

# Antibacterial Activity Of Amchur Extracts On Some Indigenous Oral Microbiota Causing Dental Caries

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## Abstract

The antibacterial activity of amchur (dried pulp of unripe *Mangifera indica*) extract (50% ethanol) was tested against ten bacterial strains causing dental plaque by agar well diffusion method. The crude extract showed a broad spectrum of antibacterial activity inhibiting both the groups of Gram-positive & Gram-negative bacteria. The extract was most effective against *Bacillus* sp., followed by *Staphylococcus mutans* and *Pseudomonas* sp., whereas *Halobacterium* sp. was found to be the most resistant. Chlorhexidine (present in mouthwashes to prevent infection of dental caries) was used as a positive control. Natural extract of amchur was found to be more effective as compared to chlorhexidine. This study shows the potential of amchur in the treatment of dental caries.

## INTRODUCTION

Dental caries is the localized destruction of the tissues of the tooth by acid produced from the bacterial degradation of fermentable sugars. Gnotobiotic animal studies showed that caries could be induced by specific bacteria, especially members of the mutans Streptococci- group (e.g. *Streptococcus mutans* & *Streptococcus sobrinus*), but only when fed a cariogenic (high sucrose) diet. These studies also showed the potential for transmission from animal to animal, & that protection could be achieved by antimicrobial agents & vaccination. Advanced lesions often have a high proportion of lactobacilli, while dentinal lesions have a diverse microflora with many fastidious Gram positive (*Actinomyces naeslundii*, *A. odontolyticus*, *Propionibacterium* spp., *Eubacterium* spp.) and Gram negative (*Fusobacterium* spp., *Capnocytophage* spp., *Veillonella* spp.) bacteria.

Many natural substances of plant origin are reported to be biologically active, endowed with antimicrobial, allelopathic and antioxidant properties (Beuchat and Golden, 1989).

Amchur (dried pulp of unripe *Mangifera indica*) is used in Indian spices as a souring agent to provide the desired acidity in the various food recipes. Very limited literature is available on the antimicrobial activity of amchur extract.

Mango is considered as a king of fruits in Indian delicacy. The roots and bark of mango *Mangifera indica*

(Anacardiaceae) are astringent, acrid, anti-inflammatory, and constipating. The leaves and flowers are refrigerant, styptic, vulnerary and constipating. Amchur (dried or dehydrated product of unripe mango flesh in the form of peeled slices or powder) is used as an acidulant or a souring agent for curries. Amchur is rich in citric acid.

Very limited literature is available on the antimicrobial activity of amchur extract. In the present study, we have investigated the antibacterial activity of dried pulp of unripe *Mangifera indica* against dental caries for the first time.

## MATERIALS AND METHODS

**Materials:** All chemicals used were of analytical-reagent grade and obtained from E. Merck (Mumbai, India). Amchur (*Mangifera indica*) was collected from local market of Meerut (Uttar Pradesh, India). Dr. C.M Govil, Professor, Botany Department, C.C.S University, Meerut, India confirmed the species.

**Bacterial Strains:** Ten bacterial strains (6 Gram positive and 4 Gram negative), involved in dental caries, were selected for the study. Gram positives were *Streptococcus mutans*, *Streptococcus salivarius*, *Lactobacillus* sp., *Bacillus* sp., *Micrococcus* sp., *Staphylococcus aureus*, *Halobacterium* sp., *Veillonella* sp., *Pseudomonas aeruginosa*, *Pseudomonas* sp. The bacterial stock cultures were obtained from the culture collection unit of Department of Microbiology, C.C.S University, Meerut, India. The viability tests for each isolate

were carried out by resuscitating the organism in nutrient agar medium.

The stock on nutrient agar medium (Hi Media, Mumbai, India) was incubated for 24h at 37°C following refrigeration storage at 4°C until required for sensitivity testing.

**Extraction:** The pulp of unripe mango (*Mangifera indica*) was dried and powdered in milling machine (Inalsa Mixer Grinder) to obtain fine dry powder called amchur. The powder was weighed using single pan electronic weighing balance (Ohaus model). The herbal extract was prepared at the rate of 1g/5ml of solvent (50% ethanol) in a 250mL Erlenmeyer flasks. The flasks were closed with cotton plug and aluminium foil. The spice powder was soaked in 50% ethanol for 48h at room temperature with intermittent shaking. The mixture was centrifuged at 3500xg for 20min and finally filtered through Whatmann filter paper No.1 (Azoro, 2000). The pellet was discarded and the supernatant was collected and concentrated under reduced pressure in a rotary vacuum evaporator (Buchi Type) until semisolid substance was obtained. This was dried inside the crucible under a controlled temperature (45°C) to obtain solid powder (Jonathan and Fasidi, 2003). The process of extraction was repeated until the weight of 500mg was obtained.

