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Antibacterial Activity of Bohera (*Terminaliabellirica*) Extract against Dental Carries Causing Bacteria *Streptococcus mutans*

M. R. B. Mizan[1], Kamrunnahar[2] and M. A. K. Azad[3*]

[1]Institute of Biological Science, University of Rajshahi, Rajshahi
[2]Department of Oral Anatomy and Physiology, Rangpur Dental College, Rangpur
[3]Institute of Environmental Science, University of Rajshahi, Rajshahi
Corresponding author: akazad_ies@yahoo.com

Abstract

Dental caries is very common to people of Bangladesh. The treatment of dental carries is very expensive so alternative low-cost option from plant products are important for the rural people. Bohera (*Terminaliabellirica*) is known to people for its medicinal values. The antibacterial activity of methanolic extracts of bohera bark and fruits were tested against dental caries causing bacteria *Streptococcus mutans*. It was found that crude extracts of both bark and fruits of bohera have the antibacterial activity against *Streptococcus mutans*. The crude extract was purified with sillica gel (230-300mesh)with gradient elution of methanol, ethanol and chloroform. The purified extract of bohera also showed strong antibacterial activity against dental caries causing bacteria *Streptococcus mutans*. The rural people of Bangladesh may use the barks and fruits of bohera to control the dental caries problem locally.

Key words: Antibacterial, Bohera, Dental caries, Methanolic extract

Introduction

Dental caries are one of major oral health problem for the people of all ages over the world. According to World Health Organization (WHO) report, dental caries are declining in many industrialized world but it is still an important public health concern to developing countries. The treatment of dental carries is very expensive for the developing countries like Bangladesh. On the other hand, the increasing resistance of pathogenic bacteria has necessitated the need for a global alternative prevention treatment options and products for oral diseases that are safe, effective and affordable (Tichy and Novak, 1998).

Streptococcus mutans belongs to the viridans group of streptococci, and is part of the normal oral flora of man, and is an etiological agent in smooth-surface dental caries (Hamada *et al*., 1980). Streptococci of the mutans group are highly acidogenic; they produce short-chain carboxylic acids which dissolve hard tissues such as dentine and enamel, and are the most carcinogenic pathogens (Shaw, 1987). In addition, they produce insoluble extracellular polysaccharides, which improve their adherence to the tooth surface and encourage biofilm formation by fermenting sucrose (Shen *et al*., 2004).

In drug discovery, most studies have examined on the antimicrobial potential of medicinal plants and other natural products measured as either killing or inhibiting the microbial growth. Natural products including medicinal plants are still major sources of innovative therapeutic agents for the various conditions of human diseases. The populations in rural developing countries rely heavily on traditional healers and medicinal plants as a basis to treat various maladies. The world health organization reported that 80% of the world populations rely mainly on traditional medicine. Herbal medicine of natives in every country forms a major part of the world heritage of the plant material medical (Al-Hussainiand Mahasneh, 2011).

Medicinal plants have been documented for prevention and cure of many systemic diseases since ancient times. With advancements in science and scientific procedures it is now known that plants have potential curative action for oral diseases such as dental caries (Kabra *et al*., 2011). The main objectives of this study were to evaluate the antibacterial activity of bohera (barks and fruits) extracts against dental caries causing bacteria *Streptococcus mutans*.

Materials and Methods

Clinical sample collection

Clinical sample for bacteria (*Streptococcus mutans*) was collected from the Outdoor Patients of Rajshahi Medical College Hospital, Rajshahi during February, 2017. The dental plaque samples (10 nos.) were obtained by swabbing all surfaces of the teeth of a subject, using sterile cotton tipped swabs (Wan *et al*., 2002). The samples were placed into in sterile tubes containing 2 ml

normal saline which was sealed tightly labeled and transported immediately to the laboratory. The isolation and identification of bacteria was performed in Environmental Microbiology Lab of the Institute of Environmental Science, University of Rajshahi.

Isolation and identification of Streptococcus mutans

The isolation and identification of *Streptococcus mutans* bacteria was done on Mitis ï Salivarius (MS) agar medium enriched with 5% sheep blood. The composition of MS agar medium is given in Table 1 (Wan *et al*., 2002).

