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Evaluation of antimicrobial activity of *Azadirachta indica*, *Syzygium aromaticum* and *Cinnamomum zeyalnicum* against oral microflora.

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Abstract :

For several years, varieties of plants have been used throughout India to treat oral care issues and are used till date in rural as well as urban areas. The inhibitory effects of aqueous extracts and essential oil of three plants, Neem (*Azadirachta indica*), Clove (*Syzygium aromaticum*) and Cinnamon (*Cinnamomum zeylanicum*) against clinical isolates identified as *Staphylococcus auricularis*, *Micrococcus* species, *Acinetobacter lwoffii* and *Candida albicans*, were studied. Cinnamon extract successfully inhibited all four organisms showing largest zones of inhibition. Clove oil inhibited *S. auricularis*, *A. lwoffii* and *C. albicans* but not *Micrococcus*. Neem oil did not show any significant activity against any organisms. Toothpastes were then formulated using effective plant extract showed the antimicrobial activity.

Key words: Aqueous extract, *Azadirachta indica*, *Syzygium aromaticum*, *Cinnamomum zeylanicum*, *Staphylococcus auricularis*, *Micrococcus species*, *Acinetobacter lwoffii*, *Candida albicans*.

Introduction

Microflora refers to the collective bacteria and other microorganisms present in a particular ecosystem. The ecosystem can be an animal or human host or a single part of its body. Our body is a host to billions of bacteria of many kinds. The mouth harbours many microorganisms and is an ecosystem of considerable complexity that has not been fully investigated yet and the mechanism/ chemistry has also not been completely understood. More than 30 genera of bacteria have been detected in human mouth. The oral microflora can be beneficial or detrimental. They supply some nutrients and digestive enzymes such as amylase, lipase and proteases and contribute to the hosts defences by protecting from exogenous microbes (Marsh *et al*., 2006). Any change in the host's oral environment or immune system can cause a number of different oral problems. Use of plants for maintaining oral hygiene is common till date in rural areas where toothpastes, mouthwashes, massage gels and other oral care products are not available. These products contain synthetic substances such as triclosan, phenol, benzydamine hydrochloride, zinc chloride, stannous fluoride etc. as antimicrobial agents. However, use of leaves, stem, fruits, seeds and bark of some plants (Neem/babool etc.,) for oral hygiene is common. *Azadirachta indica* has anti-inflammatory, antibacterial, antimalarial, antiulcer, antiparasitic, antifungal, antiprotozoal and antiviral properties (Biswas *et al.*, 1973; Siddique *et al.,* 1992; Ian *et al*., 1994). All parts of the tree have been used traditionally to cure various disorders as stated by Subapriya *et al.,* 2005. Buds of *Syzygium aromaticum* are used as an analgesic, antiseptic and a carminative. *Cinnamomum zeylanicum* is known to inhibit the growth of Candida hence preventing & curing oral thrush. It has been proven to be active against many pathogens (Suresh *et al*., 1992).

Materials and Methods

Collection of plant Material: *Azadirachta indica* twigs, *Syzygium aromaticum* buds and *Cinnamomum zeylanicum* bark were obtained from local market. Plant essential oil was obtained from commercial outlet of Dr. Urjita Jain Herbal ltd, Mumbai.

Collection and maintenance of oral flora: Mouth swabs from 6 people were collected and isolated on sterile Nutrient Agar and Sabouraud's Agar plate and incubated at 37ºC and room temperature respectively for 24hrs. The colonies obtained were transferred to sterile slants and sent to Metropolis Labs, Worli for identification.

Preparation of plant Extracts: The plant parts were sun-dried and ground into a course powder in a blender. 2 g of powdered plant material was added to 10 ml of sterile distilled water and heated to 70-80ºC for 2 hours. The mixtures were then allowed to cool, filtered through muslin cloth and centrifuged. The supernatant were then passed through a 0.45µm millipore filter to obtain sterile extracts which were stored at 4ºC (Nazia *et al.,* 2006).

Determination of antimicrobial activity: Kirby Bauer disc diffusion method was used to determine the effect of aqueous extracts and plant essential oil on the isolates. The broth culture of each of the isolate was swabbed on sterile Mueller-Hinton agar. Sterile discs were dipped in plant extracts and placed onto the agar plates. Plates were incubated at 37ºC for 24 hours. The zone of inhibition was observed after 24 hours.

Formulation of herbal toothpaste:

The herbal toothpaste was prepared as per the formulation given by Jadge, 2008. The ingredients and quantities were mixed(Table 1).

