

EFFECT OF BITTER GOURD AGAINST *S.MUTANS* AND *L.ACIDOPHILUS*-AN IN-VITRO STUDY

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ABSTRACT

Objective: To assess the effect of bitter gourd extract (using petroleum ether, ethanol, and chloroform) on *S.mutans* and *L.acidophilus*.

Methods: The present study is an experimental, in-vitro study conducted to assess the antibacterial effect of bitter gourd extract against *S.mutans* and *L.acidophilus*. Bitter gourd extract was prepared using petroleum ether, ethyl alcohol, and chloroform as solvents and was serially diluted using distilled water to obtain 1 mg/ml, 2.5 mg/ml, 5 mg/ml, 7.5% mg/ml, 10 mg/ml solutions in sterile test tubes. The antibacterial activity of bitter gourd was assessed using agar well diffusion method.

Result: Bitter gourd extract using chloroform, ethanol and petroleum ether showed antibacterial activity against both *S.mutans* and Lactobacillus.

Conclusions: Bitter gourd extract possesses antibacterial activity against both *S.mutans* and Lactobacillus.

Clinical significance: The plant /herbal product will serve as an efficient, economic, accessible and acceptable measure especially in developing countries.

Keywords: Bitter gourd, *S.mutans*, *L.acidophilus*, dental caries, cariogenic

INTRODUCTION

Oral health is vital for overall health. Oral health means being free of oral and throat cancer, oral sores, birth defects such as cleft lip and cleft palate, periodontal disease, tooth decay and other diseases that affect the mouth and oral cavity¹. Despite the advent of latest tools for oral hygiene such as tooth pastes and mouth washes, and advancement in dental science majority of population suffer from

dental caries and periodontal problems amongst which dental caries remains the most important dental health problem in the developing countries. The organisms responsible for initiation and progression of dental caries are *S.mutans* and *L.acidiphillus* respectively. *S.mutans* is a facultative, gram positive anaerobic organism and is more prevalent on pit and fissure and buccal surfaces of tooth. It is the primary causative organism for the

initiation of dental caries. *L.acidophilus* is a gram positive, facultative anaerobe associated with progression of carious lesions especially those in the coronal areas. Dental caries known to be a disease of modern civilization is prevalent in about 89% of the Indian population² of which 72% are residing in the rural areas whose economic values are low to afford the treatment of dental caries. Hence, there is a need to promote preventive measures that are acceptable, easily available, and cost effective³.

There has been a change in global thinking, with a growing tendency to go natural. Medicinal plants have also been relied upon by 80% of the world population for their basic health care needs. *Salvadora persica*(Miswak), *Newbouldia laevis*(blood root plant), *Syzgium aromaticum*(clove oil), *Azadirichta Indica*(neem), *allium sativum* (Garlic) *Nidus Vespae* (Honey Comb) have proved to pose potential antibacterial effect against cariogenic bacteria^{4,5}. Bitter gourd is one among them which is been used for centuries in various cuisine of India, China and Latin America. It is a good source of vitamin C, Vitamin A, phosphorus and iron and has been extensively used in folk medicine. It is of great use in medicine for treatment of many diseases such as piles, leprosy, diabetes, jaundice, snake bite, measles and infections. It also had antibacterial activity against *E.coli*, *Pseudomonas*, *Klebsiella*, *Bacillus subtilis* *Staphylococcus*, *Salmonella*, *Streptococcus*, *Entamoeba histolytica*⁶.

Although many studies have tested the antibacterial effect of the bitter gourd

on various organisms but there is a dearth of literature pertaining to their effect against organisms causing dental caries. Hence in the current study an attempt is made to unveil the effect of bittergourd extract (using petroleum ether, ethanol, chloroform and ethyl acetate) on *S.mutans* and *L.acidophilus*.

MATERIALS AND METHOD

The present study is an experimental, in-vitro study conducted to assess the antibacterial effect of bitter gourd extract against *Streptococcus mutans* and *Lactobacillus acidophilus*.

Preparation of bitter gourd extract:

250gms of bitter gourd (BG) was obtained from local market, thoroughly washed and sun dried. The sun dried material was powdered and divided into four parts and collected in different glass jars, to which, petroleum ether (PE), ethyl alcohol (E), and chloroform(c) were added respectively such that the powder was completely immersed in the solvent.

