Antibacterial and Phytochemical Properties of Clove Extracts on Vegetable Spoilage Bacteria

ABSTRACT

The present study was carried out to analyze the antibacterial and phytochemical properties of clove extracts on vegetable spoilage bacteria. The study seeks to determine its potential use as bio-preservative. The bacteria used for this study were isolated from infected leaves of bitter leaf, pumpkin and cocoyam using spread plate technique. Phytochemical analysis was carried out with aqueous and methanol extracts. Bacterial isolates from the leaves included *Bacillus* spp., *Micrococcus* spp., *Staphylococcus* spp. and *Actinomyces* spp. The pumpkin leaf had the highest average number of bacterial colonies of 2.5 x 10^2 while cocoyam leaf had the least with 7.0 x 10^1.*Actinomycetes* sp_2 had the highest zone of inhibition of 22.0 mm while *Staphylococcus* sp_2 had the least zone of inhibition of 13.00 mm all at 500mg/ml. For aqueous extracts, *Bacillus* $sp._3$, *Staphylococcus* $sp._1$ had the highest zone of inhibition of 16.00 mm respectively while the least zones were observed in *Staphylococcus* $sp._2$ and *Bacillus* $sp._3$ at concentration of 250 mg/ml and 125 mg/ml respectively. *Bacillus* $sp._1$, *Bacillus* $sp._2$, *Micrococcus* sp. and *Actinomyces* $sp._1$ has Minimum Inhibition Concentration (MIC) of 125 mg/ml while *Staphylococcus* $sp._1$ and *Staphylococcus* $sp._2$ has 250mg/ml respectively for methanol extract. For aqueous extract, *Bacillus* $sp._2$, *Micrococcus* sp. and *Actinomyces* $sp._1$ has MIC of 250mg/ml, respectively and *Staphylococcus* $sp._1$ and *Staphylococcus* $sp._2$ has MIC of 500 mg/ml. The Minimum Bactericidal Concentration (MBC) for the methanol extracts was 125 mg/ml for *Actinomyces* $sp._1$ while *Bacillus* $sp._2$, *Bacillus* $sp._3$, *Micrococcus* sp. and *Actinomyces* $sp._2$ were 250 mg/ml respectively while *Bacillus* sp_1, *Bacillus* sp_2, *Micrococcus* sp., *Staphylococcus* $sp._2$ and *Actinomyces* $sp._1$ all has MBC of 500mg/ml, respectively on the aqueous extracts. The phytochemical analysis of the aqueous and methanol extracts of clove shows that saponins, tannins, amino acids, proteins, reducing sugar, glycosides, alkaloids, anthraquinone and phenols are present though in varying amounts. This study therefore recommends clove extract as an alternative source for bio-preservation in food and allied products.

Keywords: Antimicrobial; phytochemical; inhibition; aqueous; concentration; dilution.

1. INTRODUCTION

Environmental and health concern of the carcinogenic effect of chemical preservatives, food safety and huge economic losses of agricultural products due to spoilage by microorganisms, has aroused the thought for adoption of natural preservatives for inhibition of bacterial activity and growth over chemical preservatives. Microorganisms such as bacteria and fungi, are the major contributors of food spoilage [1]. Spices possess antimicrobial activities [2,3] and are one of the most used natural anti-microbial agents traditionally for preservation of food. Similarly, spices have been reported as rich sources of phytochemicals which are responsible for the inhibition of bacterial growth [4]. Phytochemicals such as saponins, alkaloids, tannins, steroids and etc. are partially responsible for the antimicrobial activities of plants [5]. Cloves are brown, dried, unopened flower buds of *Syzigium aromaticum* L., an evergreen tree native to Indonesia [6] and of height, 8 – 30m, with many semi- erect branches [7]. It possess a pleasant smell and is commonly and traditionally used as a spice for seasoning of meat and a wide variety of food and drinks. Additionally, some rural dwellers prepare aqueous extract of cloves, although with no standard concentration and drink same as herbal remedy for bacterial infection and to boost general health as they believed on its antibacterial activity (self observation).

The antibacterial activity of clove has been tested and reported by many researchers. Saeed *et al*.,

[8] reported that solvent extract of clove has a great potential for the inhibition of microbial load. Likewise, Cupta and Prakash [9] reported clove extract having the potential to inhibit the growth of bacterial species and be used as a natural antibacterial agent for pathogens.
Likewise, the phytochemical analysis of clove extract revealed the presence of carbohydrates, lipids, alkaloids, flavonoids, tannins, sterols and glycosides [10]. Khatri *et al*., [11] reported an essential ingredient of clove oil to include tannins, vanillin, flavonoids etc. and tannins was found to be in high amounts in fresh clove [12].

