. The acids which affect this primary decalcification of enamel leads to its total destruction and the decalcification of dentin. The acids derived from the fermentation of starches and sugar lodged in the retaining centers of teeth. The streptococci are acidogenic and ferment glucose, lactose, sucrose and maltose. The major end product of fermentation is lactic acid, dextrans and levans. These compounds showed to make dental caries and other infections (Lehner et al. 1976). These microorganisms demineralized the calcified structures (enamel, dentin) and dissolution of the organic matrix. *S.mutans*, *S.sanguis* *S.salivarius* and *Lactobacillus acidophilus* play a major role in dental plaque formation. The causative agents of dental caries and dental plaque were isolated and identified. The morphological and biochemical tests were performed and compared with

MTCC cultures. The present study has shown that these plants are potentially a rich source of antibacterial agents. This demonstrates their importance in traditional remedies in the rural populations. The 24 plant extracts tested, inhibited the growth of all pathogens but *T.chebula*, *A.arabica* and *G.glabra* are more effective. Our results showed more antibacterial activity of *G.glabra* against *S.aureus* as reported earlier by Nimri *et al.*, 1999.

The extent of antimicrobial activity of the extracts based on inhibition zone diameter has been described as low (12-18 mm), moderate (19-22 mm) and strong activity (23-38 mm) by Ahmad *et al.* (1999). In our studies the crude extracts of *T. chebula* showed strong activity while all other plants showed moderate antibacterial activity against the pathogens. The methanolic extract is highly effective against all pathogens 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. Ahmad *et al.* (1998) screened medicinal plants to detect antimicrobial activity and clearly demonstrated that alcohol is a better solvent as compared to aqueous and hexane. Similarly water was not found to be the most effective solvent for extracting the active compounds from plants as compared to hexane and methane (Shale *et al.*,1999).

Tannins were found to be a component of plants that showed antibacterial activity. It could be one of the components responsible for the antibacterial activity since it was reported by Al Genaidy, 1993. The diameters of inhibition zone around the most active extracts are comparable with those of the standard antibiotics used as positive control.

This study is a preliminary evaluation of antimicrobial activity of the plants. It indicates that plants have the potential to generate herbal metabolites. The crude extracts demonstrating anti dental caries activity could result in the discovery of new chemical classes of antibiotics that could serve as selective agents for the maintenance of animal or human health and provide biochemical tools for the study of infectious diseases. Further phytochemical studies are required to establish the types of compounds responsible for the antimicrobial effects of these medicinal plants.

Conclusion:

Indian medicine has a long history, and is one of the oldest organized systems of medicine. Its earliest concepts are set out in the sacred writings called the Vedas, especially in the metrical passages of the Atharvaveda, which may possibly date as far back as the 2nd millennium BC. In the present study we have selected 6 medicinal plants against dental infection microorganisms including *Staphylococcus aureus*, *Streptococcus mutans*, *S. sanguis*, *S. salivarius*, *Lactobacillus acidophilus* and *Candida albicans*. The selection of medicinal plants was based on their ancient (Vedic) uses, the phytochemical analysis were carried out of potentially strong active plant extract.

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