



EFFECT OF LEAVES EXTRACT OF INDIGENOUS SPECIES OF *SYZYGium CUMINI* ON DENTAL CARIES CAUSING PATHOGENS

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ABSTRACT

Dental caries is a common disease that results in tooth loss or cavitations. Medicines available in market though effectively treat dental caries but not without imparting the harmful effects. Medicinal plants have proven to be an effective and safe alternative to synthetic medicines. In vitro antibacterial activity of leaves of *Syzygium cumini* was studied against *Streptococcus viridans*, *Streptococcus mutans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*. The aqueous, methanolic, hexane and ethyl acetate extract of leaves of *S. cumini* exhibited the antimicrobial activity against dental caries causing strains. The largest zone of inhibition was obtained with the methanolic extract against *E. coli* (20mm). Minimum Inhibitory Concentration (MIC) of extract was also determined against the selected microorganisms showing zone of inhibition \geq 8mm. The study concluded that the leaves of *S. cumini* possessed very good antibacterial activity against dental caries causing microorganisms and can be used as a potential source for making a phytomedicine that can be used to cure dental caries.

KEYWORDS: Dental caries, antibacterial, methanol, hexane, ethyl acetate extract



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INTRODUCTION

Various bacteria and fungi produce diseases that are manifested in or on the oral cavity, out of which some are produced by a specific contribution to the problem of caries¹. Highest susceptibility for caries lie in the age group of 20-40 years and among them female is more susceptible than males². Dental caries, a multifactor etiological chronic disease caused by acid produced as a by product of carbohydrate metabolism by *Streptococcus mutans*, characterized by destruction of superficial dental structures³. Teeth affected with dental caries is a source of infection and if left untreated will gradually lead to tooth loss, which in turn causes difficulties in chewing and aesthetic problems⁴. It is one of the wide spread diseases of mankind and is often epidemic among poor in developing countries⁵. Since ancient times, medicinal plants are used for prophylactic and curative purposes^{6,7} and development of resistant bacterial strains against present antibiotics necessitated the need to search for new antibacterial agents⁸. According to WHO, 2001 herbal medicines serve the health need of almost 80% of world's population specially the millions of people living in rural areas of developing countries.

Syzygium cumini Linn. (synonym *Eugenia jambolan* Linn.) a large, evergreen and tropical tree of family Myrtaceae¹⁰. It is native to India and found throughout India up to an altitude of 1800 meters and its habitat is from Myanmar extended to Afghanistan and it is also found in countries like Madagascar, Thailand and Philippines¹¹. Other names of the plant from literature are Jamun, Jambolan, black plum. The plant is well known for its pharmacological properties.

In our study, we investigated the antibacterial activity of four different solvent extracts of *Syzygium cumini* on dental caries causing bacteria.

MATERIALS AND METHODS

(i) Plant material

The plants were collected from different areas of Lahore, Rawalpindi and Islamabad,

Pakistan and were further confirmed in the Department of Botany, University of Punjab, Pakistan and a sample specimen was deposited in the herbarium library of the university.

(ii) Preparation of plant extract

Leaves were thoroughly washed first with tap water and then with distilled water and left for drying in shade for five days. 500gm of air dried and powdered material was extracted with methanol, hexane, ethyl acetate and water by cold maceration method for two days and then it was filtered using Whatman No.1 filter paper¹², it was then evaporated in rotary evaporator under vacuum at 40°C using Heidolph, (Germany)

VE-11 rota evaporator¹³. After evaporation, of solvents, extracts were stored in refrigerator at 4°C until further use.

(iii) Microorganisms used:

Dental caries causing bacteria *Streptococcus mutans*, *Streptococcus viridans*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *E. coli* were procured from the University of Veterinary and Animal Sciences, Lahore, Pakistan. These were then subcultured on Nutrient Agar and Brain Heart plates and incubated aerobically at 37°C for 24 hours.

(iv) Antimicrobial assay

The aqueous, ethanol, ethyl acetate and hexane extracts were used for the screening. Antimicrobial activity was determined using agar well diffusion method¹⁴. Pure isolates of microbes were subcultured and normal saline (0.85%) was prepared of 24 hours old culture under aseptic conditions, with density of each microbial suspension adjusted to that of 10⁶ Colony Forming Unit CFU/ml standardized by 0.5McFarland standard. It was further used as inoculum. Agar plates were prepared and 100µl of inoculum of each test microbe was spread on agar plates, they were then allowed to dry and with help of sterile borer wells were made in inoculated agar plates. Each extract was then reconstituted in 20% Dimethyl sulfoxide (DMSO)¹⁵. A 100µl of each extract was then poured in the wells

and plates were incubated at 37°C for 24h¹⁶⁻¹⁸. DMSO was used as negative control and ciprofloxacin was used as positive control. If the zones of inhibition were greater than 6mm the antimicrobial activity was recorded¹⁹. The experiments were performed in duplicates and mean values of the diameter of inhibition zones with \pm standard deviation were calculated²⁰.

