Innovations

Evaluation of Antibacterial Activity of Plant Extracts Against Bacterial Isolates from Dental Decay Infection

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Abstract: *The present study was undertaken for the isolation of bacteria associated with dental problems and to assess the in vitro antibacterial activity of different plant extracts against isolated bacteria by the agar well diffusion method. Among twelve plant extracts tested, the ethanolic extract of Clove (bud) showed maximum inhibition of 40 mm, Zone of Inhibition (ZOI) against Dental Caries bacterial isolate and 25 mm against Dental Cyst bacterial isolate. Against both Dental Caries bacterial isolate and Dental Cyst bacterial isolate, mulethi (root) extract depicted a ZOI of 35 mm and 31 mm, respectively, while for cinnamon (bark) extract, the ZOI remained at a value of 32 mm for both. Neem (leaves) extracts did not depict any inhibition against the isolated bacteria.The MIC was found to be 0.15% (w/v) for both isolates utilizing Clove (bud) ethanolic extract. Results for antibiotic susceptibility testing of the isolate, Dental Caries bacterial isolate, showed that gentamicin had the largest ZOI of about 20 mm in diameter, followed by chloramphenicol and erythromycin with 15 mm each, and then vancomycin with 10 mm. Other antibiotics like ampicillin, oxacillin, clindamycin, and cephalexin did not have any zone of inhibition.The results of the phytochemical assay of the clove extract showed no anthocyanins, saponins, phlobatannins, tannins, phenolic compounds, or cardiac glycosides, but the presence of alkaloids, flavonoids, tannins, and carbohydrates. These findings contribute to establishing natural plant extracts, especially clove extract, as substitutes to standard antibiotics.*

Keywords*: Antibacterial, Dental Caries, Dental Cyst, Phytochemical, Plant Extract*

Introduction

In the human body, there are some 100 trillion symbiotic bacteria in different areas: in the gastrointestinal system, on the skin, and in the oral cavity. The mouth, with the second most divergent microbial community besides the gut, harbors over 770 different species of bacteria (Kitamoto et al., 2020).One of the most widely spread diseases in the world, caries can quite rightfully be called the "dental enemy of humanity number one."

Whereas 93 percent of the Earth's population is afflicted with caries, the very disease was first revealed 5–6 thousand years ago in human beings and still explains almost 90 percent of tooth loss in adults. But, along with the formation of the notorious "holes," caries also arouses periodontitis when spreading the process of the disease to neighbouring tissues and, in the end, provokes the total destruction of the tooth and its root (Khabibjonova et al., 2024).

The surface of the tooth has a thin protein film, called a pellicle, on it. Although largely invisible, numerous microorganisms of the oral flora have attached to it, for example, *Streptococcus mutans* and *Lactobacillus acidophilus*. These bacteria secrete acid that, in the presence of fermentable carbohydrates, begins to demineralize the tooth's enamel, which is the outermost layer of the tooth structure. It is continuously remineralized by calcium and phosphate ions found in saliva, but demineralization results in subsurface layers of the enamel that first appear as white spots or streaks. This may progress with time and, if the process goes unchecked, result in a cavity(Mathur et al., 2017).Treating periodontal diseases involves different modes of treatments, starting from behavioural modification methods, systemic, and local chemical medications, and local and systemic chemical medications, which include materials made up of zinc oxide, chlorhexidine and materials containing silver, to various surgical procedures. Various biological functions differ in chemical structures and composition, which makes the natural materials the centre of attention this decade(Barzegar et al., 2022).In the case of *S. mutans* and other bacteria, a three-dimensional biofilm structure forms and matures as the result of an intricate process by which the organisms adhere and aggregate before being encased. More interestingly, compared with planktonic bacteria, the microorganisms in this biofilm show far higher resistance to antimicrobial treatments and harsh external conditions (Gao et al., 2024).

Traditional restorative approaches to the management of dental caries are based on the removal of extensive tooth structure for the elimination of cariogenic bacteria, cessation of decay, preparation of the tooth for mechanical retention of the restoration, resistance of occlusal forces, and removal of demineralized dentin. The creation of adhesive and bioactive dental materials that bond micromechanically to the tooth has removed the requirement for removing extensive tooth structure. These materials provide excellent peripheral seals, allowing caries arrest without complete decay excavation by isolating the carious lesion from the oral environment (Desai et al., 2021).

Modern etiology describes dental caries as the result of complex interactions involving oral microbes, fermentable carbohydrates, and host factors over time. Oral factors and general health are directly related to the outcome of disease outcome. Even with a more sophisticated caries management system, caries difficulty assessments are still necessary before initiating any treatment planning. The next step would be to introduce a caries management strategy aimed at specific lesions while, at the same time, lowering their caries risk factors (Cheng et al., 2022).A serious risk to human health is posed by bacterial infections, which are made worse by rising antibiotic resistance. The high morbidity and fatality rates associated with these illnesses highlight the urgency of promptly identifying and treating harmful germs (Deusenbery et al., 2021).

