

Original Research Article

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Antimicrobial Activity of Ethanolic and Methanolic Extract of *Artocarpus lakoocha* Wall. Ex Roxb. (Moraceae) against Five Different Oral Bacterial Strains

P.C. Nath* and Sarmila Boruah

Institutional Level Biotech Hub, Sibsagar College Joysagar, Sivasagar, Assam 785665, India

*Corresponding author

ABSTRACT

Keywords

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Antimicrobial activity of ethanolic and methanolic extract of *Artocarpus lakoocha* Wall. ex Roxb. bark were evaluated against five oral inhabiting bacteria i.e., *Escherichia coli* (ATCC 8739), *Streptococcus mutans* (ATCC 25175), *Streptococcus sanguinis* (ATCC 10556), *Porphyromonas gingivalis* (ATCC 33277) and *Fusobacterium nucleatum* (ATCC 25586) at different concentration by using agar well diffusion method. The test extract was found to be bacteriostatic in action, thus can be used as a source of antibiotic substances for drug development and in the control of these bacterial infections.

Introduction

Artocarpus lakoocha Wall. ex Roxb. (Moraceae) is a tropical deciduous plant common in Northeastern India as well as in all South East Asian nations. The plant is generally used for its durable wood for furniture and house building. The seeds are edible by roasting and the leaves are good fodder for cattle. Besides, the tender bark of the tree is used by several communities for masticatory purpose together with betel nut (Borthakur, 1981). The seeds and bark of the

plant is used to treat stomach and liver diseases. The bark is also used as astringent that shrinks or constricts body tissues (Perry, 1980). The fruits are the source of vitamins and antioxidants like vitamin C and beta-carotene. In addition to these lots of minerals such as zinc, copper, manganese and iron are present which acts as antioxidant (Jahan *et al.*, 2011). World Health Organization has reported that about 80% of the world depends on traditional herbal medicine for their primary health care (Vijayan *et al.*, 2007). The different parts and chemical constituents of

this plant can be the possible starting points for new drugs (Tijani *et al.*, 2008).

A number of studies show that using up of different tropical fruit species had helped to curb the danger of no-communicable diseases like diabetes, cancer, coronary heart disease, neurodegenerative ailment (Rajurkar *et al.*, 2012). The plant parts of *A. lakoocha* have had within flavonoids and phenolic acids, these are strong antioxidants.

There are some pharmacological activities discovered in this plant that are anti-inflammatory, antiviral, anticancer and anti-HIV properties (Luthfun Nesa, 2015). The fruit pulp of *A. lakoocha* boosts the liver functions and the seeds and the latex can utilized as laxative (Kumar, 2010). In several studies it revealed that *A. lakoocha* has many medicinal uses (Hossain *et al.*, 2016). The hydroglycolic concentrate of *A. lakoocha* heartwood showed as important antioxidant which are ordinarily utilized as antioxidant and skin whitening operators in cosmetic products (Teapaisan *et al.*, 2014). Heart wood extract of *A. lakoocha* yields a yellow colour dye (Kar *et al.*, 2008).

Oral ailments, including dental caries and periodontal sicknesses, are normally caused by an extensive variety of microorganisms related with oral biofilm or dental plaque (Socransky *et al.*, 2002, Marsh, 2010). *A. lakoocha* extract might be a valuable antimicrobial solution for endodontic treatment, because of its capacity to restrain development of *Enterococcus faecalis* (Teapaisan *et al.*, 2013).

Basic literature survey on the antibacterial, antioxidant, anthelmintic and insecticidal movement of heart wood of *A. lakoocha* provides a least information on the medicinal properties of the plant. In this manner, the present examination has been conveyed to research antibacterial movement of bark extract of *A. lakoocha*.

Materials and Methods

Experimental procedure

Plant extract preparation

The plant for the present study was collected from Kakopothar village of Tinsukia district, Assam, India that was growing adjacent to a forest area. The bark of a medium size tree of ca 1.2m girth was extracted with the help of a sharp knife and cut into small pieces and allowed to shade dried for 15 days. The dried sample was then finely grounded into powder for extraction. The extraction of the sample was done using ethanol and methanol as solvent. 100gm of the sample was taken in a beaker in 300ml of above mentioned solvents. Extraction was done for 24 hours and then filtered using Whatman filter paper No 1. Crude extract obtained was kept at 4°C until further work was carried out.

Microorganism used for Antimicrobial Assay

The bacterial strains studied are *Escherichia coli* (ATCC 8739), *Streptococcus mutans* (ATCC 25175), *Streptococcus sanguinis* (ATCC 10556), *Porphyromonas gingivalis* (ATCC 33277), *Fusobacterium nucleatum* (ATCC 25586).

Antimicrobial media preparation

15g of Nutrient Agar was added to 500ml of distilled water and autoclaved at 121°C for 15 minutes at 15lbs. After sterilization the content was poured into sterile petriplates and allowed to set at ambient temperature and used for further study.

Antimicrobial agar well diffusion preparation

About 15-20 ml of nutrient agar was poured on sterile petriplates and allowed to solidify.

