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Research Article

Antimicrobial activity of herbal extracts on oral pathogens

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Abstract

Objective- Traditional plants have been proved to be a better source in the search for novel antimicrobial compounds. In search of novel antimicrobial agents, an attempt is made to evaluate the antimicrobial efficacy of extracts of different herbs. Neem (*Azadirachta indica linn*.), green tea*(Camellia sinensis)*, stevia (*Stevia rebaudiana)* and poly-herbal extract. **Method-**The plant leaves were dried and powdered for obtaining herbal extracts. The minimum inhibitory concentration (MIC) for the active extracts was examined by broth dilution method. The minimal bactericidal concentration (MBC) was determined by colony count of more than 99.9% killing. **Results**-The results showed that the MIC of aqueous neem extracts from *B.subtilis* and *S.epidermidis* were 3.13 and 4.10 mg/ml respectively. The minimal bactericidal concentration (MBC) were 6.25 and 11.4 mg/ml for *B.subtilis and S.epidermidis* respectively. **Conclusion**-This study concludes that aqueous neem extracts can be used effectively against *S.epidermidis* and *B.subtilis* pathogens.

Keywords: Antimicrobial activity, herbal extracts, *Staphylococcus epidermidis, Bacillus subtilis* , Zone of inhibition

Introduction

For many centuries, plants have been main source for drug development. Human use of plants as medicinal agents predates recorded history (1). Ethnomedical plant-use data in many forms has been heavily utilized in the development of formularies and pharmacopoeias, providing a major focus in global health care, as well as contributing substantially to the drug development process (2). Medicinal plants contain physiologically active principles that over the years have been exploited in traditional medicine for the treatment of various ailments (3) as they contain anti-microbial properties (4).These medicinal herbs constitute indispensable components of the traditional medicine practiced worldwide due to the low cost, easy access and ancestral experience (5). The presence of nutrients, epithelial debris and secretions makes the mouth a favourable habitat for a great variety of bacteria. Oral bacteria include mainly streptococci, lactobacilli, staphylococci, coryne bacteria and a great number of anaerobes, especially bacteroides. Hundreds of different bacteria have been identified in the oral cavity although this does not imply that they are not transients. The mouth presents a succession of different ecological situations with age, and this corresponds with changes in the composition of the normal flora. At birth the oral cavity is composed solely of the soft tissues of the lips, cheeks, tongue and palate, which are kept moist by the secretions of the salivary glands. At birth the oral cavity is sterile but rapidly becomes colonized from the environment, particularly from the mother in the first feeding. *Straptococcus salivarius* is dominant and may make up 98% of the total oral flora until the appearance of the teeth (6 - 9 months in humans)(6). The eruption of the teeth during the first year leads to colonization by *S. mutans* and *S. sanguis*. These bacteria require a no desquamating (no epithelial) surface in order to colonize. They will persist as long as teeth remain. Other strains of streptococci adhere

strongly to the gums and cheeks but not to the teeth. The creation of the gingival crevice area (supporting structures of the teeth) increases the habitat for the variety of anaerobic species found. The complexity of the oral flora continues to increase with time, and bacteroides and spirochetes colonize around puberty. (7) The oral bacteria can invade compromised tissues in their hosts and produce disease outside the oral cavity. Oral bacteria invade deeper tissues they may cause abscesses of alveolar bone, lung, brain, or the extremities(8). Such infections usually contain mixtures of bacteria with *Bacteroides melaninogenicus* often playing a dominant role. If oral streptococci are introduced into wounds created by dental manipulation or treatment, they may adhere to heart valves and initiate subacute bacterial endocarditis or other heart disease. Agents that kill or inhibit microorganisms may be classified as disinfectants, antiseptics or antibiotics. Antibiotics are molecules that are produced by one microorganism that kill (bactericidal) or inhibit (bacteriostatic) other microorganisms. Antiseptics and disinfectants are commercially prepared chemicals and the distinction between them is that antiseptics can be exposed to mucosal surfaces for at least a short time and disinfectants should not as they could impart harm. Of all the types of microbes living in your mouth, bacteria are the most numerous. It has been estimated that there are over 100 million in every millilitre of saliva from more than 600 different species. Unless there are open wounds or cuts inside your mouth, most mouth bacteria will do you no harm. Some are swallowed and are killed by stomach enzymes; others perish when they are attacked by enzymes in saliva. However, mouth bacteria are responsible for some of the most common bacterial diseases in humans and they are gum disease and tooth decay (caries).