This study therefore seeks to evaluate the antibacterial and phytochemical properties of extracts of clove and to determine the concentrations of the extracts at which the antibacterial activity could be achieved. The study also seeks to assess its use as a bio-preservative of food and allied food product.

Scientific Classification of *Syzygium aromaticum* L.

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Myrtales
Family: Myrtaceae
Genus: Syzygium
Species: *S. aromaticum*

2. MATERIALS AND METHODS

2.1 Collection of Samples

Cloves were purchased from a local trader at the Oron main market, Akwa Ibom State of Nigeria and identified by a botanist at the Department of Biological Sciences, Clifford University, Owerrinta, Abia State where the research was carried out.

2.2 Sample Preparation

The Cloves were washed in tap water, dried under shade and then homogenized to fine powder using a disinfected manual blender and stored in airtight bottles. Fifty (50) grams of leaf powder was placed in 450 ml of methanol and water respectively in conical flasks and stirred on a rotary shaker at 200 rpm for 48 h. The suspension was filtered through muslin cloth and the extracts were centrifuged at 5000 rpm for 10 min. Supernatants were evaporated at 45 ^{0}Cto dry and stored at 4 ^{0}C in air tight bottles. The dried extract was re-dissolved at 10% (100 mg/ml) in DMSO for the antifungal testing, when not in use, it was stored in a refrigerator in 4 ^{0}C [13].

2.3 Determination of percentage yield

The percentage yield of the extract was calculated using the following formula:

% yield = mass of extract/mass of leaves x 100/1

2.4 Isolation of Bacterial Plant Pathogen

The infected parts of vegetables including (pumpkin), bitter leaf and cocoyam leaves were aseptically collected from the university farm with clean nylon bag. After washing the leaves thoroughly in sterile water, the infected leaves along with adjacent small uninfected leaves were cut into small pieces (2-5 mm squares) and flamed – sterilized forceps was used to transfer them into 0.1% mercuric chloride solution used for surface sterilization of plant tissues, and rinsed. A sterile mortar and pestle was used to squash the various samples into a fine homogenized form. A clean filter paper and digital weighing balance was used to weigh one gram of each of the samples into 9 ml of sterile water, they were shaken to homogenize respectively. Tenfold serial dilution was carried out by transferring 1 ml from the stock into the first test containing 9 ml of sterile water, it was equally shake to homogenize. The dilution was continued till the last tube at which 1ml aliquot was discarded. The samples (10^{-3}, 10^{-4} and 10^{-5}) were inoculated on a nutrient agar using spread plate technique [14]. The plates were incubated at 37^{0}C for 24hrs for the complete growth of bacteria.

2.5 Purification of Isolates

The colonial bacterial growths were purified by sub culturing them on sterile nutrient agar plates using the streak method. Thereafter the pure cultures were stored in agar slants.

2.6 Identification of Isolates

The bacterial isolates were identified by Gram staining and biochemical tests which include: Catalase, Coagulase, Indole, Citrate utilization, Urease, Hydrogen sulphide, sugar fermentation and Oxidase tests.

2.6.1 Gram staining

Gram staining reaction was used to identify gram status of bacteria in the culture. Heat-fixed smears of young cultures were prepared. The smears were flooded with crystal violet and allowed to stand for 30 seconds and then rinsed

with a slow running tap water for 5 seconds. Again, the smears were flooded with Gram's iodine mordant, allowed to stand for 60 seconds and rinsed. The smears were decolorized with 95% ethanol and also rinsed for 5 seconds. The smear was counterstained with safranine and left for 60 seconds and was rinsed with a slow running tap water for 5 seconds. The slides were air-dried and examined under ×100 with the use of oil immersion [14].

2.6.2 Biochemical test

2.6.2.1 Catalase test

Colonies of the test organism was immersed in three ml of 3 % hydrogen peroxide solution and allowed for 2 seconds. Active bubble indicates catalase positive.

2.6.2.2 Coagulase test

Thick suspensions of a colony of the test organism were made and a loop full of human plasma was added to the suspension and gently mixed. It was kept for 10 seconds for reaction. Clumping of the bacterial cell indicates positive result.

2.6.2.3 Oxidase test

To a three drops of freshly prepared oxidase reagent on a filter paper placed on a test tube was a colony of the test organism dropped. The development of a deep purple color indicates the production of oxidase by the organism which oxidizes the phenylenediamine in the reagent to the deep purple color.

