Global Academic Journal of Dentistry and Oral Health

Available online at https://www.gajrc.com
DOI: https://doi.org/10.36348/gajdoh.2024.v06i03.001

OPEN ACCESS

ISSN-2706-8994 (P)
ISSN-2707-8868 (O)

Original Research Article

Evaluation of Antibacterial Activities of Clove Bud Oil and Extract on Bacterial Isolates from Dental Caries

Agu, Mellisa Chinelo[1*], Tasie Floretta Omebere[1]
[1]Department of Applied Microbiology and Brewing, Enugu State University of Science and Technology, Nigeria

***Corresponding Author**
Agu, Mellisa Chinelo
Department of Applied Microbiology and Brewing, Enugu State University of Science and Technology, Nigeria

Article History
Received: 14.07.2024
Accepted: 23.08.2024
Published: 21.09.2024

Abstract: Dental caries remains one of the most prevalent oral health issues worldwide, primarily caused by bacterial activity within the oral cavity. This study investigates the antibacterial activities of clove oil bud against bacteria associated with dental caries. The clove buds were collected, identified, and processed for ethanol extraction of bioactive compounds. Also, 30 samples of dental caries were obtained using swab sticks and cultured on nutrient agar, MacConkey agar, and Mannitol salt agar, then incubated for 24 hours and identified using biochemical tests and molecular analysis. Qualitative and quantitative phytochemical analysis confirmed the presence of alkaloids (5.033%), saponins (3.50%), flavonoids (0.407%), phenols (0.4545%), tannins (5.227%), and glycosides (2.1285%) in the ethanol extract, while the clove oil contained saponins (8.70%) and a small amount of glycosides (0.0375%). The extracted compounds underwent Gas Chromatography-Mass Spectrometry (GC-MS) analysis, which identified key constituents such as eugenol, Caryophyllene oxide, and 9,12-Octadecadienoic acid. The characterization of the clove oil revealed a density of 0.86 g/cm^3, viscosity of 113.59 mm^2/s, refractive index of ≥36% Brix, saponification value of 39.19 mg KOH, iodine value of 21.488 g/ml, peroxide value of 13.4 mEq/kg, and a free fatty acid content of 1.37%. Biochemical tests on the bacterial isolates from dental caries samples identified several species, including *Actinomyces spp., Streptococcus spp., Lactococcus spp., Lacticaseibacillus paracasei, Staphylococcus aureus strain CIB, Bifidobacterium spp., Veillonella spp.,* and *Bacillus subtilis.* Antimicrobial activity assay shows that clove oil exhibited significant inhibition of microbial growth at higher concentrations: *B.subtilis* had inhibition zones of 12 mm at 100 mg/ml, 6 mm at 50 mg/ml, and 2 mm at 25 mg/ml. Similarly, *Lacticaseibacillus agile* 1365 showed inhibition zones of 14 mm at 100 mg/ml, 8 mm at 50 mg/ml, and 3 mm at 25 mg/ml. *S.aureus* strain B3A22 had inhibition zones of 10 mm at 100 mg/ml and 6 mm at 50 mg/ml. In contrast, *L. paracasei* showed minimal inhibition, indicating lower susceptibility to clove oil. On the other hand, the result also illustrates that clove extract was more effective at lower concentrations compared to clove oil. *B.subtilis* had inhibition zones of 14 mm at 100 mg/ml, 8 mm at 50 mg/ml, and 5 mm at 25 mg/ml. *L.paracasei* demonstrated inhibition zones of 16 mm at 100 mg/ml, 11 mm at 50 mg/ml, and 8 mm at 25 mg/ml. *S.aureus* strain B3A22 had inhibition zones of 10 mm at 100 mg/ml and 6 mm at 50 mg/ml. The Minimum Inhibitory Concentration (MIC) of clove oil was found to be 25 mg/ml for *B.subtilis*, 100 mg/ml for *L.paracasei*, 25 mg/ml for *L.agile* 1365, and 25 mg/ml for both *S.aureus* strain CIB and strain B3A22 respectively. In comparison, clove extract demonstrated lower MIC values: 12.5 mg/ml for *B.subtilis*, 12.5 mg/ml for *L.paracasei*, 12.5 mg/ml for *L.agile* 1365, 25 mg/ml for *S.aureus* strain CIB, and

25 mg/ml for *S.aureus* strain B3A22. The Minimum Bactericidal Concentration (MBC) of clove oil was 50 mg/ml for *B.subtilis*, for *L.paracasei*, 25 mg/ml for *L.agile* 1365, 50 mg/ml for *S.aureus* strain CIB, and 100 mg/ml for *S.aureus* strain B3A22. In contrast, clove extract showed an MBC of 25 mg/ml for *B.subtilis*, 50 mg/ml for *L.paracasei*, 25 mg/ml for *L.agile* 1365, 50 mg/ml for *S.aureus* strain CIB, and 25 mg/ml for *S.aureus* strain B3A22. These results underscore the significant antibacterial activity of clove extract, particularly due to its higher potency at lower concentrations compared to clove oil, supporting its potential use in developing alternative treatments for dental caries.

Keywords: Evaluation, Antibacterial, Activities, Clove, Bud, Oil, Bacteria, Dental, Caries.

INTRODUCTION

Cloves (*Syzygium aromaticum*) are dried aromatic unopened floral buds, belonging to the family Myrtaceae. They add flavour and freshness to food, tobacco, and betel chew. Cloves have many therapeutic uses: they control nausea and vomiting, cough, diarrhea, dyspepsia, flatulence, stomach distension and gastro intestinal spasm, relieve pain, cause uterine contractions and stimulate the nerves [1, 2]. In addition, the cloves are highly antimutagenic, antiinflammatory, antioxidant, antiulcerogenic, antithrombotic, antifungal, antiviral and antiparasitic [3].

Clove is used as an analgesic, anti-spasmodic, and as a general antiseptic in medical dental practices as a dressing in dentistry for minor wounds, as an analgesic in painful and infective diseases of the oral cavity and pharynx as well as general hygiene. Importantly, and considered also as an anti-carcinogenic agent due to its antioxidant properties [4].

Despite variations in medicinal properties across regions, the most abundant natural product in *S.aromaticum* is eugenol. For example, Cai *et al*., [5], use the headspace solid-phase micro extraction (HSPM)-gas chromatography and mass spectrometry (GC-MS) technique to detect batches of *S.aromaticum* purchased from different countries in Asia, the highest content of these volatile components are eugenol and (—)-a-selinene . Otherwise, the contents of a's-a-bisabolene, ocimene, santolinatriene, and humulene are of high percentage, which are relatively higher than that of other natural product [5].

When *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus carnosus*, and *Pseudomonas fluorescens* are first exposed to a low lethal dose of eugenol or half doses over time, eugenol shows increased antimicrobial effectiveness against *E.coli*. The population dynamics analysis result of K12 indicates that after immediate exposure to full minimum lethal concentration dose, complete cell reduction occurred, whereas sequential half doses were bacteriostatic regardless of application times [6].

Perez-Conesa *et al*., [7], indicates that, *E.coli* shows more increased susceptibility to eugenol compared with *L.monocytogenes*. The greatest antibacterial power of eugenol is seen in *E.coli* which was detected when the bacteria is exposed to eugenol for the first 10 min. Eugenol significantly inhibit the biofilm growth and increase the number of dead cells. Eugenol shows dose-dependent destructive effect on the cell integrity in Gram-positive bacteria, which is not strongly correlated with growth inhibition. However, in Gram-negative bacteria, growth inhibition is detected mostly without cell integrity loss [8]. Eugenol from clove bud oil can significantly inhibit the growth of *S.aureus* and *S.epidermidis* [9].