Table 1. Composition of Mitis -Salivarius(MS) agar medium

Ingredients	Amount
Mitissalivariusagar	90 gm
Potassium tellurite	10 gm
Sucrose	200 gm
Bacitracin	0.2 U
Distilled water	1000 ml

One hundred micro liter of undiluted sample were spread on the surface of MS-agar (Mitis -Salivarius agar) plates using sterile swabs. Cultures were incubated an anaerobically for 48hrs at 37 and count more than 250 colonies (104 cells/ml) was considered as positive samples. Samples were considered to be positive bacterial isolates about using selective MS agar (Mitis -Salivarius agar) medium. Mitis -Salivarius medium is usually used which is composed of Mitis -Salivarius agar with sucrose and potassium tellurite. It has the ability to inhibit growth of most bacteria, except streptococci, because it contains trypan blue and crystal violet which suppress the growth of gram negative organism. After examination of positive samples on the surface of MS-agar medium, small colonies were sub-cultured on the surface of blood agar (5%) plates for further purification and incubated an anaerobically for two days at 37 . After preparing 90 mm petridis plate of blood agar base added different antibiotic (azithromycin, amoxicillin, doxycycline, cefradine, tetracycline, levofloxacin) disk and incubated for 24hrs at 37 . Bigger the clear zone of antibiotic disc means higher antibiotic sensitivity to *Streptococcus mutans*. Result showed that azithromycin (clear zone 30mm) is sensitive to *Streptococcus mutans*. Finally, the bacterial identification was done by various standard techniques such as shape and size, Gram staining, hemolytic pattern, sugar fermentation test, bacitracin sensitivity test and catalase test (Beighton *et al*., 1991).

Plant sample collection and crude extract preparation

The barks and fruits of medicinal plant bohera (*Terminalia belerica)* were collected from the Botanical Garden of University of Rajshahi. The plant was authenticated by the Botanist of the Department of Botany of University of Rajshahi. The plant parts (bark and fruit) were washed first with tap water and later with distilled water. Then it was air-dried for 7-10 days. The dried plant materials were grounded to powder by grinding machine. About 100ml methanol was added to 25 gm of powder and shaking for 2-days in shaker machine. After shaking the extract was filtered and the filtrate was evaporated to dryness by Rotary Evaporator at 55 using vacuum pressure.

Purification of crude extract

The crude extract of bohera fruits was purified by sillica gel (230-300mesh particle) column using a gradient a solvent system of methanol, ethanol and chloroform. Three solvent systems were used, methanol:chloroform (6:4), methanol:chloroform (9:1) and methanol:ethanol (5:5).

Antibacterial activity test

Antibacterial activity of the extracts was determined by the agar disk diffusion method. *Streptococcus mutans*bacteria were grown on blood agar base medium anaerobically at 37 for 24 hrs. Several colonies of culture bacteria were transferred into blood agar base medium and the density was adjusted to FC Farland standard 0.5 or approximately equivalent to 108 CFU/ml. The density-adjusted bacteria were swabbed on blood agar base medium. After preparing the different concentrations of different extracts each sterile paper disc was impregnated with 10 µldiluted extracts. The disk was allowed to dry. Using a sterile forceps, the disks were placed on the inoculated blood agar medium. One paper disk on each plate was soaked in methanol as a negative control. One antibiotic (azithromycin) disk was placed on each plate for compare the clear zone of selected extract and antibiotic. Then the plates were incubated in an appropriate atmosphere at 37 for 24 hrs and the diameter of the inhibition zone was measured. Each test was done in triplicate and the average was recorded.

Results and Discussion

Plants and human are inseparable. The use of plants to alleviate human suffering is as old as the evolution of human civilization itself. From the early stages of human civilization, plants, especially medicinal plants have played a pioneering role for the welfare of human beings. Recently, dramatic changes have taken place in the primary health care system through the development of science, technology and medical science, but till today many peoples of the world are totally dependent on herbal medicine (Chitme *et al.*, 2003). It is revealed that even in the developed countries 25%, of the prescribed drugs come from plant sources, and herbal medicines are used by about 75-80% of the worldôs population for primary health care because of their better cultural acceptability, better compatibility with human body and lesser side effects (Sofowora,1982).