Each mixture was allowed to boil at 40-50⁰c in hot water bath for half an hour. This procedure was repeated 5 times with intermittent stirring of contents. The containers were tightly packed and subjected for maceration the whole or coarsely powdered crude drug is placed in a stoppered container with the solvent and allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter has dissolved. The mixture then is strained, the marc (the damp solid material) is pressed, and the combined liquids are clarified by filtration or decantation after standing. Later the solvent was separated by

filtration using whatman no.1 filter paper. The filtrate so obtained was placed over rotary evaporator to facilitate evaporation of the solvent from the filtrate to obtain a dried extract. The extracts were serially diluted using distilled water to obtain 1 mg/ml, 2.5 mg/ml, 5 mg/ml, 7.5% mg/ml, 10 mg/ml solutions in sterile test tubes.

Antibacterial activity testing:

Collection of micro-organisms

Pure strains of *Streptococcus mutans* (ATCC No.497) and *Lactobacillus acidophilus* (ATCC No.10307) were obtained from microbial type culture collection and gene bank (MTCC) Chandigarh.

Preparation of media and culture of organism

Nutrient broth was used to culture the organism. The organisms were inoculated (using inoculating loop) on the sterilized media and incubated at 37°C for 24hrs.

Testing for zone of inhibition

Zone of Inhibition Test, also called a Kirby-Bauer Test used clinically to measure the ability of a material/antibiotic to inhibit microbial growth. Once the culture of *S. mutans* and *Lactobacillus acidophilus* was obtained. The organisms were isolated and inoculated on the selective media using swabbing method (**brain heart infusion agar** and **MRS agar** for *S. mutans* and *Lactobacillus acidophilus* respectively were used). The agar well diffusion method was employed for testing the antibacterial activity of the bitter gourd extract. The plates were examined for clear zones of inhibition (presence of zone of inhibition indicated activity) around the wells which were there after measured in

mm using vernier caliper. Figure 1 shows the zone of inhibition obtained during the study.

RESULTS

Table 1: Showed there was no zone of inhibition seen at 0.5mg/ml concentration of Bitter gourd on *S.mutans*. With 1mg/ml concentration of Bitter gourd, Chloroform showed greater zone of inhibition (i.e. 14mm) when compared with ethanol and petroleum ether (i.e. 12mm each). There was a higher effect noticed with chloroform extract (16mm) when compared to Ethanol(14mm) and Petroleum ether (14mm) at 7.5mg/ml and 10mg/ml.

Table. 2: Bitter gourd showed no zone of inhibition with different solvents at 1mg/ml concentration on *lactobacillus acidophilus*. Bitter gourd extract using chloroform, ethanol, petroleum ether had an antibacterial effect of 12, 12, 11mm respectively at 5mg/ml concentration. As there was increase in concentration, the zone of inhibition was also increased. There was a high zone of inhibition noticed at concentration of 10mg/ml using various solvents.(petroleum ether 16mm, ethanol of 14mm, Chloroform 12mm).

DISCUSSION

Natural products have recently been demonstrated as an alternative to synthetic substances for prevention of tooth decay. Factors associated with cariogenicity include adhesion, acidogenicity and acid tolerance. Acidogenic bacteria such as *S. mutans* and *Lactobacillus* species produce metabolic products such as lactic acid and acetic acid which lower plaque pH below 5.5 and the critical pH for enamel demineralization, leading to the initiation and development of caries⁷. In the current study

we aimed to assess the effect of bitter gourd extract (using petroleum ether, ethanol, and chloroform) on *S.mutans* and *L.acidophilus*. Bitter Gourd (*Momordica charantia*) is tropical and subtropical climber of the family Cucurbitaceae. It is widely distributed in China, Malaysia, India and tropical Africa. The Latin name *Momordica* means “to bite” (referring to the jagged edges of the leaf, which appear as if they have been bitten). All parts of the plant, including the fruit taste very bitter, as it contains a bitter compound called momordicin.⁸ Bitter gourd is a potential herbal plant which is used as vegetable and medicine. It is a good source of various medically important biochemical's like, triterpene, protein, steroid, alkaloid, and phenolic which are responsible for its biological and pharmacological activities including anti-diabetic, antioxidant, anti-cancerous and anti-tumorous, antimicrobial, anti-fertility, anti-viral, anti-helminthic, antimalarial, anti-ulcerative and immunomodulatory etc,⁹ thus in the present study we decided to exploit its effect on oral micro-organism to know if it can be used as a major agent in common risk approach.