When *S.carnosus* is treated with eugenol, the highest antibacterial activity is detected after the addition of eugenol for 3 to 4 hours [10]. Otherwise, eugenol can cause death of vancomycin-resistant *S.aureus*, by stimulating reactive oxygen species production and damaging bacterial cell membranes [11]. Eugenol compromises biofilm stability and reduces the attachment of antibiotic-resistant *S.aureus* and does not pose a resistance development risk in a continuous mode of use [12].

Eugenol exhibits antibacterial properties against free-floating bacterial cells *S. agalactiae*, and this effect is time- dependent judged by viability analysis and the time-kill curves. Furthermore, electron microscopy reveals that eugenol impairs the survival of biofilm-associated cells during formation and in established biofilms [13]. The antibacterial activity of clove essential oil was tested against oral bacterial strains associated with dental caries using the agar disk diffusion assay [14].

Clove oil is generally considered as safe [15]. Dental caries is increasing in developing and

underdeveloping countries and treatment is expensive. Hence, there is a need to research on clove bud oil as traditional preventive measures for dental caries.

MATERIALS AND METHODS

Study Area:

This study was conducted in the Applied Microbiology Laboratory, Enugu State University of Science and Technology.

Sample Collection

A total of 50 dental caries samples were collected using sterile swab sticks from the teeth of dental caries patients from Federal College of Dental Therapy and Technology Hospital Trans-Ekulu Enugu State, Nigeria.

Collection and Preparation of Sample

A total of 800g of dried clove bud was locally purchased from Ogbete Main Market Enugu, in Enugu State. It was identified by a taxonomist in the Department of Applied Biology and Biotechnology of the Enugu State University of Science and Technology. The dried cloves were pulverized (grounded into powder) with the aid of a sterile blender and were divided equally to produce 30g of grounded samples, stored in an airtight container until required for different extraction methods.

Media Preparation

Nutrient Agar

A total of 7g of nutrient agar was weighed with a weighing balance and dissolved in 250ml of distilled water in a conical flask. The mixture was heated over a burnsen burner flame with frequent agitation and allowed to boil for 1min to completely dissolve the components. It was autoclaved at 121^0C for 15min and allowed to cool. 20ml aliquots was dispensed aseptically into petri-dishes and allowed to solidify. This was prepared according to the manufacturers guide.

MacConkey Agar (MA)

A total of 27g of macconkey agar was weighed out with a weighing balance and was dissolved in 250ml of sterile distilled water in a clean 250ml conical flask. The mixture was agitated gently to mix and placed on a burnsen flame to boil and dissolve completely. It was autoclaved at 121^0C for 15min, then allowed to cool. 20ml aliquots was dispensed aseptically into petri-dishes and allowed to solidify. This was prepared according to the manufacturer guide.

Mannitol Salt Agar (MSA)

This was prepared according to the manufacturers guide. A total of 27.5g of mannitol salt agar was weighed out with a weighing balance and was dissolved in 250ml of sterile distilled water in a clean 250ml conical flask. The mixture was agitated gently to mix and placed on a burnsen flame to boil and dissolve completely. It was autoclaved at 121^0C for 15min, then allowed to cool. 20ml aliquots was dispensed aseptically into petri-dishes and allowed to solidify.

Mueller Hinton Agar

This was prepared according to the manufacturers guide. A total of 9g of Mueller Hinton agar was weighed with a weighing balance and was dissolved in 250ml of distilled water in a conical flask. The mixture was heated over a burnsen flame with frequent agitation and allowed to boil for 1min to completely dissolve the components. It was autoclaved at 121^0C for 15min and allowed to cool. 20ml aliquots was dispensed aseptically into petri-dishes and allowed to solidify.

Preparation of the Extract

Extraction Procedure Using Soxhlet Apparatus

Total of 500g of dried and ground clove was weighed out and placed in a 500ml flask, which was submitted to hydro distillation for 4 - 6 hrs and steam distillation for 8 - 10 hrs. The volatile distillate was collected untill no oil dropped out; the distillate was saturated with sodium chloride and added with some ether [16].

Extraction Procedure Using Ethanol

The extraction was carried out according to the method of Mishra and Kalyani [17]. A total of 20g of the powdered clove samples were placed in 250ml round bottom flask. Then it was macerated using ethanol (99.7-100%vv) GPR 2.5 liter. Then it was placed on a stirrer for 24 hrs. Then the clove extract was then collected by using the rotary evaporator at (45°C) until obtaining the clove extract.

Qualitative Phytochemical Screening Tests:

Extracts from the clove bud were subjected to qualitative phytochemical screening using standard phytochemical methods as described by Harborne [18].

Test for Alkaloids

A quantity (0.4 g) of extract was stirred with 8 ml of 2% HCL and the mixture was warmed and filtered. The filtrate 2 ml was treated with potassium bismuth (Dragendroff's reagent). Turbidity or precipitation with this reagent was taken as evidence for existence of alkaloids.

Test for Saponins

The ability of saponins to produce emulsion with oil was used for the screening test. 20 mg of Extract was boiled in 20 ml of distilled water in a water bath for five minutes and filtered. 10 ml of the

filtrate was mixed with 5 ml of distilled water and shaken vigorously for froth formation. 3 drops of olive oil were mixed with froth, shaken vigorously and observed for emulsion development.

Test for Terpenoids

Presence of terpenoids in extract was carried out by taking 5 ml (1 mg/ml) of extract and mixed with 2 ml of chloroform, followed by 3 ml of con.H2SO4. A reddish brown colouration at the interface confirmed the presence of terpenoids.

Test for Steriods:

A volume of (5 drops) of concentrated H2SO4 was added to 1 ml of the extract in a test tube. A red colouration indicates the presence of steroid.

Test for Flavonoids

A quantity (50 mg) of extract was suspended in 100 ml of distilled water to get the filtrate. A 5 ml of diluted ammonia solution was added to 10 ml of filtrate followed by few drops of concentrated H2SO4. Presence of flavonoids was confirmed by yellow colouration.

Test for Tannins

A quantity (50 mg) of each of the various extracts from the stem and leaves were boiled in 20 ml of distilled H2O and filtered. A few drops of 0.1%. FeCl3 was added in filtrate and observed for colour change; brownish green or a blue-black colouration was taken as evidence for the presence of tannins.

Test for Glycosides

Glycosides are compounds which upon hydrolysis give raise to one or more sugars (glycones) and a compound which is not a sugar (aglycone or genine). To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid were added, and observed for a reddish-brown coloration at the junction of two layers and the bluish green colour in the upper layer [19].

Quantitative Phytochemical Analysis

Total Phenolic Content

Total phenolics content of the various extracts were determined using the method of McDonald *et al*., [20], with slight modifications. Calibration curve was prepared by mixing ethanol solution of Gallic acid (1 ml; 0.025 – 0.400 mg ml-1) with 5 ml Folin-Ciocalteu reagent (diluted tenfold) and sodium carbonate (4 ml, 0.7 M). Absorbance values were measured at 765 nm using a UV-VIS spectrophotometer and the standard curve was plotted using gallic acid. One milliliter of each of the extract solution in methanol (5 g/l) was also mixed with the reagents above and after 30 min the absorbance was measured to determine the total phenolic contents. The total phenolic components in the extract and fractions in gallic acid equivalents (GAE) were calculated by the following formular: T = C.V/M; where T is a total phenolic content, milligram per gram of sample extract, in GAE; C is the concentration of gallic acid established from the calibration curve, mg mL-1; V is the volume of extract, milliliter; M is the weight of sample extract (g).