2.6.2.4 Citrate utilization test

Test tubes were slanted with Simmons Citrate agar and were allowed to solidify. A colony of the test organism was inoculated in the slant by first stabbing the butt and finally streaking the slant and was incubated at 37^{o}C for 24 hours.. Medium changing from green to blue color indicates positive result.

2.6.2.5 Indole production test

The test organism was inoculated in trypton broth and incubated for 24 hours at 37^{o}C. Ten drops of kovac's reagent was added to the trypton broth and was allowed to stand for 5 minutes. Appearance of a dark-red upper layer indicates the presence of Indole.

2.6.2.6 Hydrogen Sulphide (H_2S) production and sugar fermentation test using TSI agar

TSI agar was prepared, sterilized and slanted to solidify. A colony of the test organism was inoculated in the slant by first stabbing the butt and then streaking the slope and incubated at 37^{o}C for 24 hours and observations were taken. Formation of black precipitate in the butt of the tube indicates H_2S positive [14].

2.7 Phytochemical Analysis

Phytochemical analysis of the extracts (cloves) was carried out according to the general method of Harbone [15]. Basic phytochemical screening was carried out using simple chemical tests to detect the presence of secondary plant constituents such as alkaloids, tannins, flavonoids, saponins, phenols, glycoside, reducing sugar, soluble carbohydrate etc. in the sample. The methods used were those outlined by Harbone [15] except otherwise stated.

2.7.1 Saponin (frothing test)

Extract of clove (0.5 g) was suspended in 5 ml of distilled water in a test tube. The contents were shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and again shaken vigorously. The formation of an emulsion, if any, was observed.

2.7.2 Test for alkaloids

The extract (0.1mg) was added to 6ml of dilute hydrochloric acid and boiled. After boiling, it was cooled and filtered. Few drops of Mayer's reagent were added. The formation of turbidity indicated the presence of alkaloids.

2.7.3 Glycoside

Five milligram **(**5 mg) extract was suspended in 5 ml ethanol and two drops each of 1% ferric chloride and concentrated sulphuric acid were added. The development of reddish-brown coloration at the junction of two layers and the bluish green color in the upper layer indicated the presence of glycoside.

2.7.4 Test for terpenoids

Nine milliliters (9 ml) of ethanol was added to 1 g of the extract and refluxed for a five min and filtered. The filtrate was concentrated down to 2.5 ml in a boiling water bath. Hot distilled water (5 ml) was added to the concentrated solution; the

mixture was allowed to stand for 1 hour and the waxy mater was filtered off. The filtrate was extracted with 2.5 ml of chloroform using a separating funnel. The chloroform extract was evaporated to dryness in a water bath and dissolved in 3 ml of concentrated sulphuric acid and then heated for 10 min in a water bath. A grey color indicated the presence of terpenoids.

2.7.5 Test for Steroids

A known quantity of the test sample was extracted in the chloroform and filtered. The filtrate was mixed with 2 ml of conc. H_2SO_4 carefully so that the sulphuric acid formed a lower layer. A reddish-brown color at the interphase indicated the presence of steroidal ring.

2.7.6 Reducing sugar

Extract of 0.5 mg was dissolved in 1 ml of water. To it 5 – 8 drops of Fehling's solution were added and solution was heated. Appearance of brick red precipitate indicated the presence of reducing sugar.

2.7.7 Amino acid and Protein

Extract of 0.1 mg was boiled with 1ml of 0.2% Ninhydrin solution. The appearance of violet blue color indicates the presence of protein in the extract.

2.7.8 Phenols

Extract of 0.2 mg was taken in 3 ml of 5% ferric chloride and then add 1 ml each of methyl alcohol and Folin – Ciocalteu reagent and shaken gently. Formation of deep blue or black color indicated the presence of phenol.

2.7.9 Tannins

Extract of 0.5 g of extract was boiled in 10 ml of water in a test tube and then filtered. Few drops of 0.1%ferric chloride were added. Production of brownish green or blue-black color indicated the presence of tannins.

2.7.10 Test for flavonoids

A quantity of the extract was boiled in ethyl acetate (10 ml) for 3 mins, filtered and cooled. Then the filtrate (4 ml) was shaken with 1 ml of dilute ammonia solution. An intense yellow coloration indicated the presence of flavonoids.

2.7.11 Test for soluble carbohydrate (molisch test)

The extract (0.1 g) was boiled with 2 ml of distilled water and filtered. To the filtrate, few drops of naphthol solution in ethanol (molisch's reagent) were added. Concentrated sulphuric acid in a Pasteur pipette was then gently poured down the side of the test tube to form a lower layer. A purple interfacial ring indicated the presence of carbohydrate.