The results of the present study showed that, bitter gourd extract using ethanol, chloroform and petroleum ether has an antibacterial effect against *S.mutans* and *L.acidiphillus*. the antibacterial effect can be attributed to its high trans-nerolidol.^{10,11} The result could not be compared with any other study as this is the first of its kind. Leaf extracts of *M. charantia* showed broad spectrum antimicrobial activity since various water, ethanol and methanol extracts of the leaves have exhibited antibacterial activities against *E. coli*, *Staphylococcus*, *Pseudomonas*, *Salmonella*, *Strep-*

tobacillus.¹² Besides, extract of the entire plant has shown antiprotozoal activity against *Entamoeba histolytica* and its fruit extract has demonstrated antibacterial properties against *Helicobacter pylori*, the bacteria causing stomach ulcer.¹³

CONCLUSION

Bitter gourd is used invariably in the Indian homes. It has proved to be effective against *S.mutans* and *L. acidophilus* which are responsible for initiation and progression of dental caries respectively. These plant /herbal product will serve as an efficient, economic, accessible and acceptable measure especially among the rural people which constitutes 72% of Indian population. Since they are also very beneficial for the general wellbeing of an individual, hence their use can be promoted as common approach for dual purpose (systemic and oral health).

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Table: 1 Antibacterial effect of bitter gourd on *S.mutans* using various solvents:

Concentration	Zone of inhibition with chloroform	Zone of inhibition with ethanol	Zone of inhibition with petroleum ether
0.5mg/ml	R	R	R
0.75mg/ml	R	R	12mm
1mg/ml	14mm	12mm	12mm
2.5mg/ml	14mm	12mm	12mm
5mg/ml	14mm	14mm	14mm
7.5mg/ml	16mm	14mm	14mm
10mg/ml	16mm	14mm	14mm

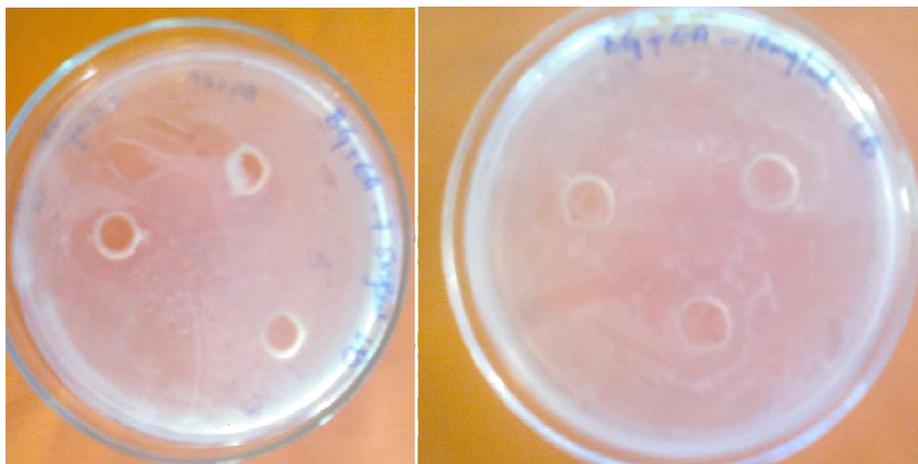
R-resistance

Table 2: Antibacterial effect of bitter gourd on *L.acidophilus* using various solvents:

R-resistance

Concentration	Zone of inhibition with chloroform	Zone of inhibition with ethanol	Zone of inhibition with petroleum ether
1mg/ml	R	R	R
2.5mg/ml	11mm	R	11mm
5mg/ml	12mm	12mm	11mm
7.5mg/ml	12mm	12mm	14mm
10mg/ml	12mm	14mm	16mm

Figure 1: Zone of Inhibition obtained by bittergourd against *S.mutans* and *Lactobacillus acidophilus*



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