Since the discovery by Alexander Fleming in 1928, antibiotics have been the keystone of modern medicine. They are both preventive and curative, protecting patients from fatal diseases and allowing major surgeries and cancer treatments that are essentially low-risk. On the other hand, increasing antibiotic resistance is rendering these very drugs ineffective, making even minor surgeries and routine operations life-threatening. In other words, it simply means standard treatments of infections are no longer working, thus making it easier for infections to stay on longer or spread out. Antibiotic resistance is one of the major threats to human health in this century, with others being climate change and global terrorism. Scarily, only a few new antibiotics are in development, and we face nearing a post-antibiotic era—when effective treatments are no longer available. This is obviously an issue that does not recognize borders; if anything warrants a global response, then it is antibiotic resistance since it affects and puts everyone at risk (Thompson et al., 2021).

When prescribing antibiotics in dentistry, the microflora that makes up the dental plaque is not considered. Dentists authorize a broad variety of systemic antibiotics, penicillin in particular, for use in outpatient treatment without taking into account the medicines' effects on the microbiota in the mouth or other parts of the body. When it comes to *Candidaalbicans* and oral *streptococci*, for example, overusing broad-spectrum antibiotics may actually accelerate the growth of *Candidaalbicans* since the medicines do not eradicate the fungus. A shift towards oral conditions may be facilitated by the increased adhesion of bacteria such as *S. mutans* brought on by an increase in *Candida albicans*. When choosing an antibiotic to administer for a certain medical treatment, it is important to have a better understanding of the interactions that biofilm bacteria have with prescription drugs as well as how these interactions work (Taylor et al.,2021).

The use of plants to promote oral hygiene and enhance dental health has a long and venerable history. Plants include phytochemicals with strong defence and therapeutic properties, such as flavonoids, tannins, alkaloids, and essential oils. India is a large country home to a diverse range of cultures and demographics. The Indian populace has long employed a wide range of species of medicinal plants from different families to treat and manage a wide range of dental conditions. In order to encourage further dental science research, traditional knowledge should be well documented (Vaishali et al., 2014). Keeping in view the above justification the present study was to screen plant extracts against bacteria from dental decal infection.

Materials and Methods

Isolation of Bacteria from Dental Decay Infection

Media Preparation

All culture media were prepared in accordance with the manufacturer's instructions, and the media was sterilized by autoclaving at 121°C for 15 minutes. The broths flasks were then kept at a temperature between 2°C – 8°C for future use.

Sample Collection

Two dental samples (Dental Caries bacterial isolate, Dental Cyst bacterial isolate) were taken in sterile Nutrient Broth from Sikh Seva MultispecialitySector 125, Mohali. Samples were brought to the laboratory for isolation and Preliminary identification of Dental Associated Bacteria.

Bacterial Isolation from Dental Samples

A loopful samples were streaked on sterile nutrient agar plates and plates were incubated at 37°C for 24-48 hours. After incubation, various bacterial colonies were carefully selected from each growth plate. These colonies were aseptically transferred to new agar plates in an attempt to obtain pure bacterial strains. The newly inoculated plates were then incubated again at 37°C for 24-48 hours thereafter cultures were maintained at 4°C till further analysis.

Collection of Plant Materials

The plant parts (Cloves bud (*Syzygium* Sp.), bark of Cinnamon (*Cinnamomum*Sp.), Mulethi Root (*Glycyrrhiza* Sp.), Neem leaves (*Azadirachta* Sp.)used in this research work were collected from a local market of Kharar, Mohali, Punjab and brought to the laboratory in controlled conditions.

Solvent Extraction

The extract is concentrated with bioactive compounds from certain parts of plants. These phytochemicals come from different botanical sources, such as clove and cinnamon, among others, with the aid of solvents like ethanol, petroleum ether, and water. This process details the extraction of a broad spectrum of plant constituents that go on to find therapeutic and aromatic applications.

The collected plant parts were washed thrice with tap water to remove impurities. The washed plant parts were spread loosely on an aluminium sheet and dried in an oven at 60°C for 12 hours. After drying, the plant parts were immediately crushed with mortar and pestle. For extraction, three solvents were used namely, distilled water, ethanol, and petroleum ether.20 gram of dried plant powder was mixed with 100ml of each solvent (20% w/v) and flasks were incubated overnight at room temperature.The extracted liquid was filtered through Whatman Grade 1 Qualitative Filter Paperand collected in 20 ml Tarson tubes. (Enejiyon et al.,2020)

Antibacterial Activity of Plant Extract by Agar Well Diffusion

The agar well diffusion technique was used for antibacterial studies (Sharma et al., 2018). Sterile plates were prepared with a standardized suspension of (10^6 cells/ml) spread on them. The wells were cut using a sterile borer, and 50-100 µL of each extract that was previously prepared was added into the wells using a micropipette. The plates were incubated at 37°C for 24 hours, and inhibitory activity of the plant extract against bacteria was observed in terms of the diameter of the inhibition zone in mm.

Antibiotic Susceptibility Pattern of Bacterial Isolate by Disc Diffusion Method

Antibiotic susceptibility of test microorganisms was assessed through the use of the Kirby-Bauer Disc Diffusion Assay (Qodrati et al., 2022). Sterile plates were prepared, and a standardized suspension of 10^6 cells/mL spread over their surface. Antibiotic discs were placed using sterile forceps on the inoculated nutrient agar, after which the plates were incubated at 37°C for 24 hours. Once the incubation period was completed, the diameter of the inhibition zones around each disc was measured in millimetres. The obtained values were compared with standardized tables to determine the susceptibility, intermediate response, or resistance of the bacteria to the tested antibiotics.(Yao et al.,2021).