Evaluation of toothpaste's activity against the isolates

Agar cup Method was used for testing the antimicrobial activity of the toothpastes prepared using the essential oil. The broth culture of the isolates was swabbed on Mueller-Hinton agar plates. Wells were bored using sterile borer. Toothpaste formulations were added in the wells and plates were incubated at 37ºC for 24 hours. The zone of inhibition was observed after 24 hours**.** For each strain a control was maintained using a formulation without the active ingredient.

Results and discussion

Asikainen and Chen (2000) stated that the oral ecology is different from person-to-

person in transmission of *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*

Smith (1993) studied the oral streptococcal colonization of infants while Pearce (1995) found viridans streptococci in the oral cavity of human neonates. In the present study the isolates form the oral cavity were identified as *Staphylococcus auricularis*, *Micrococcus species*, *Acinetobacter lwoffii* and *Candida albicans.*

Aqueous extract of *Azadirachta indica* and *Syzygium aromaticum* could not inhibit any of the culture. Essential oil of *Azadirachta indica* showed the activity only against *S. auricularis.* The essential oil of *Syzygium aromaticum* was found to be effective against *Staphylococcus auricularis, Acinetobacter lwoffii* and *Candida albicans.* Aqueous extract and the essential oil of Cinnamomum *zeylanicum* were found to be effective against all the four isolates. These results have been compiled in Table 2.

The toothpastes formulated were found to be effective against the isolates. The zone sizes observed after 24 hrs incubation are tabulated in Table 3.The activity of cinnamon is due to the presence of cinnamaldehyde that inhibits amino acid decarboxylase activity as suggested by Wendakoon and Sakaguchi (1995). Cinnamon oil contains benzoic acid, benzaldehyde and cinnamic acid whose lipophyllic part is responsible for its antimicrobial properties (Ramos-Nino *et al*., 1996). Both clove and cinnamon contain eugenol, which is known to be bacteriostatic and bactericidal depending on the concentration used (Pelczar *et. al.*, 1988). Essential oil from cinnamon bark also contains cinnamyl acetate (8.7%), which increases the activity of the parent compound (Gupta *et al.,* 2008). The mechanisms or modes of action of the compounds on the bacteria and fungi include cytoplasmic granulation, cytoplasmic membrane rupture and inhibition of intracellular and extracellular enzymes. The mechanism of oils is generally hydrophobicity leading to partition in the lipid bilayer of the cell membrane, leading to alteration in the permeability and consequent leakage of cell contents. It also inhibits respiration in the cell and causes potassium ion leakage.

The present study suggests that the aqueous extracts were not able to inhibit the isolates. However, essential oil can inhibit the growth at larger extent. Further study of the components responsible for the biological activity is required and possibly a combination of these components would yield better results. The tooth pastes also were found to be effective in controlling the growth of these organisms hence such an herbal product may have a potential to replace the standard formulations.

Table 1: Formulation of Toothpaste

Ingredient	Quantity in %
Calcium Carbonate	35.00
Sodium Lauryl Sulphate	01.50
Glycerin	30.00
Sodium Alginate	01.00
Sodium Benzoate	00.12
Sodium saccharine	00.30
Plant Extract	02.50
Purified water	q.s.

Table 2: Evaluation of antimicrobial activity of plant extracts

Plant	Average Zone size in mm							
	S.auricularis		*Micrococcus spp*		*A.lwoffii*		*C.albicans*	
	A.E	**E.O**	**A.E**	**E.O**	**A.E**	**E.O**	**A.E**	**E.O**
A. indica	No Zone	13.30 ± 1.5	No Zone	No Zone	No Zone	No Zone	No Zone	No Zone
S. aromaticum	No Zone	26.0 ± 1	No Zone	No Zone	No Zone	18.0 ± 1	No Zone	26.3 ± 0.5
C. zeylanicum	11.00 ± 1	45.3 ± 3	11.6 ± 0.5	23.6 ± 1.5	11.3 ± 0.5	22.3 ± 2.5	11.0 ± 0	45.6 ± 1
Key: AE: Aqueous extract, EO: Essential oil								

Table 3: Evaluation of toothpaste's activity

Plant	Average Zone size in mm			
	S.auricularis	*Micrococcus spp*	*A.lwoffii*	*C.albicans*
A. indica	11.6 ± 0.5	-	10.6 ± 0.5	11 ± 1
S. aromaticum	16 ± 1	-	15 ± 1	11.6 ± 1.5
C. zeylanicum	13.3 ± 0.5	13.6 ± 1.5	13.6 ± 0.5	13 ± 0
Control	No Zone	No Zone	No Zone	No Zone

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