Bacillus subtilis produces bacitracin, which is an antibiotic often used to treat cuts and wounds. Although generally considered non-pathogenic and harmless, it can cause lung and blood infections in people who have weak immune systems (5) .It has also been occasionally involved in food poisoning incidents. Regardless, *Bacillus subtilis* is a good bacterium to work with in the laboratory because it is, for the most part, non-pathogenic and harmless. In addition, *B. subtilis* is a facultative aerobe, meaning that it requires oxygen in order to grow. The human mouth is the place where we inhale oxygen, which means that *B. subtilis* can grow well in our mouths because of the abundance of oxygen. This makes it a good bacterium candidate for this experiment. In addition, *B. subtilis* is present in small amounts in most samples of flour .*Staphylococcus epidermidis* is a bacterium that lives in skin and mucous membranes. This fact makes *S. epidermidis* a good bacterium for this experiment because of its accessibility to our mouths. When we eat, our skin comes in contact with our mouths numerous times, such as when we eat foods with our hands. Thus, *S. epidermidis* has many opportunities to come in contact with our mouths. *S. epidermidis* has been known to cause endocarditis, bacteremia, and urinary tract infections. However, it is non-pathogenic, which makes it a good candidate for laboratory work and for inclusion in this experiment Herbal extracts as the name suggests, is the extract of herbs. Herbal extracts are an ancient methodology as its references have been discovered in holy Vedas and in Unani scriptures. Herbal extracts have unending health benefits so, it is expected that they will soon revive the era of healthy mind and body. (9) With the realization that chemical medicines are not always "magic bullets" and may carry serious side effects. Today, in this whole world there is turn to return towards the herbalism and use of herbal products and to adopt more natural way of life.Herbal extracts are primarily added to the cosmetic preparations due to several associated properties such as antioxidant propertie. These antioxidant botanicals are generally classified into three categories depending upon the nature of their constituents as carotenoids, flavonoids and polyphenols. (8,9) Flavonoids, impart the UV protection and metal chelating properties. The polyphenols is a large class and contains various molecules like rosemarinicacid(rosemary). Apart from these, the herbal extracts have also been used for the topical anti-inflammatory properties.(10) These agents block the inflammatory changes that result during cutaneous ageing and thus may be helpful in reversing the signs of ageing ·Although the term herbal extract inherently purports to have beneficial and benign properties, these extracts may have adverse reactions in individuals. For example, they can be a possible source of allergenicity in patients presenting with contact dermatitis. We therefore, suggest that regulatory authorities should attend the issue of ensuring quality and safety of herbal cosmetic products immediately before embarking on more arduous task of ensuring efficacy (11)

Materials and Methods

Plant Materials

Leaves of Neem (*Azadirachta indica linn*.) , Green Tea*(Camellia sinensis)* and leaves of stevia (*Stevia rebaudiana)* were collected from Maharshi Ayurveda Products Pvt. Ltd New Delhi, 110044

Preparation of Plant Extracts

For maceration, each herb was dried, reduced to powder then macerated with 95% ethanol for 2 days, filtered the extract and repeated for 2 times. Concentrated all the collected filtrated by Rotary evaporator (Eyela N-N series) and stored at 2-8°C until further use. (12) For continuous extraction, hexane, chloroform, 95% ethanol and distilled water were prepared for extraction solvents. Each herb was reduced to powder. Each solvent was added to each herb with 10-fold weight, and extracted until exhausted(13). Each filtrate was concentrated under vacuum except water filtrate was concentrated by freeze dryer. For reflux, each herb was minced then reflux for 4 hrs by using water as a solvent. The filtrate was collected and concentrated by freeze dryer.

Test Organisms

The Pathogenic strains of, *Staphylococcus epidermidis (MTCCNo.740) and Bacillus subtilis(MTCC No. 121)*were used. These strains were the swab. To ensure that the growth is uniform and confluent (or semi confluent) the swab is passed three times over the entire surface, by repeating the procedure, taking care the second and third time to turn the plate through leaf extract and which were prepared using Dimethylsulfoxide: Methanol (1:1) solvent to dissolve the plant extract and then placed on the inoculated agar surface using sterile forceps. Colonies were diluted to provide a final inoculum of approximately 106 CFU/ml.