Determination of Minimum Inhibitory Concentration of Clove Ethanolic Extract

MIC assay is thus important in assessing antimicrobial potency, determining the minimum concentration of an agent that prevents visible microbial growth in a liquid medium. Eight dilutions of Clove ethanolic plant extract ranging from 20% to 0.07% were prepared through serial dilution with ethanol, and the dilutions were tested by a macrodilution agar plate method in this study. Three different agar plates were used: Plate 1 with 20%, 10%, and 5%; Plate 2 with 5%, 2.5%, and 1.25%; and Plate 3 with 0.625%, 0.31%, and 0.15%. The inhibition zone diameter was then measured after 18-24 hours of incubation at 37-40°C. The MIC was identified in the form of the lowest concentration showing visible inhibition (Parmar et al., 2022).

Phytochemical Analysis of Clove Ethanolic Extract

The phytochemicals are biochemical compounds produced by plants; the term is derived from the Greek phyto, meaning "plant." Plants that are medicinal have an active principle which gives them powers of significant medicinal value (Chanda et al., 2019). The present study focused on the phytochemical analysis of most potent antibacterial clove ethanolic extract. The following Phytochemical analysis methods were used as per modified methods from Shaikh et al., (2020).

Testing for various phytochemicals was done using the following procedures:

1. Alkaloids Test

Picric Acid Test:

Added a few milliliters of the filtrate with 3-4 drops of 2% picric acid solution.

Result: Orange colour indicates the presence of alkaloids.

2. Test for Flavonoids

Alkaline Reagent Test:

1 mL of the extract is mixed with 2 mL of 2% NaOH solution and a few drops of diluted HCl added.

Result: A strong yellow color that turns colorless by the addition of the acid shows the presence of flavonoids.

3. Test for Tannins

Braymer's Test:
Mix 1 mL of the filtrate with 3 mL of distilled water; then add 3 drops of a 10% ferric chloride solution.
Result: A blue-green color indicates the presence of tannins.

4. Test for Saponins

Foam Test:
Mix 0.5 g of the plant extract and 2 mL of water, and then shake vigorously.
Result: The persistent foam for 10 minutes indicates the presence of saponins.

5. Detection of Phlobatannins

HCl Test:
Mix 2 mL of the aqueous extract with 2 mL of a 1% HCl solution and boil.
Result: A red precipitate indicates the presence of phlobatannins.

6. Detection of Phenolic Compounds

Ferric Chloride Test:
Add a few drops of a 5% ferric chloride solution to the aqueous extract.
Result: Dark green or bluish-black color indicates the presence of phenolic compounds.

7. Detection of Cardiac Glycosides

Keller-Killani Test:
Mix 1 mL of the filtrate with 1.5 mL of glacial acetic acid, add 1 drop of a 5% ferric chloride solution and carefully add concentrated H_2SO_4 along the side of the test tube.
Result: Blue-colored solution in the acetic acid layer indicates the presence of cardiac glycosides.

8. Detection of Resins

Acetic Anhydride Test:
Mix 1 mL of plant extract with acetic anhydride solution, then add 1 mL of concentrated H_2SO_4.
Result: Specific colour changes will indicate the presence of resins.

9. Detection of Anthocyanins

HCl Test:
Mix 2 mL of the plant extract with 2 mL of 2N HCl and then add a few milliliters of ammonia.
Result: A pink-red colored solution becomes blue-violet on the addition of ammonia, indicating anthocyanins.

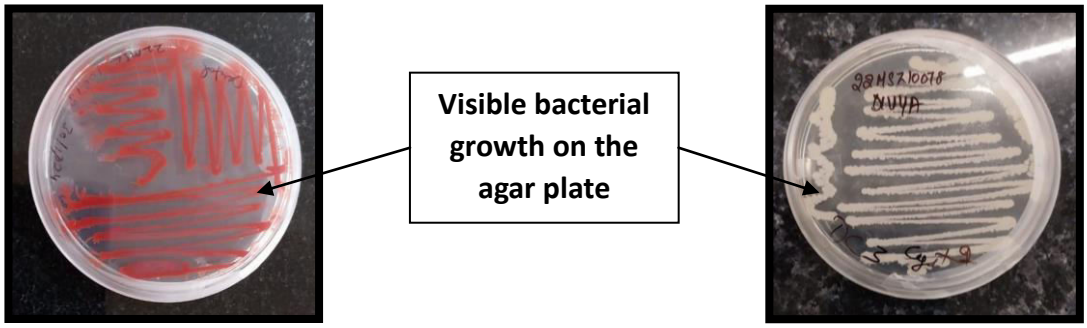
10. Detection of Carbohydrates

Fehling's Test:
Add 1 mL each of Fehling's solutions A and B to 1 mL of the spice extract and boil in a water bath.
Result: A red precipitate indicates the presence of carbohydrates.

