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A study on the antibacterial effect of selected medicinal plants of Western Ghats against dental caries bacteria

Shafi Thompson T[1*], Arunima Ashok[2], Sukesh K[2]

***Corresponding author:**

Shafi Thompson T

[1] Department of Biotechnology, Mar Ivanios College, Thiruvananthapuram Dist-695 015, Kerala, India

[2] Department of Microbial Technology, Malankara Catholic College, Kanyakumari Dist- 629 153,Tamilnadu, India

Abstract

This study was conducted to reveal the antibacterial effect of selected medicinal plants from Western Ghats against dental caries bacteria and aware the populace about the importance of using phytomedicines. In this study, the antibacterial effects of nine well known medicinal plants were checked against the dental caries bacteria using well diffusion method. The highest zonation was reported in acetone and methanol extracts. The Chloroform and ethanol extracts revealed sensible activity and water extracts were produced least inhibitory activity. Acetone extract of *Eucalyptus* gained high inhibitory activity against *Bacillus megaterium*. While *Pseudomonas aeruginosa* showed resistance to all the extracts of *Syzygium aromaticum*.

Keywords: Dental caries, Western Ghats, Medicinal plants, Antibacterial activity, Phytochemical investigation

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Introduction

Dental decay is the most prevalent disease affecting humanity. Teeth get decayed due to a combination of causes that include bad oral hygiene, stagnation of food on or around the teeth, presence of plaque on the tooth structure and the presence of caries causing microorganisms [1]. The presence of a certain types of microorganism was discovered during the last decade in dental plaques. The nucleating role of the microorganisms in the formation of dental calculus shows similarities to that of nanobacteria in calcification [2]. Periodontal disease has long been recognized as a chronic disease, but literature describes it as a disease derived entirely from the effects of a microbial colonization of the gingival crevice. If this were so, it would mean that periodontal disease is unique among chronic diseases, all of which represent the long-term cumulative effects of interaction between a host biologic system and the surrounding environment [3]. Antibiotic resistance is the ability of a microorganism to withstand the effects of an antibiotic. It is a specific type of drug resistance. Antibiotic resistance evolves naturally via natural selection through random mutation, but it could also be engineered by applying an evolutionary stress on a population. Once such a gene is generated, bacteria can then transfer the genetic information in a horizontal fashion (between individuals) by plasmid exchange. The patterns of antibiotic usage greatly affect the number of resistant organisms which develop. Overuse of broad-spectrum antibiotics, such as second- and third-generation greatly hastens the development of

resistance. Other factors contributing towards resistance include incorrect diagnosis, unnecessary prescriptions, improper use of antibiotics by patients [4]. Antibiotic resistance in microorganisms recovered from the acute dental abscess has been reported to be increasing (with the exception of Metronidazole) in some populations studied over the last few decades. The resistance problem demands that a renewed effort be made to seek antibacterial agents effective against pathogenic bacteria resistant to current antibiotics. One of the possible strategies towards this objective is the rational localization of bioactive phytochemicals. Plants have a limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen substituted derivatives such as tannins. Many of the herbs and spices used by humans to season food yield have useful medicinal compounds including those having antibacterial activity [5]. Plant derived drugs remain an important resource especially in developing countries to combat serious diseases.

Materials and Methods

Selection of Bacterial Strains

Bacterial strains of six different species (Pseudomonas aeruginosa, Streptococcus salivarius, Streptococcus viridans, Streptococcus mutans, Bacillus megaterium, and Neisseria catarrhalis) were selected from Microbial Technology Laboratory, Malankara Catholic College, Mariagiri, Kaliakkivilai, Tamil Nadu.

Collection of Medicinal Plants

The medicinal plant samples were collected from the Maruthuvarmalai region of Western Ghats of Kanyakumari district. The different parts such as root, stem, leaves and inflorescence of nine well-known plants were selected for testing its antibacterial studies and characterization of secondary metabolites of effective ones.

Preparation of Plant Extracts

Plant samples were shade dried and ground well. 10 gram of powdered sample was filled in screw cap bottles with 10 ml of different solvent systems (acetone, ethanol, chloroform, methanol and water). It was kept at 22^0 c for fifteen days.

Antibacterial Effect Checking of Medicinal Plant Extracts

Antibacterial effect of medicinal plant extracts were checked by Well- diffusion method.

Well Diffusion Method

The bacterial isolates were effectively swabbed on the Mueller-Hinton agar plates. After allowing the inoculums to dry at room temperature and six millimeter wells were bored on it. The extract was introduced (50 µl of a 100mg/ml concentration) into three duplicate wells. The plates were allowed to stand at room temperature for one hour for the extract to diffuse into the agar and then they were incubated at 37^0 c for 18 hours. After incubation the plates were observed for the results.