Total Flavonoid Content

Total flavonoid content of the extracts was determined. In a10 ml test tube, 0.3 ml of extracts 3.4 ml of 30% methanol, 0.15 ml of NaNO2 (0.5 M) and 0.15 ml of AlCl3.6H2O (0.3M) were mixed. After 5 min, 1ml of NaOH (1 M) was added. The solution was mixed well and the absorbance was measured against the reagent blank at 506 nm. The standard curve for total flavonoids was made using rutin standard solution (0 to 100 mg/l) under the same procedure as earlier described. The total flavonoids were expressed as milligrams of rutin equivalents per g of dried fraction.

Determination of Total Alkaliods

Five (5) g of each of the extracts were weighed into a 250 ml conical flask and 200ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4hrs. This was filtered and the extract was concentrated on water bath to one – quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed [18].

Determination of Total Saponins

Each of the samples were ground and 20g of each were put into a 250ml conical flask and 100ml of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4hrs with continuous stirring at 55°c. The mixture was filtered and the residue re-extracted with another 200ml of 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90°c. The concentrate was transferred into a 250ml separating funnel and 20ml of diethyl ether was added and shaken vigorously, the aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60ml of n- butanol was added, the n-butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath after evaporation, the samples were dried in an oven to a constant weight, and the saponin content was calculated [21].

Determination of Total Glycosides

Quantities (5g) of each extract were weighed into a beaker and add 100 ml of distilled water. Soak for 3 hour and filter to get the filtrate. Then pipette 1 ml of filtrate into a test tube, followed by 2 ml of 3,5-Dinitro- salicylic acid and boil in a water bath for 10 - 15 minutes, then cool the test tubes and add 10 ml of distilled water. Read the absorbance at 540 nm. Glycoside (mg/ml) = [Absorbance ×Volume of Extract ×100]/ [1000×Weight of Sample]

GC-MS Analysis

For the Gas Chromatography-Mass Spectrometry (GC-MS) analysis, a system equipped with a capillary column (e.g., HP-5 MS, 30 m x 0.25 mm x 0.25 µm film thickness) and a mass selective detector was utilized. The GC conditions included setting the injector temperature to 250°C and using helium as the carrier gas with a constant flow rate of 1.0 mL/min. A 1 µL injection volume of the ethanol extract, diluted in ethanol if necessary, was introduced in splitless mode. The oven temperature program was initiated at 50°C with a hold time of 2 minutes, followed by an increase to 150°C at a rate of 10°C/min, and further increased to 300°C at a rate of 5°C/min, holding for 10 minutes. The MS conditions involved setting the ion source temperature to 230°C and the quadrupole temperature to 150°C, operating the MS in electron ionization (EI) mode at 70 eV, and scanning the mass range from 50 to 550 m/z.

Data analysis of GC-MS

The identification of compounds was achieved by comparing the mass spectra of the eluted compounds with those in the NIST/EPA/NIH Mass Spectral Library or other relevant spectral libraries, and the relative concentrations of the identified compounds were determined using the area under the peak in the chromatograms.

Isolation of Organisms using Culture Media

The swab sticks were streaked on Nutrient, MacConkey and Mannitol salt agar. The plates were incubated at 37°C for 24hrs. After incubation the representative colonies on the plates were counted and recorded.

Characterization and Identification of Bacteria

The inoculated plates were examined and different colonies observed using physical, biochemical morphological differences as well as molecular analysis. They were sub cultured to obtain pure cultures. The pure cultures were sub-cultured into an agar slant in bijour bottle and thereafter kept in refrigerator at 4^0C to serve as stock culture.

Biochemical Characterization of Bacterial Isolates

The isolates were identified and characterized using different biochemical tests such as; catalase, coagulase, oxidase, Indole, sugar fermentation test and molecular analysis [22].

Catalase

Emulsify the test organism with 1 or 2 drops of hydrogen peroxide on a glass slide. An observed bubbling of air effect indicated oxygen gas production and such bacteria is said to be catalase positive [24].

Coagulase

Make a dense suspension of the organism using 1-2 drops of distilled water on a glass slide (Observe for auto agglutination). Mix with a loopful of EDTA plasma and observe for clumping or agglutination [24].

Citrate Test

A loop full of the test organism was picked from the nutrient agar plates, using a sterile glass rod and lightly inoculated on Simon citrate agar slants and incubated at 370c for 24 hours. A positive test result was looked out for with the development of a blue colour in the medium [25].

Indole

Inoculate a bijou bottle containing peptone water with the test organism. Incubate at 35-37^0C for 24-48 hours. Then add 0.5mls of kovac's reagent. Shake gently and examine for a red colour on the surface. The formation of pink to red ring colour indicates positive test while no colour change indicates negative test [25].

Oxidase Test

Filter papers were soaked with tetramethyl-p-phenylenediaminedihydrochloride substrate. The filter papers were moistened with sterile distilled water. The isolates were collected with a wooden stick and smeared on the filter paper [26].

Methyl Red Test

A loop full of the test organism was inoculated into a tube containing 1% glucose phosphate was incubated at 37^0C for 24h. A volume of 2.5ml of this suspension was transferred into a sterile tube and few drops of methyl red reagent were added. Absence of color change indicates negative result [27].

Sugar Fermentation Test

A total of 5ml of 2% sugar solutions of glucose, fructose, lactose, sucrose and mannitol which has already been sterilized with membrane filter were dispensed into 5 test tubes containing 5ml of peptone water and a colony of test organism was

inoculated into the test tubes, 3 drops of methyl red was added. Durham's tubes were inverted into the test tubes and incubated for 48 hours. The yellow color observed indicated acid formation, yellow color plus a gas accumulation in Durham's tube indicated acid gas formation while red color indicated no fermentation.

Standardization of Inoculum

Pure cultures of identified bacterial isolates from a 24hour plate culture was selected. Sterile wire loop was used to pick 2 to 3 colonies of each isolate and emulsified into test tubes containing 5ml of sterile saline, they were vortexed thoroughly. Adjustment was made with extra inoculums or diluents, until 0.5 McFarland turbidity standards were obtained [22].

Antibacterial Assay

A total of 0.1ml of the organism already matched to McFarland turbidity standards were inoculated into Mueller Hinton agar plates. The inoculated plates were air dried at 37^0C to allow for any excess surface moisture to be absorbed before applying the antibiotic discs. After the inoculation, the antibiotic discs for bacteria were placed on the inoculated media with the different isolates respectively using sterile forceps. The disc was gently pressed down to ensure complete contact with the agar surface. The plates were inverted and incubated at 37^0C for 18 hours. Where there were zones of inhibition, they were measured using caliper to determine the level of sensitivity of the drugs. Results were recorded as sensitive, intermediate or resistant [28].

Molecular Identification:

Some bacterial isolates were further characterized by PCR. This was done by Bioinformatics Ibadan, Oyo State, Nigeria.