2.7.12 Test for anthraquinone

Diluted H_2SO_4 was added to 0.5 g of the extract; the solution was then boiled and filtered. The filtrate was cooled and to it equal volume of benzene was added. The solution was shaken well and the organic layer was separated. Equal volume of dilute ammonia solution was added to the organic layer. If the ammonia layer turns pink, then it indicates the presence of anthraquinone.

2.8 Preliminary Antimicrobial Screening of the Extract

The Mueller Hinton agar was prepared according to the manufacturer's instruction and poured aseptically into Petri-dishes and was allowed to gel. The surface of the plates was then streaked with inoculums of the test organisms using an inoculating loop. Thereafter, a sterilized 6mm long cork-borer with depth 3-4 mm, was used to create holes on the agar plates. The extract was then serially diluted in test-tubes containing 1ml of the solvents. In the first test-tube containing the weighed plant extract, 2 ml of 10% Dimethyl sulphoxide (DMSO) was used to dissolve the extract (the stock solution of 500 mg/4ml), then 1 ml of the solvent was added into the other test-tubes. From the first test-tube, 1000ul was pipette and put in the second test-tube until it got to the last one (500 mg/4ml, 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml, 15.625 mg/ml, 7.8 mg/ml). This was done for the methanol and aqueous extracts respectively. After the dilution, 20 µl of the extracts were pipette according to their concentration and put in the hole. The Petri-dishes were allowed to sit for 30 min for pre-diffusion of the plant extracts and the plates were incubated at 37 ^{0}C for 24hours. The inhibition zones were then measured after incubation by using a transparent meter rule in millimeters (mm). This was also done for the test drug (Chloramphenicol 200mg) which served as the positive control while 10% DMSO alone served as the negative control.

2.8.1 Minimum Inhibitory Concentration (MIC) Determination

MIC is the lowest concentration of extracts that inhibited noticeable growth of the organisms after 24 hours using the tube dilution method as described by the Clinical and laboratory standards institute one (1 ml) milliliter of different concentrates (500 mg/ml, 250mg/ml, 125 mg/ml, 62.5 ml/ml and 31.25 mg/ml, 15.625 mg/ml) of the various extracts. Sterile pipette was used to transfer 20 µl into each well, with the bacterial colonies streaked on the media surface. The MHA plates were incubated at 37^0C 24hours. After the incubation the visible zone of inhibition was measured [16].

2.8.2 Minimum Bactericidal Concentration (MBC) Determination

The MBC was determined by inoculating from the MIC plate showing a visible inhibition and was incubated at 37^0C for 24 hours.

3. RESULTS AND DISCUSSION

The results of the antibacterial and phytochemical properties of extracts of clove are shown in Tables 1 – 8 and Figs. 1 – 3.

Table 1. Percentage (%) yield of extracts

Extracts	Percentage (%) Yield
Methanol	44.68
Aqueous	14.32

A higher percentage yield of methanol extract compare to the aqueous extracts was observed. This is presented in Table 1. This suggests the ability of alcohol to extract more of the bioactive compounds in clove than water. Therefore, alcohol could be recommended as a better option for extraction of active and chemical compounds in cloves than water. To achieve best result in its use as bio preservatives as well as herbal remedy for bacterial infections, alcohol extract will be more active and preferable to aqueous extracts.

Table 2. Phytochemical analysis of clove extracts

S/n	Phytochemical	Aqueous	Methanol
1.	Saponins	++	+++
2.	Tannins	+	++
3.	Amino acid and proteins	++	++
4.	Reducing sugar	+	++
5.	Glycosides	+	+++
6.	Alkaloids	+	++
7.	Anthraquinone	+	+
8.	Phenol	+	++

Key: + = Positive test

The phytochemical analysis of aqueous and methanol extracts of clove shows that saponins, tannins, amino acids, proteins, reducing sugar, glycosides, alkaloids, anthraquinone and phenols were present though in varying amounts as shown in Table 2 This is in agreement with the previous report of Sanusi *et al*., [17], who equally has all the phytochemicals present. The phytochemical result is contrary to the report of Ajobiewe*et al*., [18], where tanins, glycosides, alkaloids and saponins were absent in ethanol and aqueous extracts respectively.