RESULTS

Isolation of Bacteria from Dental Decay Infection

Two bacterialisolates were isolated namely, Dental Caries bacterial isolate and Dental Cyst bacterial isolate. The Pure Culture of isolated bacteria are shown in figure 1.The bacterial cultures were observed under microscope, Preparations of slides "Dental Caries bacterial isolate" and "Dental Cyst bacterial isolate" were done, and Gram staining was carried out to help in the identification of isolated bacteria.



(A) **(B)**

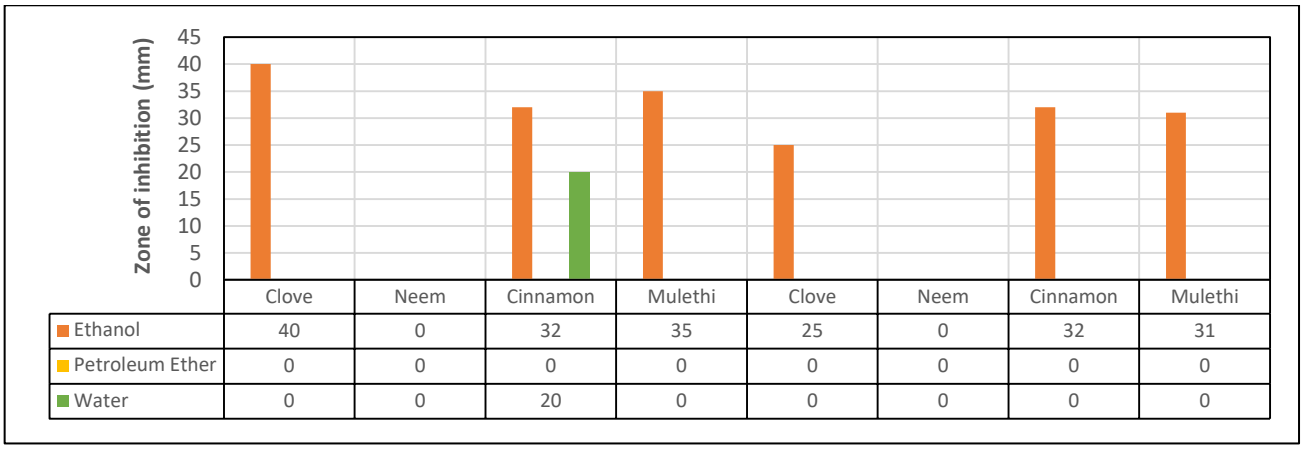
Figure1: Pure Culture of Isolated Bacteria(A)Dental Caries Bacterial Isolate and (B) Dental Cyst Bacterial Isolate

Antibacterial Activity of Plant Extracts by Agar Well Diffusion

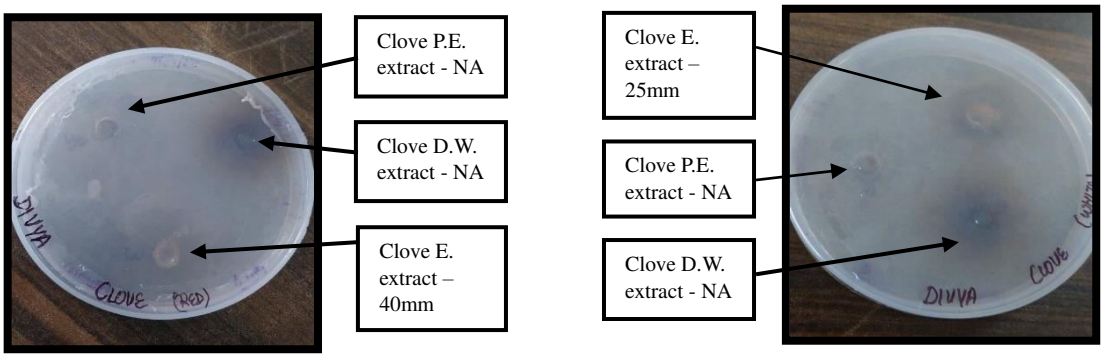
Of the 4 plant extracts clove (bud) ethanolic extract was found most effective against dental caries bacterial isolate with zone of inhibition 40 mm as well as against Dental cyst bacterial isolate with zone of inhibition 20 mm. Followed by mulethi (root) ethanolic extract with zone of inhibition 35 mm in Dental caries bacterial isolate and zone of inhibition 31mm in dental cyst bacterial isolate. The extract of cinnamon bark with ethanol also showed significant antibacterial activity against both types of bacteria with ZOI 32 mm in dental caries bacterial isolate and 32 mm in dental cyst bacterial isolate also in aqueous extract of water 20mm in Dental caries bacterial isolate. No inhibitory activity was observed in Petroleum Ether against Dental Caries bacterial isolate and Dental Cyst bacterial isolate. The results are shown in Table 1, Graph 1 and Figure 2.