RESULTS

Table 1: Qualitative Phytochemical Composition of Clove Extract and Oil

S/N	***Constituent***	***Experimental method***		
Secondary Metabolites			**Ethanol Extract**	**Clove oil**
1	Saponins	Foam Test	++	++
2	Tannin (Catecholic)	Ferric Chloride Test	++	ND
3	Flavonoids	Lead acetate test	++	ND
		Alkaline Test	+	ND
		Shinoda test	ND	ND
		Pew'sTest	ND	ND
		Conc H_2SO_4	++	ND
		Ferric chloride	ND	ND
4	Alkaloids	Wagner's Test	++	ND
		Mayer's Test	++	ND
		Hager's test	+	ND
		Iodine Tess	ND	ND
5	Cardiac Glycosides	Keller-kilani test	++	++
6	Phenol	Iodine test	ND	ND
		5% $Fecl_3$ test	++	ND
		Lead acetate test	++	ND
		Ellagic acid test	+	ND
		Potassium dichlromate test	ND	ND
7	phlobatannins	Hcl test	+	ND
8	phytosteriods		++	++
9	Terpinoids		+	++
10	Triterpinoids	Salkowski's test	++	+
11	Diterpenes	Copper acetate	+	ND
12	Quinones	Conc Hcl test	ND	ND
13	Anthraquiones	Borntrager's test	ND	ND
14	Leuconthocyanins test	Isoamyl acohol test	ND	ND
15	Resins	Acetic anahydride test	ND	ND
16	Fixed oil and fat	Spottest/ stain test	+	++
17	Carbohydrates and Redcing sugars	Molish's test	+	ND
		seliwanoff's tset	ND	ND
		Iodine test	ND	ND
		Benedict's test	+	ND

		Barfford test	ND	ND
18	Proteins and Amino acids	Biuret test	+	ND
		Ninhydrin test	+	ND
		Xanthoprotei test	ND	ND

+ Present in trace concentration; Present in moderately high concentration ++,
+++ Present in very high concentration; ND- (not detected), NT (not tested).

Table 2: Quantitative Phytochemical Composition of Clove Extract and Oil

S/N	Sample Id	% Alkaloids	% Saponins	% Flavonoids	% Phenols	% Tannins	% Cardiac Glycosides
1.	Ethanol Extract	5.033	3.50	0.407	0.4545	5.227	2.1285
2.	Clove oil	ND	8.70	ND	ND	ND	0.0375

Table 3: Phytochemical Constituent in Ethanol Extract of *Clove* Extract in GC-MS analysis

S/N	Name of compound	Molecular Formula	Molecular Weight (g/mol)
1	Vanillin	$C_8H_8O_3$	152.047344
2	Caryophyllene	$C_{15}H_{24}O$	204.1878
3	Phenol, 4-(2-propenyl)-	$C_9H_{10}O$	134.073165
4	Phenol, 2-methoxy-3-(2-propenyl)-	$C_{10}H_{12}O_2$	164.08373
5	Methyl propyl ether	$C4H_{10}$	74.073165
6	Humulene	$C_{15}H_{24}$	204.1878
7	Phenol, 2-methoxy-4-(2-propenyl)-, acetate	$C_{12}H_{14}O_3$	206.094295
8	1,Z-5,E-7-Dodecatriene	$C_{12}H_{20}$	164.156501
9	Bicyclo[6.1.0]nonane, 9-(1-methylethylidene)-	$C_{12}H_{20}$	164.156501
10	10,10-Dimethyl-2,6-dimethylenebicyclo[7.2.0]undecan-5β-ol	$C_{15}H_{24}O$	220.182715
11	Caryophyllene oxide	$C_{15}H_{24}O$	220.182715
12	Cedranoxide, 8,14-	$C_{15}H_{24}O$	220.182715
13	Heptadecane, 2-methyl-	$C_{16}H_{32}O_2$	256.24023
14	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.24023
15	3-Allyl-6-methoxyphenol	$C_{10}H_{12}O_2$	164.08373
16	Phenol, 2-methoxy-4-(1-propenyl)-	$C_{10}H_{12}O_2$	164.08373
17	1-Bromoeicosane	$C_{20}H_{41}Br$	360.239164
18	Eugenol	$C_{10}H_{12}O_2$	164.08373

Table 4: **Percentage Yield of Clove Oil**

S/N	Weight of Clove Powder (Grams)	Weight of Oil (grams)	Volume of Oil (mls)	Percentage Yield
1	465	13.87	14	2.98

Table 5: Characterization Result of Clove Oil Extract

S/N	Parameter	Values	Units
1	Density	0.86	g/cm^3
2	Viscosity	113.59	Mm^2/s
3	Refractive Index	≥36	% brix
4	SAPONIFICATION Value	39.19	Mg/KOH
5	Iodine Value	21.488	g/ml
6	Peroxide Value	13.4	mEq/kg
7	Free Fatty Acid	1.37	%

Table 6: Morphological and Biochemical Identification of Isolates from the Samples

S/N	Growth Appearance on Media	Gram reaction	Cat test	Oxi test	Cit test	Coa test	Methyl red test	Indole test	Glu	Fru	Mal	Suc	Lac	Suspected Organisms
1	Small, irregular, gray or yellowish colonies on NA	+v short rod in pairs	+v	-v	+v	-v	-v	-v	A	A	A	A	A	*Actinomyces spp.*
2	Small, smooth, circular, creamy-white colonies on NA	+v cocci	+v	-v	+v	-v	-v	-v	A	A	AG	G	A	*Streptococcus spp.*
3	Creamy-white circular, convex colonies	+v cocci	-v	-v	-v	-v	-v	-v	A	A	A	A	A	*Lactococcus spp.*
4	Small, whitish to cream-colored colonies on Nutrient Agar	+v short Rod	-v	-v	-v	-v	-v	-v	A	A	A	A	A	*Lacticaseibacilluspa racasei*
5	Creamy yellow, small, round and opaque colonies	+v cocci	+v	-v	-v	+v	-v	-v	A	A	A	A	A	*Staphylococcus aureus strain CIB*
6	Cream-colored colonies	+v Rod	-v	-v	+v	-v	-v	-v	A	A	A	A	A	*Bifidobacteriumspp*
7	Yellow, small, round and opaque colonies	+v cocci	+v	-v	-v	+v	-v	-v	A	A	A	A	A	*Staphylococcus aureus strain B3A22*
8	Whitish small, pinpoint, and translucent colonies	-v cocci	-v	-v	+v	-v	-v	-v	-v	-v	-v	-v	-v	*Veillonellaspp*

9	Very large whitish cottony colony on nutrient agar.	+v short rod	-v	-v	+v	-v	-v	-v	A	A	A	-v	-v	*Bacillus subtilis*
10	Small, whitish to cream-colored colonies on Nutrient Agar	+v short Rod	-v	-v	-v	-v	-v	-v	A	A	A	A	A	*Lacticaseibacilluspa racasei*

KEY: Cat=Catalase test, Cit= Citrate test, Coa=Coagulase test, Ind=Indole test, Oxi=Oxidase test, Gl=Glucose, F=D-Fructose, Ml=Maltose, Suc= Sucrose, La=Lactose, +=positive, **-** =negative, A=Acidic, AG=Acidic and Gas, G=Gas, +ve=positive, -ve=negative

Table 7: Antimicrobial Activity of Clove Oil Against Microbes Isolated from Samples Infected with Dental Caries