Eight bacterial isolates were obtained from the infected leaves of Bitter leaf, pumpkin and Coco yam leaf. This is enlisted in Table 3. The isolates includes *Bacillus* $sp._1$, *Bacillus* $sp._2$, *Bacillus* $sp._3$, *Micrococcus* sp.,*Staphylococcus* $sp._1$, *Staphylococcus* $sp._2$, *Actinomyces* $sp._1$ and *Actinomyces* sp_2. These are common infections bacteria causing in humans. There is tendency that high cases of infections in rural areas may also be due to consumption of infected or spoiled vegetables. The application of clove powder or extracts on these vegetables before or after harvest could inhibit the activities of these bacteria on the vegetables and thus preserve them from their attack and possibly from economic loss. Therefore, it could save a lot of economic waste and combat cases of infectious diseases among the rural dwellers.

The pumpkin leaf has the highest average number of bacterial colonies of 2.5×10^2 followed by bitter leaf which had 1.5×10^2 while Coco yam has the least with 7.0×10^1 as indicated in Fig.1 The result of the bacteriological load agrees with Okafor [19] whose bacterial load on nutrient agar ranged from $6.7x\ 10^4$ to 1.02×10^5 and 7.2×10^4 to 1.64×10^5 from pumpkin, bitter leaf and scent leaf surfaces. The organisms isolated were *Bacillus* sp., *Erwinia* spp., *Pseudomonas* spp. and *Streptococcus* spp. It also agrees with the work of Muhammad *et al*., [20] who reported the isolation and identification of bacteria responsible for the spoilage of fluted pumpkin and bitter leaf. The isolates include *E*. *coli*, *Klebsiella* and *Proteus mirabilis* from the fluted pumpkin and *Proteus mirablis*, *E*. *coli* and *Bacillus cereus* from bitter leaf.

The zone of inhibition of the bacteria in both aqueous and methanol extracts of clove are shown in Tables 4 – 6. *Actinomyces* sp.$_2$ has the highest zone of inhibition of 22.0 mm followed by *Bacillus* sp.$_1$ which has 20.00 mm, *Bacillus* sp.$_2$ has 18.00 mm *Micrococcus* sp. has 17.00 mm, *Actinomyces*sp.$_1$ has 19.00 mm, *Staphylococcus* sp.$_1$ has 15 mm, *Bacillus* sp.$_3$ has 14.00 mm while *Staphylococcus* sp.$_2$ has the least zone of inhibition of 13.00 mm all at 500mg/ml. The organisms were more susceptible at higher concentrations than the lower concentrations for both the methanol and aqueous extracts of clove flower bud. For aqueous extracts *Bacillus* sp.$_3$and*Staphylococcus* sp.$_1$ has the highest zone of inhibition of 16.00 mm, respectively followed by *Bacillus* sp.$_1$ and *Bacillus* sp.$_2$ which has 12.00 mm, respectively at concentrations of 500 mg/ml. The least zones were observed in *Staphylococcus* sp.$_2$ and *Bacillus* sp.$_3$ and concentration of 250 mg/ml and 125 mg/ml, respectively. This work is in line with the previous report of Sanusi *et al*., [17] whose inhibition zone diameter of clove bud extracts for *S. aureus* was 21.00 mm and 22.00 mm for the aqueous and ethanol extracts. Ajobiewe *et al*., [18] had zone of inhibitions of 27, 22 to 9 mm on cold water extracts of clove plant extracts on *E. coli* and 28, 25 to 8mm,respectively on ethanol extract at concentrations of 500mg/ml to 15.63 mg/ml.

Tables 7- 8 and Figs. 2 - 3 present the MIC and the MBC of the isolates. *Bacillus* sp.$_1$, *Bacillus* sp.$_2$, *Micrococcus* sp. and *Actinomyces* sp.$_1$ has MIC of 125 mg/ml while *Staphylococcus* sp.$_1$ and *Staphylococcus* sp.$_2$ has 250mg/ml, respectively for methanol extract while *Bacillus* sp.$_2$, *Micrococcus* sp. and *Actinomyces* sp.$_1$ has MIC of 250mg/ml. respectively and *Staphylococcus* sp.$_1$ and *Staphylococcus* sp.$_2$ has MIC of 500 mg/ml for aqueous extracts. The Minimum Bactericidal Concentration (MBC) of the Clove extract of methanol is lower than the aqueous extracts across all the bacterial isolates. This is shown in Table 8. The MBC for the methanol extracts is 125 mg/ml for *Actinomycts*sp.$_1$ while *Bacillus* sp.$_2$, *Bacillus* sp.$_3$, *Micrococcus* sp. and *Actinomyces*sp.$_2$ are 250 mg/ml, respectively while *Bacillus* sp.$_1$, *Bacillus* sp.$_2$, *Micrococcus* sp., *Staphylococcus* sp.$_2$ and *Actinomyces* sp.$_1$ all has MBC of 500mg/ml, respectively on the aqueous extracts. The MIC result above is contrary to the MIC result obtained by Sanusi *et al*., [17] were 6.25 mg/ml was obtained for the isolates on ethanol extract. The isolates includes *E. coli*, *Salmonella* sp., *Staphylococcus aureus* and *Pseudomonas* sp. while for aqueous extracts *E. coli* has the highest MIC of 12.5mg/ml followed by *Salmonella* sp., *S. aureus* and *Pseudomonas* sp. which has 6.25 mg/ml, respectively.