Results

Antimicrobial Effect of Medicinal Plant Extracts

The antimicrobial activity of nine selected medicinal plant's (*Syzygium aromaticum, Piper betle,* Areca catechu, *Camellia sinensis, Eucalyptus* globules , *Zingiber officinale,* Gymnema sylvestre, *Azadirachta indica,* and *Chrysopogon zizanioides*) ethanol, acetone, chloroform, methanol and water extracts against bacterial isolates (Pseudomonas aeruginosa, Streptococcus salivarius, Strptococcus viridans, Strptococcus mutants, Bacillus megaterium, Neisseria catarrhalis) were tabulated.

The acetone extract of Eucalyptus globulus showed high inhibitory activity against Streptococcus mutans, Streptococcus viridans and Bacillus megaterium with 30mm, 31mm and 36mm respectively. Whereas the ethanol extract revealed activity against Bacillus megaterium and Streptococcus salivarius.. It was observed that

Table.1. Zone of Inhibition of Different Extracts of *Eucalyptus globulus* against Bacteria

Bacteria	Acetone (mm)	Chloroform (mm)	Ethanol (mm)	Methanol (mm)	Water (mm)
Neisseria catarrhalis	18	10	25	17	13
Streptococcus mutans	30	12	23	17	_
Streptococcus salivarius	18	10	25	17	_
Streptococcus viridans	31	10	_	22	27
Bacillus megaterium	36	13	26	20	14
Pseudomonas aeruginosa	11	9	13	_	_

Table.2. Zone of Inhibition of Different Extracts of *Zingiber officinale* against Bacteria

Bacteria	Acetone (mm)	Chloroform (mm)	Ethanol (mm)	Methanol (mm)	Water (mm)
Neisseria catarrhalis	25	16	23	17	_
Streptococcus mutans	21	15	20	17	_
Streptococcus salivarius	12	15	15	16	_
Streptococcus viridans	16	14	20	14	_
Bacillus megaterium	12	16	16	11	_
Pseudomonas aeruginosa	_	13	11	_	_

Table.3.Zone of Inhibition of Different Extracts of *Syzygium aromaticum* against Bacteria

Bacteria	Acetone (mm)	Chloroform (mm)	Ethanol (mm)	Methanol (mm)	Water (mm)
Neisseria catarrhalis	30	18	23	25	_
Streptococcus mutans	23	20	24	24	24
Streptococcus salivarius	22	15	21	20	11
Streptococcus viridans	21	18	22	18	11
Bacillus megaterium	22	17	18	20	12
Pseudomonas aeruginosa	_	_	_	_	_

Table.4. Zone of Inhibition of Different Extracts of *Camellia sinensis* against Bacteria

Bacteria	Acetone (mm)	Chloroform (mm)	Ethanol (mm)	Methanol (mm)	Water (mm)
Neisseria catarrhalis	25	_	15	18	_
Streptococcus mutans	20	_	_	_	_
Streptococcus salivarius	20	_	18	15	_
Streptococcus viridans	22	_	30	24	_
Bacillus megaterium	18	_	25	23	_
Pseudomonas aeruginosa	15	8	18	9	_

Table.5. Zone of Inhibition of Different extracts of *Piper betle* against Bacteria

Bacteria	Acetone (mm)	Chloroform (mm)	Ethanol (mm)	Methanol (mm)	Water (mm)
Neisseria catarrhalis	10	11	17	11	_
Streptococcus mutans	12	21	13	12	_
Streptococcus salivarius	11	9	18	9	7
Streptococcus viridans	10	23	15	20	_
Bacillus megaterium	22	15	23	20	_
Pseudomonas aeruginosa	18	20	14	18	_

Table.6. Zone of Inhibition of Different extracts of *Gymnema sylvestre* against Bacteria

Bacteria	Acetone (mm)	Chloroform (mm)	Ethanol (mm)	Methanol (mm)	Water (mm)
Neisseria catarrhalis	10	8	13	_	_
Streptococcus mutans	13	_	13	9	_
Streptococcus salivarius	10	10	13	18	_
Streptococcus viridans	13	_	15	11	13
Bacillus megaterium	20	14	14	20	_
Pseudomonas aeruginosa	16	_	10	_	_

Table.7. Zone of Inhibition of Different extracts of *Azadirachta indica* against Bacteria

Bacteria	Acetone (mm)	Chloroform (mm)	Ethanol (mm)	Methanol (mm)	Water (mm)
Neisseria catarrhalis	17	9	13	20	_
Streptococcus mutans	7	12	10	9	_
Streptococcus salivarius	15	14	11	11	_
Streptococcus viridans	12	9	15	10	_
Bacillus megaterium	15	9	8	_	7
Pseudomonas aeruginosa	_	6	6	_	_

Table.8. Zone of Inhibition of Different extracts of *Chrysopogon zizanioides* against Bacteria