S/N	Isolates	Concentrations (mg/ml)					
		100	50	25	12.5	6.25	AZ (mm)
1	*Bacillus subtilis*	12	6	2	0	0	15
2	*Lacticaseibacillus paracasei*	6	0	0	0	0	20
3	*Lactobacillus agile* 1365	14	8	3	0	0	20
4	*Staphylococcus aureus* strain CIB	12.5	7.5	3	0	0	20
5	*Staphylococcus aureus* strain B3A22	10	6	0	0	0	20

Key: ≤ 7 - ≤ 9 = Resistance, ≥ 10 - ≥ 20 = Susceptible, AZ - Azithromycin

Table 8: Antibacterial Activity of Clove Extract Against Microbes Isolated from Samples Infected with Dental Caries

S/N	Isolates	Concentrations (mg/ml)					
		100	50	25	12.5	6.25	AZ (mm)
1	*Bacillus subtilis*	14	8	5	2	0	15
2	*Lacticaseibacillus paracasei*	16	11	8	4	0	20
3	*Lacticaseibacillus agile* 1365	15	10	6	2.5	0	20
4	*Staphylococcus aureus* strain CIB	10	7	3	0	0	20
5	*Staphylococcus aureus* strain B3A22	10	6	3.2	0	0	20

Key: ≤ 7 - ≤ 9 = Resistance, ≥ 10 - ≥ 20 = Susceptible, AZ - Azithromycin

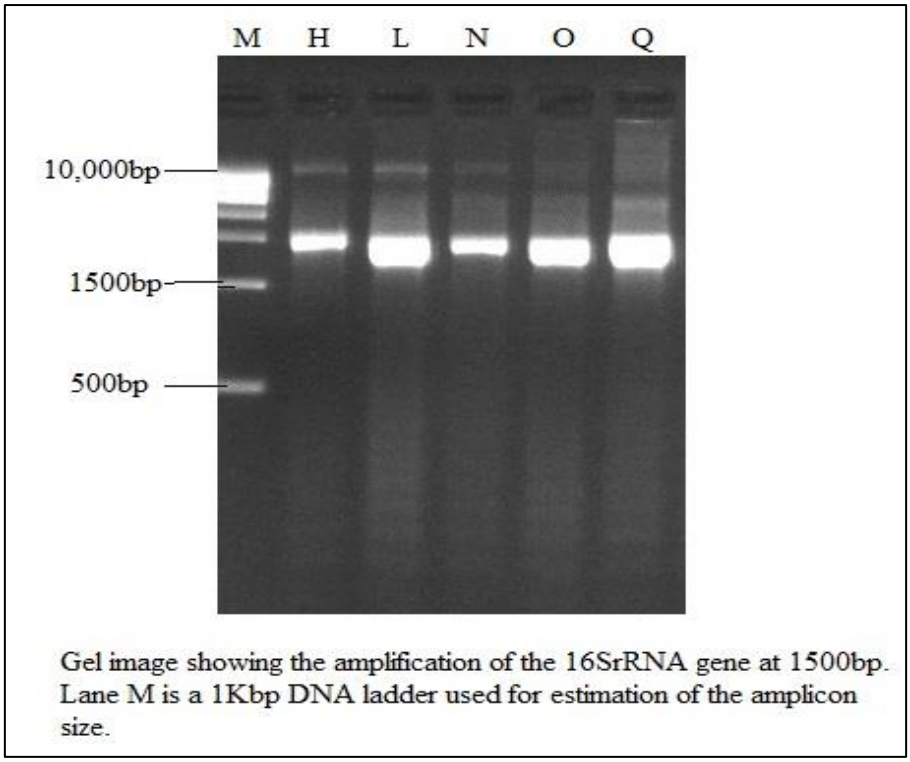
Table 9: Minimum Inhibitory Concentration of Clove Oil and Extracts against Test Organisms

Isolates	Oil	Extract
Bacillus subtilis	25mg	12.5mg
Lacticaseibacillus paracasei	100mg	12.5mg
Lacticaseibacillus agile 1365	25mg	12.5mg
Staphylococcus aureus strain CIB	25mg	25mg
Staphylococcus aureus strain B3A22	50mg	25mg

Table 10: Minimum Bactericidal Concentration of Clove Oil and Extracts against Test Organisms

Isolates	Oil	Extract
Bacillus subtilis	50mg/ml	25mg/ml
Lacticaseibacillus paracasei	-	50mg/ml
Lacticaseibacillus agile 1365	25mgml	25mg/ml
Staphylococcus aureus strain CIB	50mg/ml	50mg/ml
Staphylococcus aureus strain B3A22	100mg/ml	25mg/ml

Key: - = no activity



Gel image showing the amplification of the 16SrRNA gene at 1500bp. Lane M is a 1Kbp DNA ladder used for estimation of the amplicon size.