Tests for Antimicrobial Activities, Well Diffusion Method

Antimicrobial activities of all test substances against bacteria were screened by well diffusion method (Lee MJ, Lambert JD 2004). A 0.1 ml volume of broth organism cultured was seeded into 10 ml of molten and cool (45°C) agar then poured in plate that has been poured with 10 ml agar and placed four sterile 12-mm diameter cups prior. Cups were taken off and 0.05 ml of test substances were added into each well with duplicate experiments. The plates were allowed to stand for 30 minutes at room temperature, and then incubated aerobically for both bacteria at 37°C. The diameter of inhibition zone was measured, including well size, after 24 hrs incubation for *S.epidermidis* and 72 hrs incubation for *Bacillus subtilis*

Minimal Inhibitory Concentration (MIC) Test, Broth Dilution Method

Antimicrobial activities against both bacteria were evaluated by broth dilution method. (14).Cultures of bacteria were diluted to density of 105 CFU/ml in MHB and TSB, respectively. The test tubes containing 2-folds dilution of each test substance were inoculated by the diluents. *S. epidermidis* was incubated aerobically at 37°C for 24 hrs and *B.subtilis* was incubated anaerobically at 37°C for 72 hrs. The MICs were determined by detecting the turbidity.

Minimal Bactericidal Concentration (MBC) Test

All clear tubes from MIC test were streaked on TSA plate using 10 µl loop. *S. epidermidis* was incubated aerobically at 37°C for 24 hrs and *B.subtilis* was incubated anaerobically at 37°C for 72 hrs. The MBC were determined by colony count of more than 99.9% killing.

Results and Discussion

The study strongly suggest that the aqueous extract of Neem leaves exhibits *in vitro* antibacterial activity against both *Bacillus subtilis* , *Staphylococcus epidermidis* ***as*** shown in Table 1. It appears that the antibacterial activity follows a dose-dependent pattern with the greatest zone of inhibition noted at 100% concentration(15). Although ethanolic and methanolic extract of Neem leaf powder also showed the moderate antibacterial activity. Methanolic extract of neem leaves has shown activity more on *staphylococcus epidermidis* than *Bacillus subtilis* on different concentrations. To sum up the antimicrobial activity of neem , we can say that aqueous extract , methanolic extract and ethanolic extract show the activity(16) . While aqueous extracts are more effective against particular bacteria. Green Tea also have antibacterial activity with ethanolic extract .

The results in form of Zone of Inhibition showed that green tea is most effective on staphylococcus s at different concentrations as shown in Table 2.Stevia is a natural sweetener but it has also notable antimicrobial activity on different concentrations. Experimental datas are showing that stevia extract is more effective against bacillus subtilis species in Table 3.Poly Herbal Extracts had also reported antimicrobial activity. Results from the experiments are showing that mixture of herbs can also be effective against series of microbes . Various mix extracts in different concentration are showing the optimal activity. High concentrations of poly herbal extract is showing high zone of inhibition which is proving its antimicrobial activity as shown in Table 4.Graphs are showing the combined activity of herbal axtracts on both test organisms in Figure 1 and 2.

Table 1 - Antimicrobial Activities of Neem (*Azadirachta indica linn*.) Extracts

Sample	Concentration (µl)	ZOI(mm) *(B.subtilis)*	ZOI(mm) *.(S.epidermidis)*
Aq. Neem extract	30	12	16
	40	14	17
	50	14	17
	60	15	15
Methanolic Extract	30	14	16
	40	12	10
	50	12	12
Ethanolic Extract	30	10	12
	40	12	12
	50	14	14
Distilled water	40	-	-

Table 2 - Antimicrobial Activities of Green Tea (*Camellia sinensis*) Extracts

Sample	**Concentration (µl)**	**ZOI(mm) *(B.subtilis)***	**ZOI(mm) *(S.epidermidis)***
Ethanolic Green Tea extract	30	10	12
	40	8	10
	50	12	10