The powder was weighed and reconstituted in dimethyl sulfoxide (DMSO). These were stored in the refrigerator at 4°C for testing antimicrobial sensitivity. The extract was exposed to UV rays for 24h and checked for sterility by streaking on NAM.

**Antibacterial assay:** The antimicrobial activity of amchur extract was determined by agar well diffusion method against different bacteria as described by Okeke et al., 2001. In this method, pure isolate of each bacterium was sub-cultured in nutrient broth at 37°C for 24h. One hundred microlitres (about 106CFU/mL, standardized by 0.5 Mac-Farland) of each test bacterium was spread with the help of sterile spreader on to a sterile Muller-Hinton Agar plate (Hi Media, Mumbai, India) so as to achieve a confluent growth. The plates were allowed to dry and a sterile cork borer of diameter 6.0mm was used to bore wells in the agar plates. Subsequently, a 50µL volume of the extract was introduced in triplicate wells into Muller-Hinton Agar plate. Sterile DMSO served as negative control. Chlorhexidine (standard chemotherapeutic agent in mouth washes) was also used as positive control. The plates were allowed to stand for 1h or more for diffusion to take place and then incubated at 37°C for 24h. The zone of inhibition was recorded to the nearest

size in mm (Norrel and Messely, 1997).

## RESULTS AND DISCUSSION

Following the extraction of the dried unripe pulp of *Mangifera indica* (Amchur) using 50% ethanol by maceration method, the antimicrobial activity of the extract was determined by agar well diffusion method. Table 1 shows the antimicrobial activity of the amchur extract on the indigenous oral microbiota that cause dental caries. The extract was effective against both Gram positive and Gram negative bacteria. However the ethanolic extract was most effective against *Bacillus* sp. with diameter of zone of inhibition 19.0mm followed by *Streptococcus mutans* (main causative organism of dental caries). Chlorhexidine, on the other hand was less effective producing an inhibition zone of diameter 14mm. Amongst the Gram negative bacteria, the extract showed highest activity against *Pseudomonas* sp. with diameter of zone of inhibition 18.0mm and was least effective against *Halobacterium* sp. with diameter of zone of inhibition 10.0mm.

**Figure 1**

Table1: Zone of inhibition (mm) of ethanolic extract of amchur () on selected bacteria that cause dental plaque

S.No.	Bacterium	Amchur extract	Positive control	Negative Control
1.	<i>Bacillus</i> sp.	19.0	15.0	0.0
2.	<i>Halobacterium</i> sp.	10.0	9.0	0.0
3.	<i>Lactobacillus</i> sp.	11.0	11.0	0.0
4.	<i>Micrococcus</i> sp.	12.0	10.0	0.0
5.	<i>Pseudomonas aeruginosa</i>	11.0	10.0	0.0
6.	<i>Pseudomonas</i> sp.	18.0	12.0	0.0
7.	<i>Staphylococcus aureus</i>	12.0	11.0	0.0
8.	<i>Streptococcus mutans</i>	18.0	14.0	0.0
9.	<i>Streptococcus salivarius</i>	14.0	14.0	0.0
10.	<i>Veillonella</i> sp.	16.0	15.0	0.0

Incubation temperature: 37°C; Incubation period: 24h

Negative control- Dimethyl sulfoxide

Positive control- Chlorhexidine

Volume of extract in each well = 50µL

From this investigation, it was observed that amchur extract was more effective than chlorhexidine against both groups of bacteria. It may possibly be due to the change of pH of the

medium due to amchur which cause the pH to bring down in acidic range. pH is known to control the growth, development and sporulation of all microbes including bacteria.

Amchur contains citric acid related compounds which is responsible for its sour taste. Several terpenes (ocimene, myrcene, limonene), aldehydes and esters have been found in dried unripe mango fruit. They also contain proteolytic enzymes (Gernot Katzer's Spice Pages- An encyclopedia of Spices).

Investigations into the effects of terpenoids upon isolated bacterial membranes have suggested that their activity is a function of the lipophilic properties of the constituent terpenes, the potency of their functional groups and their aqueous solubility (Knobloch et al., 1989, Elgayyar et al., 2001). Their site of action is at the phospholipid bilayer, & the biochemical mechanisms include the inhibition of electron transport, protein translocation, phosphorylation steps and other enzyme – dependent reactions (Knobloch et al., 1989). These activities suggest their potential use as food preserving agents, chemotherapeutic agents and disinfectants.

**CONCLUSION:** In conclusion, amchur extract can be used as an inexpensive source for the treatment of dental caries caused by the bacteria. Further research on the use of other botanical extracts can be rewarding to pursue in hunt for new

herbal therapeutic agent.

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