Figure 1: Gel Image showing the Amplification of the 16SrRNA gene at 1500bp

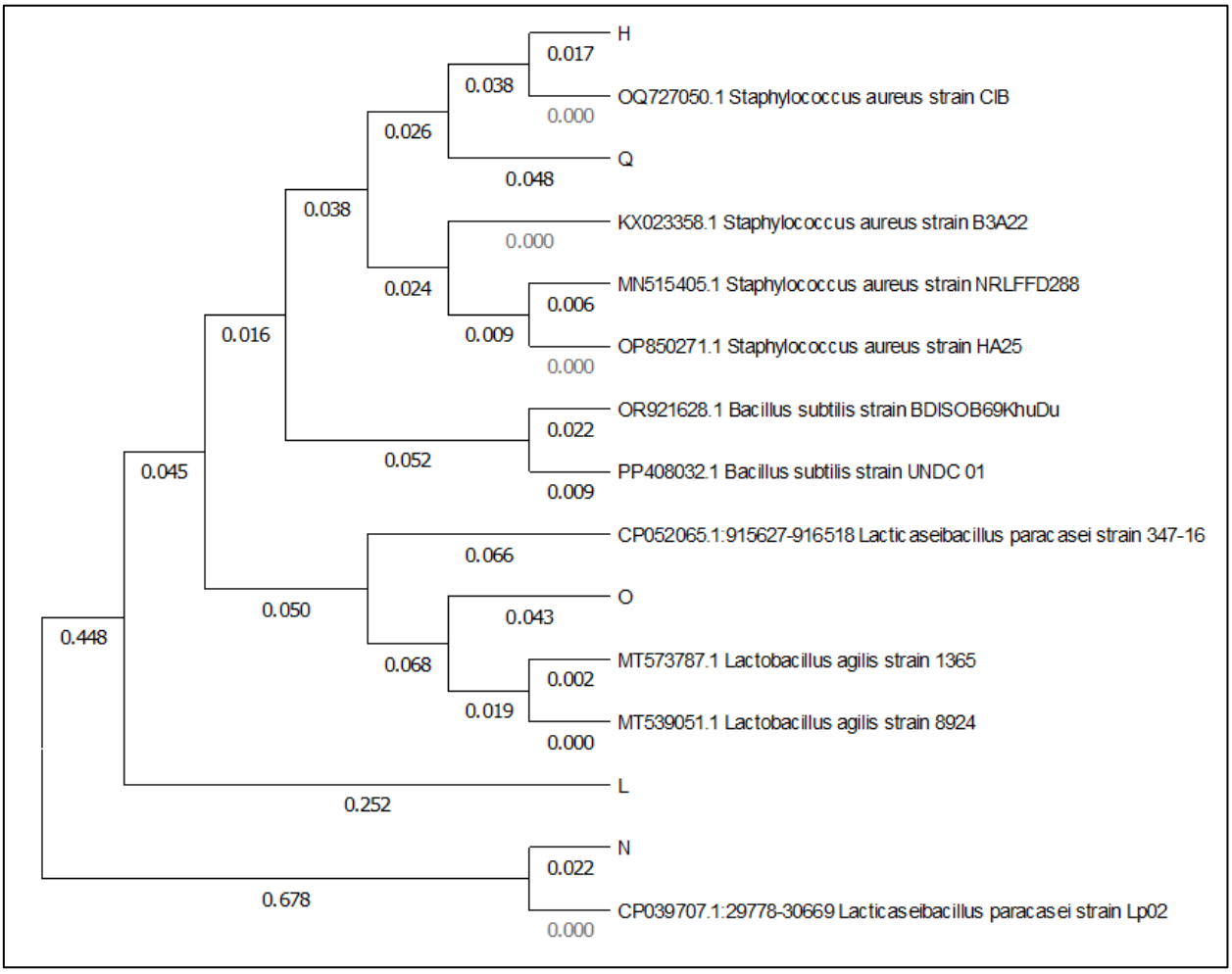


Figure 2: The Phylogenetic Relatedness of the Isolates Isolated from Dental Caries

DISCUSSION

Table 1 provides a qualitative analysis of the phytochemical constituents present in the ethanol extract of clove. The analysis reveals a broader spectrum of phytochemicals in the ethanol extract compared to the clove oil, highlighting the presence of compounds such as flavonoids, alkaloids, phenols, and tannins. The broader range of phytochemicals observed in the ethanol extract can be attributed to the solvent properties of ethanol, a polar solvent known for its efficacy in extracting a wide variety of both polar and non-polar compounds. Ethanol's ability to dissolve diverse phytochemicals, including flavonoids, alkaloids, and phenols, has been well-documented in various studies [29]. These compounds are more soluble in polar solvents, which explains their presence in the ethanol extract. The findings of this qualitative analysis align with existing literature on the phytochemical composition of clove extracts which reported the presence of substantial amounts of phenolic compounds, including tannins and flavonoids, in clove extracts, corroborating the detection of these compounds in the ethanol extract in this study [30].

Table 2 presents the quantitative phytochemical composition of clove extract and clove oil which highlights distinct differences in the concentration of various bioactive compounds. The ethanol extract of clove shows a diverse range of phytochemicals with significant quantities, including 5.033% alkaloids, 3.50% saponins, 0.407% flavonoids, 0.4545% phenols, 5.227% tannins, and 2.1285% cardiac glycosides. In contrast, the clove oil primarily contains saponins at a concentration of 8.70%, with very low levels of cardiac glycosides (0.0375%) and an absence of alkaloids, flavonoids, phenols, and tannins. The higher concentration of saponins in clove oil (8.70%) compared to the ethanol extract (3.50%) suggests that saponins are more effectively extracted by the distillation process used to obtain the oil. Saponins are amphiphilic compounds, meaning they have both hydrophilic and hydrophobic properties, which can make them more amenable to extraction in oil. The absence of phytochemicals such as flavonoids, phenols, and tannins in clove oil is notable, as these compounds are often less volatile and more polar, making them less likely to be extracted during steam distillation, a process that typically favors the extraction of volatile and hydrophobic compounds like terpenoids [31]. The implications of these findings are significant for understanding the potential applications of clove extracts and oils. The high saponin content in clove oil suggests robust surfactant and antimicrobial properties, which could be particularly useful in topical formulations or as natural preservatives. Saponins are known for their ability to disrupt microbial cell membranes, thereby exerting strong antibacterial effects [32].

Table 3 shows the GC-MS analysis which reveals a range of phytochemicals, predominantly phenolic compounds and terpenes, known for their bioactive properties. Notable compounds include Vanillin ($C_8H_8O_3$, 152.047344 g/mol), Caryophyllene ($C_{15}H_{24}O$, 204.1878 g/mol), Eugenol ($C_{10}H_{12}O_2$, 164.08373 g/mol), and several phenolic compounds such as Phenol, 4-(2-propenyl)- ($C_9H_{10}O$, 134.073165 g/mol). The table highlights the diversity of bioactive compounds present in clove extract, showcasing its potential for various applications. This is in agreement with research that showed the presence of multiple phenolic compounds, such as Phenol, 4-(2-propenyl)-, and Phenol, 2-methoxy-4-(1-propenyl)-, suggests a significant contribution to the overall antioxidant potential of the clove extract. Eugenol, Caryophyllene, and Humulene as prominent constituents, reinforcing their role in the bioactivity of clove oil [33]. Eugenol's ability to inhibit bacterial growth and alleviate pain makes it a valuable ingredient in dental care products, tropical analgesics, vanillin and eugenol, both phenolic compounds, are recognized for their antioxidant and antimicrobial activities [34].

Table 4 presents the percentage yield of clove oil, which was calculated to be 2.98%. This yield was obtained by extracting oil from 465 grams of clove powder, resulting in 13.87 grams of oil. The yield of 2.98% falls within the typical range of 2-4% for clove oil extraction, depending on factors such as the method used and the geographical origin of the clove [35]. The consistency of this result with other studies suggests that the extraction process employed is effective and that the clove material used was of good quality [35]. A yield within the expected range ensures that the process can be replicated on a larger scale, maintaining both quality and cost-effectiveness [35]. This level of yield also suggests that the clove oil extracted is likely to contain a significant concentration of its active components [35]. Singh *et al*., [35], reported similar yields when extracting clove oil using comparable methods. This consistency not only validates the current study findings but also highlights the potential for standardizing clove oil extraction processes across different settings to achieve consistent results.

Table 5 characterizes the clove oil extract, highlighting several key parameters: a density of 0.86 g/cm^3, a viscosity of 113.59 mm^2/s, a refractive index of ≥36% Brix, a saponification value of 39.19 mg KOH/g, an iodine value of 21.488 g I_2/100g, a peroxide value of 13.4 mEq/kg, and a free fatty acid content of 1.37%. The physical properties of clove oil, such as its density and viscosity, are crucial in

determining its stability and application. The high viscosity suggests that clove oil can act as a protective barrier in topical formulations, which is advantageous in preventing microbial infections when applied to the skin [30]. These findings are consistent with previous studies of Gülçin *et al*., [30], who reported similar characterization results, particularly highlighting the antioxidant potential of clove oil due to its phenolic content. Bakkali *et al*., [31], discussed the implications of peroxide and free fatty acid values in essential oils, corroborating the findings in this study regarding the stability and shelf-life of clove oil.