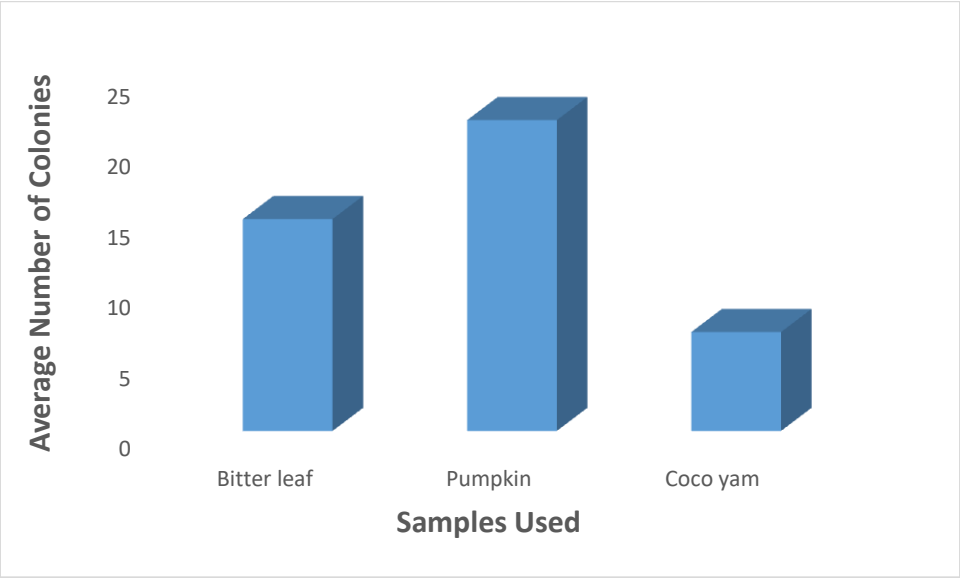

Fig. 1. Colonial Distribution of Isolates

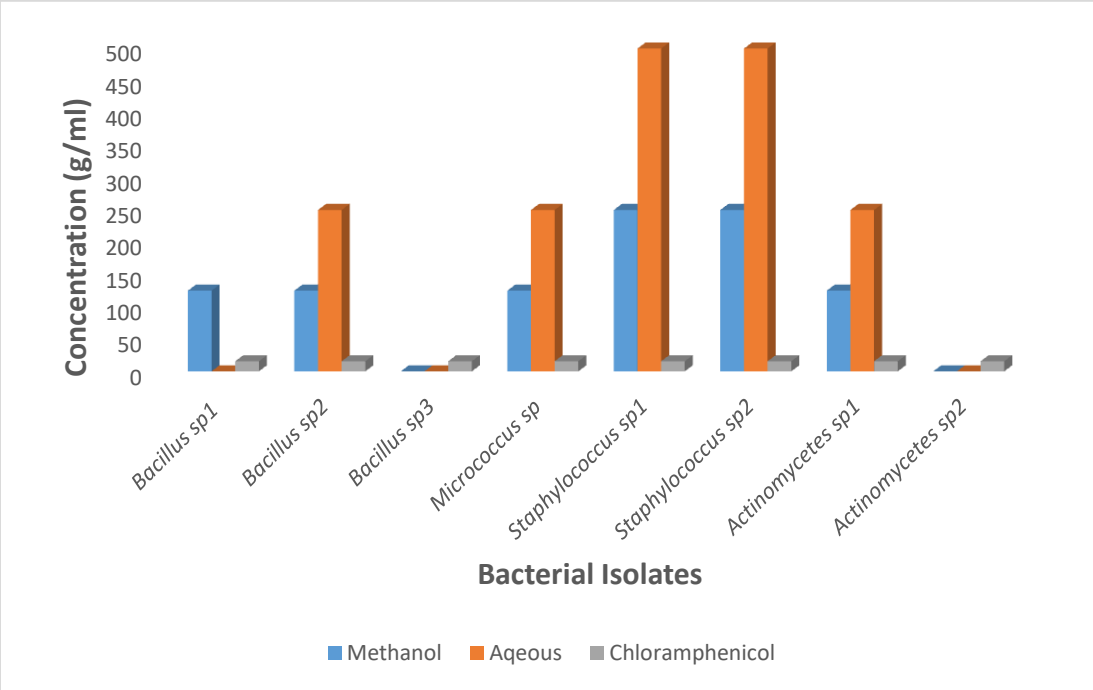

Fig. 2. Minimum Inhibitory Concentration of Clove Extracts

Table 3. Microbiological and Biochemical Characteristics of Bacterial Isolates and Their Possible Identity