Table I: Antibacterial Activity of Plant Extracts by Agar Well Diffusion against Dental Caries Bacterial Isolate and Dental Cyst Bacterial Isolate

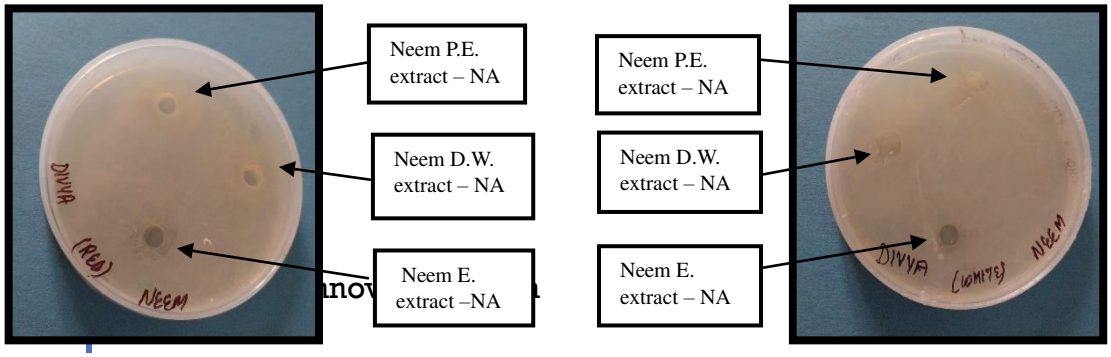
S. No.	Plant Extract	Dental Caries bacterialisolate			Dental Cyst bacterial isolate		
		Ethanol	Petroleum Ether	Water	Ethanol	Petroleum Ether	Water
		Zone of Inhibition in mm			Zone of Inhibition in mm		
1	Clove	40	NA	NA	25	NA	NA
2	Neem	NA	NA	NA	NA	NA	NA
3	Cinnamon	32	NA	20	32	NA	NA
4	Mulethi	35	NA	NA	31	NA	NA



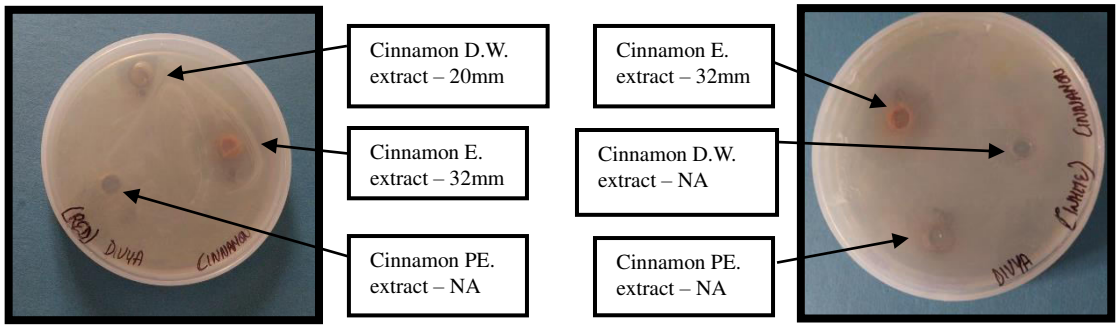
Graph 1- Antibacterial Activity of Plant Extracts by Agar Well Diffusion against Dental Caries Bacterial Isolate and Dental Cyst Bacterial Isolate.



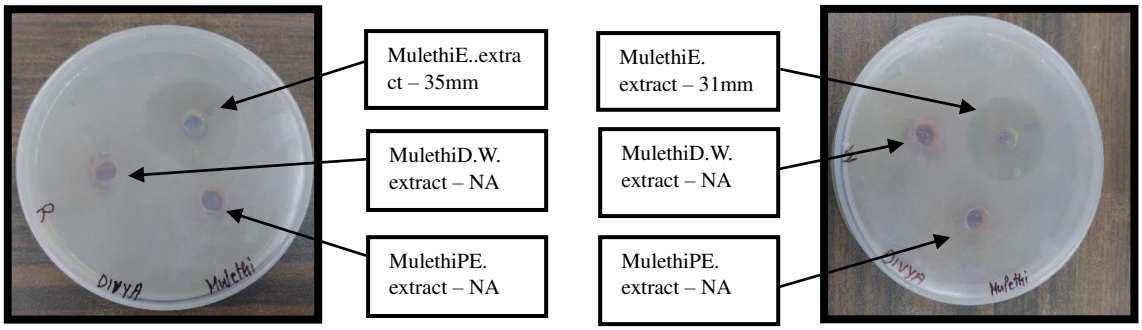
a) Antibacterial activity of Clove extract against Dental Caries bacterial isolate and Dental Cyst bacterial isolate



b) Antibacterial activity of Neem extract against Dental Caries bacterial isolate and Dental Cyst bacterial isolate



c) Antibacterial activity of Cinnamon extract against Dental Caries bacterial isolate and Dental Cyst bacterial isolate



d) Antibacterial activity of Mulethi extract against Dental Caries bacterial isolate and Dental Cyst bacterial isolate (PE: Petroleum Ether, E: Ethanol; D.W.: Distilled Water (Aqueous)

Figure 2: Images (a-d) showing Antibacterial Activity of Various Plant Extract by Agar Well Diffusion Against Dental Caries bacterial isolate and Dental Cyst bacterial isolate

Antibiotic Susceptibility Pattern of Bacterial Isolates

Gentamicin showed the very large zone of inhibition of 20 mm against the dental caries bacterial isolate, followed by chloramphenicol and erythromycin with 15 mm, and vancomycin with 10 mm. All these exhibited their effectiveness as antibacterial agents. However, ampicillin, oxacillin, clindamycin, and cephalexin did not show any inhibition against this strain. These findings suggest that the dental caries bacterial isolate has developed resistance to most of the tested antibiotics, underlining the need for new and natural alternatives with activity against bacteria causing dental caries. No inhibitory activity was determined against Dental Cyst bacterial isolate. Theresults are shown in Table II.