Table 6 provides detailed information on the morphological and biochemical characteristics of bacterial isolates from dental caries samples. The isolates include *Actinomyces spp., Streptococcus spp., Lactococcus spp., Lacticaseibacillus paracasei, Staphylococcus aureus strains CIB* and *B3A22, Bifidobacterium spp., Veillonella spp.,* and *Bacillus subtilis.*

Table 7 displays the antimicrobial activity of clove oil against various microbial isolates from dental caries, measured as inhibition zones at different concentrations (100, 50, 25, 12.5, 6.25 mg/ml). At 100 mg/ml, inhibition zones are notably large, with *Lacticaseibacillus agile* 1365 showing the highest at 14 mm, and *B.subtilis* and *S.aureus* strain CIB showing substantial inhibition at 12 mm and 12.5 mm, respectively. At lower concentrations (50 mg/ml and below), inhibition zones decrease significantly, with many isolates showing minimal or no inhibition at 12.5 mg/ml and 6.25 mg/ml. This suggests that clove oil is most effective at higher concentrations in Inhibiting the growth of these microbes. The control antibiotics disc Azithromycin showed that clove oil was susceptible against *B. subtilis, L. agile 1365, S. aureus strain CIB,* and *S. aureus strain B3A22.* This. This concentration-dependent effect aligns with findings from other studies, such as that by Bakkali *et al*., [31], which also observed enhanced antimicrobial properties of essential oils, including clove oil, at higher concentrations. Similarly, Singh *et al*., [35], found that clove oil's antimicrobial activity is enhanced with increasing concentrations, corroborating the findings of the result.

Table 8 presents the antimicrobial activity of clove extract against various microbial isolates from dental caries, measured as inhibition zones in millimeters at different concentrations (100, 50, 25, 12.5, 6.25 mg/ml). At the highest concentration of 100 mg/ml, clove extract demonstrates substantial inhibition, with *L.paracasei* showing the largest inhibition zone of 16 mm, followed by *L.agile* 1365 at 15 mm, and *B.subtilis* at 14 mm. The effectiveness decreases with lower concentrations: at 50 mg/ml, inhibition zones are reduced for most isolates, and at 25 mg/ml and below, the inhibition is minimal or absent for several microbes. The data reveal a clear concentration-dependent antimicrobial activity pattern for clove extract. At the highest concentration of 100 mg/ml, the extract shows the largest inhibition zones: *L.paracasei* has the highest at 16 mm, followed by *L.agile 1365* at 15 mm, and *B.subtilis* at 14 mm. Inhibition decreases significantly at lower concentrations; at 50 mg/ml, the inhibition zones reduce to 11 mm for *L.paracasei*, 10 mm for *L.agile* 1365, and 8 mm for *B.subtilis*. At 25 mg/ml, inhibition zones further decrease, and at 12.5 mg/ml and 6.25 mg/ml, the zones are minimal or absent for most isolates.

Table 9 presents the Minimum Inhibitory Concentration (MIC) values of clove oil and clove extract against various microbial isolates. For *B.subtilis*, clove oil shows an MIC of 25 mg/ml, while clove extract is more potent, with an MIC of 12.5 mg/ml. *L.paracasei* exhibits a much higher MIC of 100 mg/ml for clove oil, but the MIC for clove extract is significantly lower at 12.5 mg/ml. For *L.agile 1365*, both clove oil and clove extract have an MIC of 25 mg/ml and 12.5 mg/ml, respectively. *S.aureus* strain CIB shows an MIC of 25 mg/ml for both clove oil and clove extract. Similarly, *S.aureus* strain B3A22 has an MIC of 50 mg/ml for clove oil and 25 mg/ml for clove extract. The data demonstrate that clove extract generally exhibits lower MIC values compared to clove oil, indicating higher potency. *B.subtilis* has an MIC of 25 mg/ml for clove oil but 12.5 mg/ml for clove extract.

Table 10 provides the Minimum Bactericidal Concentration (MBC) values of clove oil and clove extract against various test organisms. For *B.subtilis*, clove oil has an MBC of 50 mg/ml, while clove extract is more effective with an MBC of 25 mg/ml. *L.paracase*i shows no activity with clove oil, but the extract has an MBC of 50 mg/ml. For *L.agile 1365*, both clove oil and clove extract have the same MBC of 25 mg/ml. *S.aureus* strain CIB also shows an MBC of 50 mg/ml for both clove oil and extract. Notably, *S.aureus* strain B3A22 has a much higher MBC of 100 mg/ml for clove oil compared to 25 mg/ml for the extract. The data highlight a trend where clove extract generally exhibits lower MBC values compared to clove oil, indicating higher bactericidal potency. *B.subtilis* requires an MBC of 50 mg/ml for clove oil but only 25 mg/ml for the extract, showing that the extract is more effective in killing this bacterium. *S.aureus strain B3A22* shows a significant difference with an MBC of 100 mg/ml for clove oil versus 25 mg/ml for the extract.

Figure 1 presents the gel electrophoresis results of the 16SrRNA gene amplification from

various bacterial isolates. This technique enables the identification of bacteria at the genus and species levels based on the sequence of the 16SrRNA gene, which is highly conserved among bacteria.

Figure 2 depicts the phylogenetic tree of the bacterial isolates. The control antibiotics disc Azithromycin showed that clove extract was susceptible against all the bacteria identified. The bacteria identified were H has 98.78% pairwise identity with *S.aureus* strain CIB, L has 86.18% pairwise identity with *B.subtilis* strain BDISOB69KhuDu, N has 91.41% pairwise identity with *L.paracase*i, O has 98.16% pairwise identity with *L.agilis* strain 1365, Q has 93.83% pairwise identity with *S.aureus* strain B3A22. This illustrate their evolutionary relationships based on genetic similarities. The tree is constructed from 16S rRNA gene sequences, which are used to determine the relatedness of the isolates. Each branch represents a different bacterial species, with closer branches indicating higher genetic similarity. The isolates are grouped according to their pairwise identity percentages, showing their proximity to well characterized reference strains. This visual representation helps to identify the isolates' taxonomic classifications and provides insight into their genetic diversity and evolutionary history.

CONCLUSION

This study demonstrates the significant antibacterial properties of clove bud and its oil against bacteria associated with dental caries. The results reveal that both clove oil and clove extract exhibit strong antimicrobial activity, with clove extract being more potent at lower concentrations than clove oil. The phytochemical analysis confirmed the presence of several bioactive compounds, including eugenol, which likely contributes to the observed antimicrobial effects. The clove extract was particularly effective against various bacterial isolates, suggesting its potential as an alternative treatment for dental caries.

Recommendation

Given the potent antibacterial activity of clove extract, especially at lower concentrations, it is recommended that clove extract be further explored as a natural additive in dental care products. Its inclusion could provide an effective means of preventing and treating dental caries. Future research should focus on optimizing the formulation and concentration of clove-based products to ensure both efficacy and safety in clinical applications. Additionally, studies on the long-term effects and potential side effects of using clove extract in oral health care should be conducted to ensure its suitability for widespread use.