Organism code	Cultural characteristics	Gram rxn	Cat	Coa	Ind	Cit	M	Ox	B	S	G	H2s	Possible organisms
PLM1	Milky, round, flat, smooth and shiny	+ve cocci	+	-	-	+	-	+	+	-	-	-	*Micrococcus* sp.
PLM2	Milky, rough, flat, sticky and shiny	+ve rod	+	-	-	+	-	+	+	-	-	-	*Actinomyces*sp.$_1$
PLY1	Yellow, circular, smooth, shiny and non-sticky.	+ve cocci	+	+	-	+	-	-	-	-	-	-	*Staphylococcus* sp.$_1$
PlO1	Orange, sticky ,round, smooth and shiny	+ve rod	+	+	-	+	-	+	+	-	-	-	*Bacillus* sp.$_1$
BLO2	Orange, sticky, round, smooth and shiny	+ve rod	+	-	-	+	+	-	+	+	+	-	*Bacillus* sp.$_2$
BLM3	Milky, filamentous, non-sticky and shiny	+ve rod	+	-	-	+	+	+	+	+	+	+	*Bacillus* sp.$_3$
BLY2	Yellow, round, non-sticky, raised and shiny	+ve cocci	+	+	-	+	+	-	+	+	+	-	*Staphylococcus* sp.$_2$
CLM4	Milky, rhizoid shiny and non-sticky	+ve rod	+	-	-	-	-	+	+	+	-	-	*Actinomyces*sp.$_2$
CLW1	White, non-sticky and flat	+ve rod	-	+	-	+	-	-	+	+	+	+	*Bacillus* sp.$_4$

Key: Cat: catalase test, Coa: coagulase test, Ind: indole test, Cit: Citrate utilization test, MR: Methyl red test, OX: Oxidase test, SFT: Sugar fermentation test, B=butt, S= slope, G= gas production, H_2S: Hydrogen Production.

Table 4. Zone of Inhibition of Bacterial Isolates by Methanol Extract of Clove

Conc. (Mg/ml)	***Bacillus* sp.$_1$**	***Bacillus* sp.$_2$**	***Micrococcus* sp.**	***Bacillus* sp.$_3$**	***Staph.*Sp.$_1$**	***Actinomycetes* sp.$_1$**	***Actinomycetes* sp.$_2$**	***Staph* sp.$_2$**
Zone of Inhibition (mm)								
500	20.00	18.00	17.00	14.00	15.00	19.00	22.00	13.00
250	13.00	14.00	12.00	12.00	12.00	11.00	14.00	10.00
125	12.00	0.00	0.00	11.00	0.00	0.00	0.00	0.00
62.25	10.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
31.125	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15.625	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 5. Zone of Inhibition of Bacterial Isolates by Aqueous Extract of Clove

Conc. (Mg/ml)	*Bacillus* sp.$_1$	*Bacillus* sp.$_2$	*Micrococcus* sp.	*Bacillus* sp.$_3$	*Staph* sp.$_1$	*Actinomyces* sp.$_1$	*Actinomyces* sp.$_2$	*Staph.* Sp.$_2$
Zone of Inhibition (mm)								
500	12.00	12.00	0.00	16.00	16.00	0.00	0.00	10.00
250	0.00	8.00	0.00	10.00	12.00	0.00	0.00	5.00
125	0.00	0.00	0.00	5.00	0.00	0.00	0.00	0.00
62.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
31.125	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15.625	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