Bacteria	Acetone (mm)	Chloroform (mm)	Ethanol (mm)	Methanol (mm)	Water (mm)
Neisseria catarrhalis	12	8	18	12	11
Streptococcus mutans	18	_	13	10	_
Streptococcus salivarius	15	_	_	11	_
Streptococcus viridans	32	_	18	18	_
Bacillus megaterium	25	10	25	25	7
Pseudomonas aeruginosa	12	8	13	9	_

Table.9. Diameter of Zone of Inhibition of Different extracts of *Areca catechu* against Bacteria

Bacteria	Acetone (mm)	Chloroform (mm)	Ethanol (mm)	Methanol (mm)	Water (mm)
Neisseria catarrhalis	20	11	11	18	_
Streptococcus mutans	18	9	12	13	_
Streptococcus salivarius	17	9	12	12	_
Streptococcus viridans	20	_	12	12	_
Bacillus megaterium	18	9	16	19	_
Pseudomonas aeruginosa	12	19	11	_	_

water extract inhibited the growth of Streptococcus viridans with a zone of 27mm.

The acetone extract of Zingiber officinale inhibited the growth of Neisseria catarrhalis and produced zone of 25mm. it was observed that the ethanol extract has the ability to inhibit the growth of Neisseria catarrhalis and Streptococcus mutans.

The chloroform extract of Syzygium aromaticum showed activity against both Neisseria catarrhalis and Streptococcus viridans with a clear zone of 18mm each. Whereas its acetone extraction revealed high activity against Neisseria catarrhalis with a zone of 30 mm. The methanol extract produced high activity against Streptococcus mutans with a zone of 24mm. It was observed that the water extract inhibited the growth of Streptococcus mutans with a clear zone of 24mm.

Neisseria catarrhalis was sensitive to acetone extract of Camellia sinesis with a growth inhibitory zone of 25mm. whereas the chloroform extract showed activity only against Pseudomonas aeruginosa with a zone of 8mm. Water extract did not showed activity on any organism.

The crude acetone extract of Piper betle showed high inhibitory activity against Bacillus megaterium and Pseudomonas aeruoginosa with a zone of 22mm, 18mm respectively. Chloroform extract revealed activity against Streptococcus viridans with a clear zone of 23mm. Whereas ethanol extract showed inhibitory activity against Bacillus megaterium with zone of 23mm. it was observed that water extract has ability to inhibit Streptococcus salivarius with a zone of 7mm.

The acetone extract of Gymnema sylvestre showed highest activity against Bacillus megaterium and Pseudomonas aeruginosa with 20mm, 16mm respectively. Whereas the water extract showed the maximum activity only against Streptococcus viridans with a clear zone of 13mm.

Acetone extract of *Azardirachta indica* showed maximum inhibitory activity against Neisseria catarrhalis and Streptococcus salivarius with 17mm, 15mm respectively. Whereas the methanol extract showed high activity of 20mm zone against Neisseria catarrhalis and 11mm on Streptococcus salivarius. It was observed that water extract has activity against Bacillus megaterium alone.

Acetone extract of Chrysopogon zizanioides showed high activity against Streptococcus viridans (32mm) and ethanol extract expressed high activity against Bacillus megaterium (25mm). Whereas the methanol extract showed high inhibitory activity against Bacillus megaterium and Streptococcus viridans with 25mm, 18mm respectively.

The crude acetone extract of Areca catechu showed maximum inhibitory activity against Neisseria catarrhalis (20mm) and chloroform extract was highly inhibited Pseudomonas aerugonisa with a zone of 19mm. The methanol extract expressed high activity against Bacillus megaterium and Neisseria catarrhalis with 19mm, 18mm respectively.

Discussion

Nowadays the degree of dental caries and related problems are increasing with severe effects. As part of search for effective phytoderivatives against dental pathogens, nine medicinal plants (*Syzygium aromaticum, Piper betle, Areca catechu, Camellia sinensis, Eucalyptus globules, Zingiber officinale, Gymnema sylvestre, Azadirachta indica, Chrysopogon zizanioides*) were collected from the Maruthuvar Malai region of Western Ghats in Kanyakumari district and its solvent extracts were applied against bacterial isolate. The result evidenced that all the selected plants are with acceptable levels of bactericidal activity. The highest zonation was reported in acetone and methanol extracts. The Chloroform and ethanol extracts showed moderate activity and water extracts were showed relatively least inhibitory activity. Perhaps the variations may be due to the polarity of solvents which determines the type of reaction and solubility of compounds.
The acetone and methanol have better extracting capacity which may be attributed to the ability to extract the natural antimicrobial compounds such as alkaloids, flavanoids, terpinoids and phenolic compounds from the plant. Acetone extract of *Eucalyptus* showed high inhibitory activity against *Bacillus megaterium*. Whereas *Pseudomonas aeruginosa* was resistant to all the extracts of *Syzygium aromaticum*. These results strengthen the possibility of application of phytoderivatives against human oral flora.

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