Table II: Antibiotic Susceptibility Pattern of Bacterial Isolates

S. No.	Antibiotic	*Dental Caries bacterial isolate*	*Dentalcyst bacterial isolate*
		Zone of Inhibition	
1	**Ampicillin (Amp)**	NA	ND
2	**Vancomycin (VA)**	10	ND
3	**Oxacillin (OX)**	NA	ND
4	**Gentamicin (GEN)**	20	ND
5	**Erythromycin (E)**	15	ND
6	**Clindamycin (CD)**	NA	ND
7	**Chloramphenicol (C)**	15	ND
8	**Cephalexin (CEP)**	NA	ND

NA: No activity; ND: Not Determined

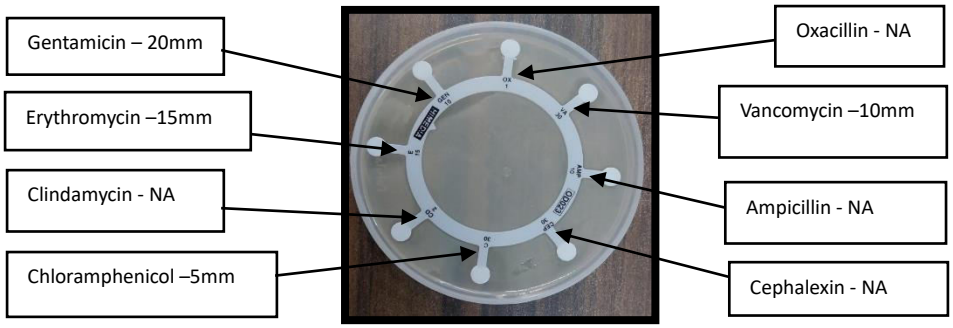


Figure 3:Antibiotic Susceptibility Pattern of Dental Caries bacterial isolate (NA: No activity)

Minimum Inhibitory Concentration of Clove Ethanolic Extract

	Concentration of Clove ethanolic extract									
	0.07 %	0.15 %	0.31 %	0.625 %	1.25 %	2.5 %	5 %	10 %	20 %	MIC
Dental Caries bacterial isolate	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	0.15 %

Dental Cyst bacterial isolate	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	0.15 %

The *Syzygium* Sp. ethanolic extract demonstrated a Minimum Inhibitory Concentration (MIC) of 0.15% against both the bacteria Dental Caries bacterial isolate and Dental Cyst bacterial isolate (producing a zone of 10 mm in inhibition zone in Dental Caries bacterial isolate and 5mm in Dental Cyst bacterial isolate). The detailed results of MIC are given in table III.

Table III: MIC result for Dental Cyst Bacterial Isolate Ethanolic Extract Clove Species