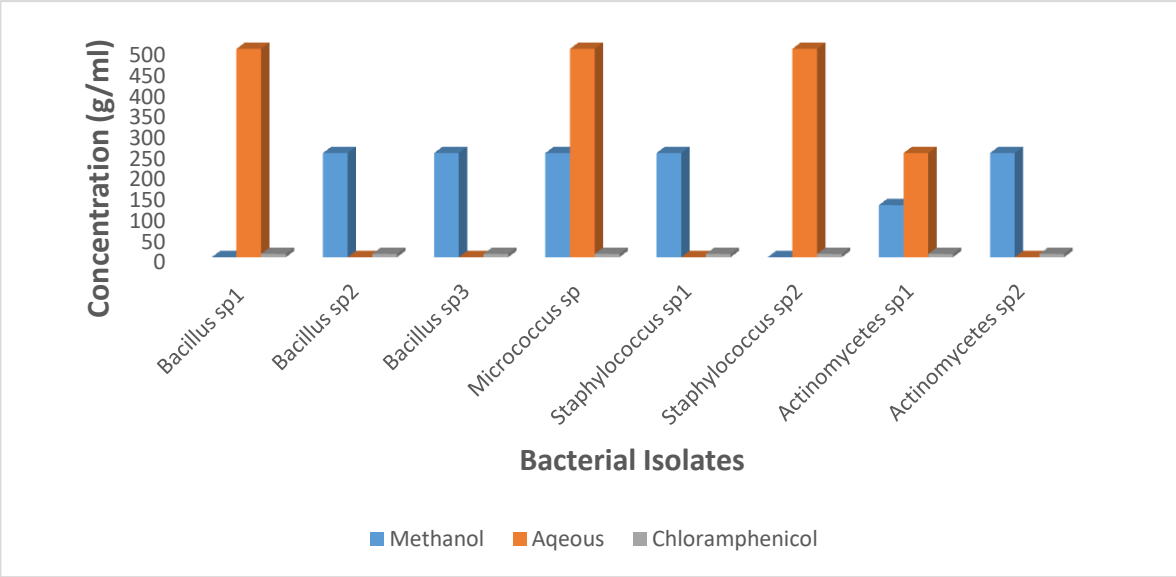

Fig. 3. Minimum Bactericidal Concentration of Clove Extracts

Table 6. Zone of Inhibition of Test Drug (Chloramphenicol)

Conc. (250mg/ml).	*Bacillus* sp_1	*Bacillus* sp_2	*Micrococcus*sp	*Bacillus* sp_3	*Staph*. sp_1	*Actinomyces* sp_1	*Actinomyces*sp_2	*Staph.* sp_2
Zone of Inhibition (mm)								
250	28.00	28.20	30.00	27.00	24.00	30.00	27.00	30.00
125	20.00	23.30	28.00	25.00	18.00	25.00	25.00	27.00
62.25	17.00	20.10	26.00	23.00	15.00	23.00	23.00	25.00
31.125	13.50	18.00	24.00	20.00	12.00	20.00	20.00	22.00
15.625	11.10	15.00	20.00	16.00	10.00	15.00	18.00	20.00
7.813	8.00	7.00	15.00	10.00	8.00	10.00	14.00	15.00

Table 7. Minimum Inhibitory Concentration (MIC) of the Clove Extracts and the Test Drug (Chloramphenicol)

Extract and Test drug	*Bacillus* $sp._1$	*Bacillus* $sp._2$	*Bacillus* $sp._3$	*Micrococcus* sp.	*Staph.*$Sp._1$	*Staph* $sp._2$	*Actinomyces* $sp._1$	*Actinomyces*$sp._2$
Methanol	125	125	-	125	250	250	125	-
Aqueous	-	250	-	250	500	500	250	-
Chloramphenicol	15.625	15.625	15.625	15.625	15.625	15.625	15.625	15.625

Table 8. Minimum Bactericidal Concentration (MBC) of the Cloves Extract

Extract and Test drug	*Bacillus* $sp._1$	*Bacillus* $sp._2$	*Bacillus* $sp._3$	*Micrococcus* sp.	*Staph.* $sp._1$	*Staph* $sp._2$	*Actinomyces*$sp._1$	*Actinomyces*$sp._2$
Methanol	-	250	250	250	250		125	250
Aqueous	500	500	-	500	-	500	500	-
Chloramphenicol	7.813	7.813	7.813	7.813	7.813	7.813	7.813	7.813

4. CONCLUSION

This study has revealed that the clove extracts possess significant amount of phytochemicals such as saponins, tannins, reducing sugar, amino acids, proteins, phenols and glycosides. The extracts also show antimicrobial properties against *Bacillus* spp., *Micrococcus* sp., *Staphylococcus* spp., and *Actinomyces* sp. These findings are interesting and essential at the present time where the problems of emerging and re -emerging resistant strains of microorganisms are becoming the order of the day and scarcity of food due to bacterial infection on plants.

5. RECOMMENDATION

This study is recommending clove extract as an alternative source for bio- preservation in food and allied products.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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