Table 3 - Antimicrobial Activities of Stevia (*Stevia rebaudiana)* Extracts

Sample	**Concentration (µl)**	**ZOI(mm) *(B.subtilis)***	**ZOI(mm) *(S.epidermidis)***
Ethanolic Stevia extract	30	8	12
	40	10	8
	50	12	8

Table 4 - Antimicrobial Activities of Poly herbal extract Extracts

Sample	**Concentration(µl)**	**ZOI(mm) *(B.subtilis)***	**ZOI(mm) *(S.epidermidis)***
Poly Herbal extract	30	12	14
	40	14	16
	50	14.5	16

Table 5 -The MIC and MBC value of herbal extracts

Herbal Extacts	MIC (mg/ml) *(B.subtilis)*	MIC (mg/ml) *(S.epidermidis)*	MBC (mg/ml) *(B.subtilis)*	MBC (mg/ml) *(S.epidermidis)*
Aq. Neem extract	3.13	4.10	6.25	11.4
Methanolic Neem Extract	2.57	3.01	2.09	8.34
Ethanolic Neem Extract	1.93	2.59	4.6	1.32
Ethanolic Green Tea extract	1.56	2.07	1.02	2.01
Ethanolic Stevia extract	1.85	1.78	3.13	3.95
Poly Herbal extract	3.10	3.22	4.79	3.75
Clindamycin	0.006	0.002	0.012	0.002

It is tested by well diffusion method.

Figure 1- Graph of MIC

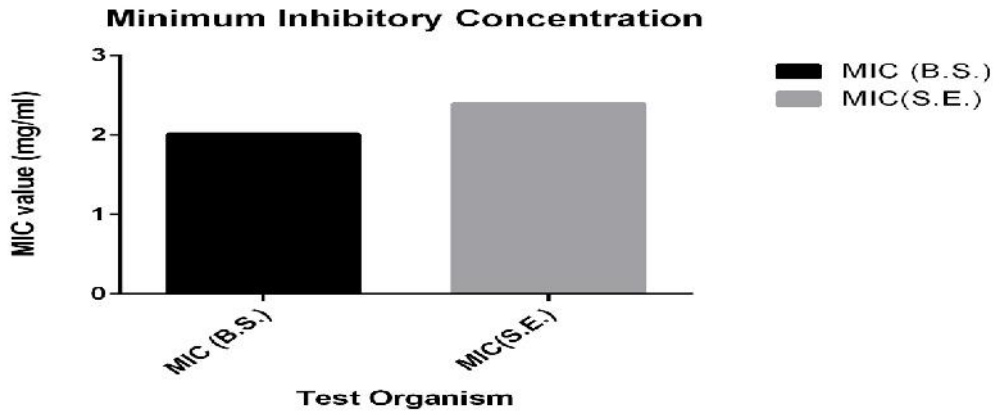
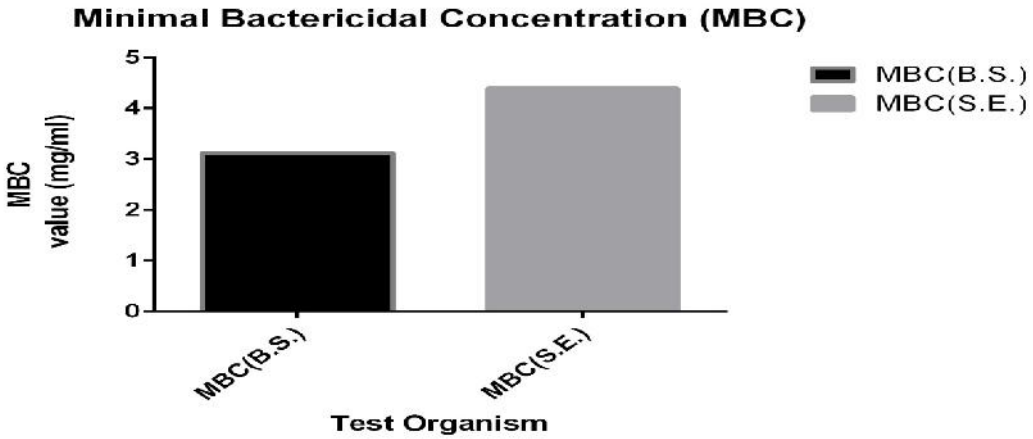


Figure 2 – Graph of MBC



Conflict of interest

The authors declare no conflict of interest.

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