(v) Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) is defined as the lowest concentration of a compound/extract/drug that completely inhibits the growth of the microorganism in 24 hour¹⁹. MIC for the extracts was determined by modified agar well diffusion method²¹. Twofold serial dilutions of extracts were prepared to get the decreasing concentration range of 4mg/ml. 100 μ l of each dilution was then introduced into wells in the specific media agar plates with inoculum (10^6 CFU/ml) of the test microbial strain. All test plates were incubated aerobically at 37°C for 24hrs and observed for zone of inhibition. The lowest concentration of each extract showing a clear zone of inhibition (>8mm) was considered as the MIC, and it was recorded accordingly.

(vi) Phytochemical analysis

Phytochemical analysis was carried out by the method defined by^{16, 22-25}

RESULTS AND DISCUSSIONS

Medicinal plants are being used for treating numerous human diseases for years and in rural parts of developing countries they are the primary source of medicines²⁶. Almost 80% people of developing countries use traditional medicines²⁷.

In present study, the antibacterial activity and phytochemical screening were performed with four different extracts of leaves of *S. cumini* and the study was made against 6 available pathogenic bacteria that cause dental caries. The results of antimicrobial assay of four different leaves extracts of *Syzygium cumini* as well as the positive control ciprofloxacin (for bacteria) have been shown in Table 1. The zone of inhibition was produced against all the oral bacteria. The maximum zone of inhibition was observed in methanolic extract (20mm) against *E. coli*. The MIC of the extracts against the test pathogens are presented in Table 2. The antimicrobial activity on the agar plates varied greatly in different solvents. The positive control (ciprofloxacin) produced significantly sized inhibition zone against the test bacteria. However, the negative control produced no observable inhibitory effect. The leaves of *Syzygium cumini* were rich in flavonoids, alkaloids, saponins and tannins, which confer the antibacterial activity on the leaf extracts. (Table3).

Table 1
Antibacterial activity of leaf extracts against dental caries causing oral pathogens by agar well diffusion method

Name of plant	Conc/mg	<i>Streptococcus viridans</i>	<i>Streptococcus mutans</i>	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>
<i>Syzygium cumini</i>	Aqueous	-	16 ± 1.142	16 ± 0.707	19 ± 0.707	17 ± 0.494	16 ± 1.484
	Methanol 20%	16 ± 1.272	13.5 ± 0.707	13 ± 1.414	16 ± 1.414	16 ± 1.414	16 ± 1.414
	60%	-	15 ± 1.41	18 ± 1.414	20 ± 1.414	18 ± 1.06	8 ± 1.414
	Hexane 20%	14 ± 0.707	10.8 ± 1.06	14 ± 1.69	-	15 ± 0.424	16 ± 0.707
	60%	17 ± 0.282	15.8 ± .212	16 ± 0.494	-	16 ± 0.707	18 ± 1.130
	Ethyl acetate 20%	9.5±0.707	12±0	11.5±0.707	14±0.707	-	-
	60%	18±0.707	13.5±0.707	12.5±0.707	17±0.707	12±0	15±1.41
	Ciprofloxacin		30mm	24mm	20mm	17mm	25mm
Dimethyl sulfoxide(DMSO)		-	-	-	-	-	-

-: No activity. Values, including the diameter of the well (6 mm), are means of three replicates ± standard deviation. Ciprofloxacin (positive control), DMSO (negative control)

The methanol, hexane, aqueous and ethyl acetate extract proved to be active against all the bacterial strains. The bactericidal activity of the *S. cumini* leaves is may be due to their flavonoid and tannin contents²⁸. Flavonoids, because of their natural ability to modify the body's reaction to allergies and viruses show antiallergic, antimicrobial and anti-cancer activities²⁹. Tannins were reported to have antibacterial and antitumor activities³⁰.

Leaves of *S. cumini* are also rich in gallic acid, ellagic acid polyphenol derivative^{31, 32}. The results obtained in the present study indicate the presence of antimicrobial compounds in the crude extracts of this plant, which show a correlation between the reported uses of these local plants against different microbial pathogens. The presence of phyto-constituents in the leaf extracts may be responsible for the antibacterial activity as reported by Marjorie³³.

Table 2
Minimum Inhibitory concentration (mg/ml) of extracts against dental caries causing microorganisms

Microorganism	<i>Syzygium cumini</i>		
	Hexane	Methanol	Ethyl acetate
<i>Streptococcus mutans</i>	20	4	100
<i>Streptococcus viridians</i>	100	4	100
<i>Bacillus subtilis</i>	-	12	100
<i>Staphylococcus aureus</i>	100	20	100
<i>Pseudomonas aeruginosa</i>	20	20	100
<i>E. coli</i>	100	4	20

Table 3
Phytochemical analysis of leaves of *Syzygium cumini*

Name of test	Methanolic extract	Ethyl acetate extract
Test for saponins	+	-
Test for tannins	+	++
Test for alkaloids	+	-
Test for flavonoids	+	+

+: Present, -: Absent

Plants that have antimicrobial compounds present in them have tremendous potential to be used for therapeutic purposes without any undesirable effects that are usually observed while using synthetic compounds³⁴.

CONCLUSION

This study has evaluated the antibacterial potential of selected species of *Syzygium cumini* against dental caries causing bacteria.

The results obtained in the present study suggest another potential application of leaves of *S. cumini* for treatment of dental caries, further purification and toxicological studies of the plant and its *in vivo* trials should be carried out for the development of a phytomedicine to act against dental caries causing microbes. Its antibacterial activities can be further enhanced if the active components are purified and proper dosage for administration is determined.

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