REFERENCES

1. Elujoba, A. A., Awoniran, O. M., & Soriyan, H. A. (2015). A framework for knowledge capture in African traditional treatment of malaria. *Nigerian Journal of Natural Products and Medicine, 1213*, 56.
2. Suliman, G. M., Alowaimer, A. N., Al-Mufarrej, S. I., Hussein, E. O., Fazea, E. H., Naiel, M. A., & Swelum, A. A. (2021). The effects of clove seed (Syzygium aromaticum) dietary administration on carcass characteristics, meat quality, and sensory attributes of broiler chickens. *Poultry Science, 100*(3), 1009-04.
3. Yang, Z., Xie, Y., Cao, D., Rong, F., Ding, H., & Zhang, D. (2015). Antitermitic and antifungal activities of eugenol and its congeners from the flower buds of Syzgium aromaticum (clove). *Industrial Crops and Products, 77*, 780-786.
4. Cai, J., Lu, J., Li, Q., Guo, S., & Dai, Y. (2015). Analysis on volatile components of Caryophylli Flos from different habitats. *Plant Science Journal, 33*, 251–258.
5. Cai, L., & Wu, C. D. (2016). Compounds from Syzygium aromaticum possessing growth inhibitory activity against oral pathogens. *Journal of Natural Products, 59*(10), 987-990.
6. Manrique, Y., Gibis, M., Schmidt, H., & Weiss, J. (2016). Antimicrobial efficacy of sequentially applied eugenol against food spoilage micro-organisms. *Journal of Applied Microbiology, 121*, 1699–1709.
7. Pérez-Conesa, J., Cao, L., & Chen, L. (2011). Inactivation of Listeria monocytogenes and Escherichia coli O157: H7 biofilms by micelle-encapsulated eugenol and carvacrol. McLandsborough, J Weiss. *Journal of Food Protection, 74*(1), 55-62.
8. Thapa, D., Losa, R., Zweifel, B., & Wallace, R. J. (2012). Sensitivity of pathogenic and commensal bacteria from the human colon to essential oils. *Microbiology, 158*, 2870–2877.
9. Morán, A., Gutiérrez, S., Martínez-Blanco, H., Ferrero, M. A., Monteagudo-Mera, A., & Rodríguez-Aparicio, L. B. (2014). Non-toxic plant metabolites regulate Staphylococcus viability and biofilm formation: A natural therapeutic strategy useful in the treatment and prevention of skin infections. *Biofouling, 30*, 1175–1182.
10. Manrique, Y., Gibis, M., Schmidt, H., & Weiss, J. (2017). Influence of application sequence and timing of eugenol and lauric arginate (LAE) on survival of spoilage organisms. *Food Microbiology, 64*, 210–218.
11. Das, B., Mandal, D., Dash, S. K., Chattopadhyay, S., Tripathy, S., Dolai, D. P., & Roy, S. (2016). Eugenol provokes ROS-mediated membrane damage-associated antibacterial activity against clinically isolated multidrug-resistant Staphylococcus aureus strains. Infectious Diseases (Auckland), 9, 11–9.
12. Apolónio, J., Faleiro, M. L., Miguel, M. G., & Neto, L. (2014). No induction of antimicrobial resistance in Staphylococcus aureus and Listeria

monocytogenes during continuous exposure to eugenol and citral. *FEMS Microbiology Letters, 354*, 92–101.
13. Perugini Biasi-Garbin, R., Saori Otaguiri, E., Morey, A. T., Fernandes da Silva, M., Belotto Morguette, A. E., Armando Contreras Lancheros, C., ... & Yamada-Ogatta, S. F. (2015). Effect of eugenol against Streptococcus agalactiae and synergistic interaction with biologically produced silver nanoparticles. *Evidence-Based Complementary and Alternative Medicine*, *2015*(1), 861497.
14. Marchese, A., Barbieri, R., Coppo, E., Orhan, I. E., Daglia, M., Nabavi, S. F., ... & Ajami, M. (2017). Antimicrobial activity of eugenol and essential oils containing eugenol: A mechanistic viewpoint. *Critical reviews in microbiology*, *43*(6), 668-689.
15. Choudhary, R., Mishra, K. P., & Subramanyam, C. (2006). Interrelations between oxidative stress and calcineurin in the attenuation of cardiac apoptosis by eugenol. *Molecular and cellular biochemistry*, *283*, 115-122.
16. Golmakani, A., Fatemi, S., & Tamnanloo, J. (2017). Investigating PSA, VSA, and TSA methods in SMR unit of refineries for hydrogen production with fuel cell specification. *Separation and Purification Technology*, *176*, 73-91.
17. Mishra, R. P., & Kalyani, S. (2014). Antimicrobial activities of Syzigium aromaticum L.(Clove). *Int Res JBiological Sci*, *3*(8), 22-25.
18. Harborne, A. J. (1998). Phytochemical methods a guide to modern techniques of plant analysis. springer science and business media. Hostettmann, K., & Marston, A. (2005). Saponins. Cambridge University Press.
19. Siddiqui, A. A., & Ali, M. (1997). *Practical Pharmaceutical Chemistry*. CBS Publishers and Distributors.
20. McDonald, S., Prenzler, P. D., Antolovich, M., & Robards, K. (2001). Phenolic content and antioxidant activity of olive extracts. *Food Chemistry, 73*(1), 73-84.
21. Obadoni, B. O., & Ochuko, P. O. (2002). Phytochemical studies and comparative efficacy of the crude extracts of some haemostatic plants in Edo and Delta States of Nigeria. *Global Journal of Pure and Applied Sciences, 8*(2), 203-208.
22. Meena, V. S., Maurya, B. R., Verma, J. P., Aeron, A., Kumar, A., Kim, K., & Bajpai, V. K. (2015). Potassium solubilizing rhizobacteria (KSR): isolation, identification, and K-release dynamics from waste mica. *Ecological Engineering, 81*, 340-347.
23. Tasie, F. O., & George-Okafor, U. O. (1999). Laboratory methods in microbiology. *Colours Communication Publishing Company Enugu, 5*(11), 24-26.
24. Nwadioha, S. I, Odimayo, M. S., & Abayomi, F. (2023). Laboratory Practical Manual for Medical Microbiology and Parasitology Laboratory, Second Edition, Chapter Five; staining and staining techniques in Microbiology. Chapter Ten, biochemical test for identification of bacteria isolates (pp 40, 54-57)
25. Cappuccino, J. C., & Sherman, N. (2005). Negative staining. Microbiology: *A Laboratory Manual, 3*, 125-179.
26. Shields, P., & Cathcart, L. (2010). Oxidase test protocol. *American Society for Microbiology, 4*, 1-9.
27. Cheesbrough, M. (2006). *District laboratory practice in tropical countries*, part 2. Cambridge university press.
28. National Committee for Clinical Laboratory Standards. (2004). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. Approved Standard 6th Edition: M07-A6, Clinical and Laboratory Standards Institute, Wayne, PA.
29. Harborne, A. J. (1998). *Phytochemical methods a guide to modern techniques of plant analysis*. springer science and business media.
30. Gülçin, İ., Elmastaş, M., & Aboul-Enein, H. Y. (2012). Antioxidant activity of clove oil–A powerful antioxidant source. *Arabian Journal of Chemistry, 5*(4), 489-499.
31. Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils–a review. *Food and Chemical Toxicology, 46*(2), 446-475.
32. Sparg, S. G., Light, M. E., & Van Staden, J. (2004). Biological activities and distribution of plant saponins. *Journal of Ethnopharmacology, 94*(2-3), 219-243.
33. Kamatou, G. P. P., Vermaak, I., & Viljoen, A. M. (2012). Eugenol—from the remote Maluku Islands to the international market place: A review of a remarkable and versatile molecule. *Molecules, 17*(6), 6953-6981.
34. Chaieb, K., Hajlaoui, H., Zmantar, T., Kahla-Nakbi, A. B., Rouabhia, M., Mahdouani, K., & Bakhrouf, A. (2007). The chemical composition and biological activity of clove essential oil, Eugenia caryophyllata (Syzigium aromaticum L. Myrtaceae): A short review. Phytotherapy Research: *An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 21*(6), 501-506.
35. Singh, G., Maurya, S., de Lampasona, M. P., & Catalan, C. (2007). A comparison of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils, oleoresins and their constituents. *Food and Chemical Toxicology, 45*(9), 1650-1661.