Agar surface of each plate was streaked with the 100µl of reference bacterial strain. Punch the wells with A sterilized improvised cork borer of 6 mm in diameter was used to bore holes on the already prepared plates, the plates were bored to accommodate 6 holes of 6 mm diameter each which has to impregnated with 0.5 ml of the 4 different concentrations of each sample was prepared in 10% DMSO [2mg/ml, 4mg/ml, 6mg/ml] (for ethanol and methanol extract) was loaded into wells at equal distance with control on each plates. As positive control streptomycin (antibiotic) is used. The plates were allowed to standby for 30 minutes and seal with paraffin. Then the plates were incubated at 37°C for 24 hours.

Results and Discussion

A. lakoocha extract was evaluated for antimicrobial potential against oral pathogens by an agar diffusion assay. The antimicrobial activity of ethanol and methanol extract of *A. lakoocha* is shown in Table 1 and 2 respectively. Results were recorded as formation of zone of inhibition around the

well. Both extract (methanol and ethanol) were found to be effective against all five oral bacteria in case of all concentration of extract (2mg/ml, 4mg/ml, 6mg/ml). The reasons for this could be that the components from the plant active against microorganisms are most often obtained through solvent extraction In case of ethanolic extract concentration of 6mg/ml shows highest antimicrobial activity against *Fusobacterium nucleatum* (27mm). But methanolic extract shows highest antimicrobial activity with both bacteria *i.e* *Streptococcus sanguinis* (25.4mm) and *Fusobacterium nucleatum* (25.8mm) at concentration of 6mg/ml. Standard antibiotic caused more inhibition of test bacteria than methanolic and ethanolic extract.

The study of various antimicrobial properties of plants has been of great interest in the last few decades. Some of the infectious diseases are known to have been treated with antimicrobial compounds which are derived from various masticatory plants (Kongkona Borborah *et al.*, 2014).

Table.1 Antimicrobial activity shown by bark extract of *Artocarpus lakoocha* (ethanolic) against different bacterial stains

Concentration level	(2 mg/ml)	(4 mg/ml)	(6 mg/ml)	Streptomycin (control)
Bacterial stains	Diameter of zone of inhibition (in mm)			2mg/ml
<i>Eschericia coli</i>	17.67	19.67	22.17	29.60
	±1.11	±0.75	±0.97	±0.97
<i>Streptococcus mutans</i>	20	21.6	23.4	30.2
	±1.29	±0.66	±0.88	±0.48
<i>Streptococcus sanguinis</i>	21.6	23.2	25.4	29.4
	±1.33	±1.11	±1.20	±0.88
<i>Fusobacterium nucleatum</i>	22.4	25.2	27	30.2
	±0.66	±0.75	±0.82	±0.86
<i>Porphyromonas gingivalis</i>	19.2	22.8	25.2	31.8
	±0.75	±0.48	±0.75	±0.86

± SE, n=5

Table.2 Antimicrobial activity shown by bark extract of *Artocarpus lakoocha* (methanolic) against different bacterial stains

Concentration level	(2 mg/ml)	(4 mg/ml)	(6 mg/ml)	Streptomycin (control)
Bacterial stains	Diameter of zone of inhibition (in mm)			2mg/ml
<i>Eschericia coli</i>	18.33	20.67	23.33	29.60
	±0.97	±1.22	±0.75	±2.11
<i>Streptococcus mutans</i>	20.4	22.4	23.6	29.2
	±1.29	±1.05	±1.20	±0.75
<i>Streptococcus sanguinis</i>	21.6	23.2	25.4	29.4
	±1.33	±1.11	±1.20	±0.88
<i>Fusobacterium nucleatum</i>	23	24.8	25.8	29.4
	±0.91	±0.75	±1.11	±0.66
<i>Porphyromonas gingivalis</i>	19.4	22	24.2	31.4
	±0.66	±0.91	±0.75	±0.97

± SE, n=5

Results of our study clarified that *A. lakoocha* extract revealed good antibacterial agent activity against all five oral pathogens. (e.g. *S. mutans*, *S. sanguinis*, *P. gingivalis*, *F. nucleatum*, *E. coli*). Our results are in agreement with Teanpaisan *et al* (2010) who demonstrated the In-vitro antimicrobial activity of *A. lakoocha* extract against some gram positive and gram negative bacteria. However, Gram negative bacteria were found to be more susceptible than Gram positive bacteria to *A. lakoocha* extract in that study (Teanpaisan *et al.*, 2014). In our study, methanolic extract of *A. lakoocha* is more sensitive than ethanolic extract. Because methanolic extract have phyto-constituents such as tannins and alkaloids (Kumar, 2010).

In conclusion, this investigation recommends that both ethanolic and methanolic extract of *A. lakoocha* extricate compounds with potential antimicrobial properties that might be valuable for treatment of oral irresistible sickness caused by certain oral pathogens. In our study highest antibacterial activity shows against *Fusobacterium nucleatum*. Our results indicate that *A. lakoocha* extract acts as a potent antibiotic agent that has potential to prevent oral infection caused by some bacterial pathogens. The demonstration of activity of the both extract against bacteria is an indication of the

broad spectrum of antimicrobial activity and thus it can be used as source of antibiotic substances for drug development.

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