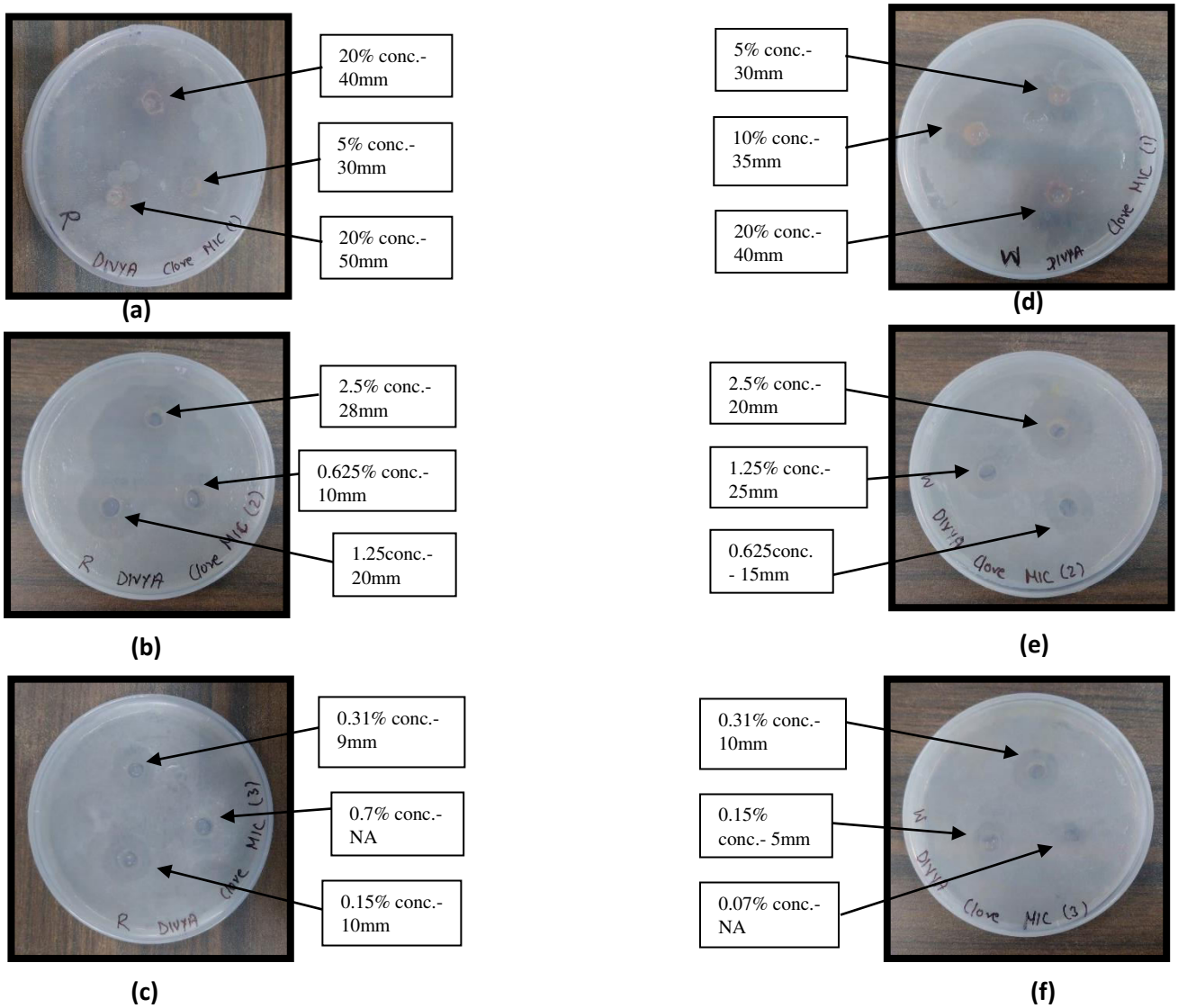


Figure 4: Imagesshowing different concentrations of Clove Ethanolic extract against *Dental Caries bacterial isolate*(a – c) and *Dental Cyst bacterial isolate* (d – f)

Photochemical Analysis of Clove Ethanolic Extract

The Phytochemical analysis of Clove extract in ethanol solvent revealed the presence of Alkaloids, Flavonoids, Tannins, Saponins, Phlobatannins, Phenolic compound, Cardiac Glycosides, Resins, Anthocyanins and Carbohydrates. The detailed results of phytochemical analysis are presented in table IV.

Table IV: Phytochemical Analysis of the best selected Plant that isused against the *Dental Caries bacterial isolate* and *Dental Cyst bacterial isolate*

S.no	Bioactive Compounds	Indication for positive result	Result
1.	**Alkaloids**	A reddish brown colour	**Present**
2.	**Flavonoids**	When diluted acid is added to a bright yellow colour, it turns colourless.	**Present**
3.	**Tannins**	Blue-green colour	**Present**
4.	**Saponins**	Stable honeycomb like froth	**Absent**
5.	**Phlobatannins**	Red precipitate	**Absent**
6.	**Phenolic Compound**	Dark green/Bluish-black colour	**Present**
7.	**Cardiac Glycosides**	Blue colour	**Present**
8.	**Resins**	Turbidity	**Absent**
9.	**Anthocyanins**	Blue violet Colour	**Absent**
10.	**Carbohydrates**	Red Precipitate	**Present**

Discussion

Dental caries is a leading global health burden, and its numerous systemic diseases relate to various current public health problems.Our results are in line with the previous literature; where our study results are supported by the previous studies, herbal plants have been used against the varieties of microbes. Antibiotics are used extensively for the cure of dental caries and other dental-related disorders, therapeutically and prophylactically, so further research studies on the topic are needed and necessary to verify the obtained results in this study.

In this study antibacterial activity of different plants was tested on *Dental Caries bacterial isolate* and *Dental Cyst bacterial isolate,* Antibioticsusceptibility pattern was examined against isolated bacteria*,* along with MIC of the best selected plant and phytochemical analysis was also carried out to determine the bioactive compounds present in the selected plant.

Ruhal et al. (2021) showed that Gram-positive and Gram-negative bacteria both form matrix-enclosed aggregates, variously referred to as biofilms. As such, these biofilms are not only responsible for primary industrial biofouling but are also related to increased antimicrobial resistance during infections. Although most of the studies of biofilms have been on single Gram type cultures, most biofilm communities in nature are composed of both Gram-negative and Gram-positive bacteria. Therefore, understanding of the conserved mechanisms of biofilm formation would be very instrumental in designing broad-spectrum therapeutics.In a study conducted by Alghamdi et al. (2022), the oral flora is described as continuously evolving due to its interaction with the external environment. The microbial species produce bacteriocins to compete against each other for nutrients in this micro-ecosystem.

In a recent study conducted by Bin et al., (2020) revealed that clove ethanolic extract showed ZOI of 8 mm against *S.sanguis* and ZOI of 17 against *S. mutans*. Both the bacteria are associated with dental caries. However, in our study it was revealed that clove exhibited a zone of 40 mm against both the dental associated bacteria that is *Dental Caries bacterial isolate* and *Dental Cyst bacterial isolate*. Also, in the previous study revealed that clove aqueous extract and other extracts showed some activity while in our study no activity was shown by other extracts such as petroleum ether and distilled water. The previous study also concluded that ethanol being an organic solvent has been frequently used as the solvent for extraction of phytochemicals.