In Bangladesh, bohera is recognized as a medicinal plant for its disease healing properties. Therefore, systematic and scientific study of bohera barks and fruits on dental caries causing bacteria are important to justify the medicinal value of bohera plant. From this study, it was found that methanolic extracts of bohera barks and fruits have strong antibacterial activity against dental caries causing bacteria *Streptococcus mutans*, zone of inhibition 12.5 mm for fruits and 10.5 mm for barks (Table 2). Tazeena *et al.* (2012) tested the antimicrobial activity of ethanolic extracts of leaf and bark of *Azadirachta indica*, bark of *Vitex negundo*, leaves of *Spinacia oleracea*, fruits of *Momordica charantia*, *Phyllanthus embilica*, *Piper nigrum*, and *Tamarindus indica*, rhizome of *Curcuma longa* and *Zingiber officinale* against *Streptococcus mutans* and found considerable zone of inhibition for three extracts of *Curcuma longa*, *Tamarindus indica* and *Phyllanthus embilica*.

Table 2. Anti-bacterial activity of crude bohera (*Terminalia bellirica*) extract against *Streptococcus mutans*

Name of plants	Scientific name	Concentration of plantextracts/disk (µg)	Zone of inhibition (mm)		
			Extract (10 µl)	Antibiotic 15 µg	Negative control (10 µl methanol)
Bohera fruits	*Terminalia bellirica*	2000	12.5	30	0
Bohera Bark	*Terminalia bellirica*	2000	10.5	29	0

After preliminary findings of antibacterial activities of high dose (2000 µg/disk) of crude extract of bohera, a dose-dependent (250 µg ï 2000 µg) activity test was carried out to find out the lowest concentration of crude extract against *Streptococcus mutans*. Antibacterial activity was found to decrease with decreasing of extract concentration (Table 3). Prashanth *et al.* (2007) reported dose dependent zone of inhibition for *Mangifera indica* against *Streptococcus mutans* at 10% and 50% concentrations were 1.5 mm and 2.9 mm, respectively.

Table 3. Dose-effect of crude bohera (*Terminalia bellirica*) extract against *Streptococcus mutans*

Name of plants	Scientific name	Concentration of plant extracts/disk & zone of inhibition				Antibiotic 15 µg	Negative control (methanol) 10µl
		2000 µg	1000 µg	500 µg	250 µg		
Bohera (fruit)	*Terminalia bellirica*	22mm	20mm	19 mm	15mm	30mm	0mm
Bohera (bark)	*Terminalia bellirika*	21 mm	19 mm	15mm	11mm	29.5 mm	0mm

Bohera fruits extract showed higher antibacterial activity against *Streptococcus mutans* than barks. Therefore, crude extract of bohera fruits (without seeds) was purified by sillica gel (230-300 mesh particle) column using a gradient a solvent system of methanol, ethanol and chloroform. Out of three solvent systems, methanol: chloroform (9:1) was found suitable for the purification of bioactive compounds from the crude extract of bohera fruits. The collected fraction in T4 and T5 tubes of this solvent system showed strong antibacterial activity against *Streptococcus mutans* with zone of inhibition 20-22 mm (Table 4).

Table 4. Anti-bacterial activity of column purified extract of bohera (*Terminalia bellirica*) fruits (without seeds) against *Streptococcus mutans*

Solvent	Ratio	Test tube nos.	Extract solvent ml	Weight of extract, mg	Concentration/disk µg	Zone of inhibition (mm)	Control (methanol)
Methanol:Chloroform	6:4	T1	10	257.0	250	0	0
		T2	10	150.0	250	0	0
		T3	10	579.0	250	0	0
Methanol:Chloroform	9:1	T4	10	452.0	250	20	0
		T5	10	789.0	250	22	0
		T6	10	246.0	250	0	0
Methanol:Ethanol	5:5	T7	10	116.0	250	0	0
		T8	10	117.0	250	0	0
		T9	10	209.0	250	0	0

Conclusions

The extracts of bohera (*Terminalia bellirica)* showed strong anti-bacterial activity against dental caries causing bacteria *Streptococcus mutans*. Therefore, the peoples of Bangladesh are suggested to use the barks and fruits of bohera to control the dental caries problem locally.

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