In a study of antibacterial activity of cinnamon ethanol against different bacteria responsible for dental plaques conducted by Waty et al., (2018) revealed that cinnamon ethanolic extract has a ZOI of 11.68 in 25% ethanolic extract against the dental associated bacteria. While in the present study cinnamon ethanolic extract exhibited a ZOI of 32 mm against *Dental Caries bacterial isolate* and a ZOI of 30 mm against *Dental Cyst bacterial isolate* which is quite better than the previous studies conducted. The difference in results may be due to different species used at different concentrations and also the plant used belongs to different localities in both the studies

In a study conducted by Shaheen et al., (2022) revealed that mulethi cleanses oral cavity and prevent mouth ulcers and stomatitis. Similarly in our study we observed that mulethi exhibited an appreciable ZOI of 35mm against *Dental Caries bacterial isolate* and 31mm against *Dental Cyst bacterial isolate* which depicts that multehi actually work as an antibacterial agent for dental caries.

In a study conducted by Nagrajappa et al., (2018) it was revealed that neem aqueous extract exhibited a ZOI of 11.4mm against *streptococcus mutants* while in our study none of the extracts of neem showed any activity against any of the bacteria. The difference in both the studies may be due to the resistance shown by both the unidentified bacteria

and their different strains. Also, it can be due to different analytical methods applied.Matsumoto-Nakano (2018) studied the association between antibiotic sensitivity and the biofilm formation of several *Streptococci* isolated from the oral cavity. The study's findings indicate that 71% of the isolates are *S. viridans*, which is associated with the *Streptococci* group. *S. mutans*, however, is more adept at producing the biofilm. The *Streptococci* have higher resistance to erythromycin and amoxicillin than to the other medications, according to profiling of antibiotic resistance in the bacteria.Lemos et al., (2019) performed a number of susceptibility tests on 207 isolates of oral *streptococci*, including *S. mutans*, *S. mitis*, *S. salivarius*, and *S. oralis*. According to the results, only *S. mutans* showed a substantial susceptibility to the antibiotic penicillin.According to a study by Lamont et al. (2018) on oral *Streptococci* in healthy Greek children, 67 (33.5%) and 77 (38.5%) of the 200 isolates were resistant to clarithromycin and erythromycin, respectively. In this present study, antibiotic susceptibility test was conducted against *Dental Caries bacterial isolate* and it revealed thatbacteriawas susceptible to Gentamicin (20mm), Erythromycin (15mm), Chloramphenicol (15mm) and Vancomycin(10mm) and was resistant to Clindamycin, Oxacillin, Ampicillin and Cephalexin. This proves that there is an urgent need to discover an alternate to antibiotics as dental caries bacteria is becoming resistant to almost very antibiotics as shown in previous studies and this study.

A study conducted by Bin et al., (2020) stated that the MIC value for *S.mutans* were found to be 20±2mg/ml. While a study conducted by Elbestawy et al., (2023)concluded that the MIC of EEO (extracted from clove) ranged from 23 to 51 μg/mL against both. *H. pylori*. However, a study conducted by Pradhan et al. (2024) aqueous extract of *S. aromaticum* shows MIC of 31.25 mg/ml against S. mutans. In this study, it was revealed that MIC value for both Dental Caries bacterial isolate and Dental Cyst bacterial isolate bacteria was 1.5mg/ml. The difference in results may be due to different analytical methods used at different concentrations. A study conducted by Sawant et al., (2022) revealed that clove ethanolic extract contained alkaloids, terpenoids, saponins, flavonoids, steroids, and tannins, phenolic compounds, carbohydrates while there was absence of saponins, phlobatannins, anthocyanins and resins.However, in our study we found thatclove ethanolic extracts showed presence of alkaloids, flavonoids, tannins, phenolic compounds, cardiac glycosides and carbohydrates while there was absence of saponins,phlobatannins, resins and anthocyanins. This comparison shows the similarity may be due to the consistent genetic makeup of clove, same solvent used and similar analytical techniques.

Conclusion

This research work was able to isolate bacterial strains from dental caries and dental cyst samples. On the search for antibacterial activity in plant extracts, it was revealed that the ethanolic extract of clove (*Syzygium* sp.) showed the greatest antibacterial effect, recording the largest zones of inhibition against both dental caries and dental cyst isolates (40 mm and 20 mm, respectively). The ethanolic extracts of both the root and cinnamon bark also showed significant antibacterial activity. While petroleum ether extract did not exhibit any inhibitory effect.Gentamicin, chloramphenicol, and erythromycin still had good activities, with a maximum zone of inhibition produced by

gentamicin of 20 mm. On the other side,no inhibitory activity was determined against Dental Cyst bacterial isolate, hence an indication of the need for alternative treatment.It was observed that the Minimum Inhibitory Concentration (MIC) of clove ethanolic extract was 0.15% against dental caries and dental cyst bacterial isolates. Phytochemical analysis of the clove extract unveiled the presence of alkaloids, flavonoids, tannins, phenolic compounds, and cardiac glycosides that may attribute to the derived antibacterial properties.The present study demonstrates that clove and the rest of the plant extracts may have a stronger hand in being potential natural antibacterial agents against bacterial strains that are resistant and causes infections in the oral cavity. With a growing concern of antibiotic resistance, these results are highly significant in research on plant antimicrobials against dental pathogens.

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