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Efficacy of Clove (*Syzygium Aromaticum*) Extracts on Food Borne Pathogens

Ali M[1]*, Nas FS[2], Yahaya A[3] and Ibrahim IS[4]

[1]Department of Microbiology, Kano University of Science and Technology Wudil Kano, Nigeria

[2]Department of Biological Science, Bayero University Kano, Nigeria

[3]Department of Biological Science, Kano University of Science and Technology Wudil Kano, Nigeria

[4]Department of Pharmaceutical Technology, School of Technology, Kano, Nigeria

***Corresponding author:** Muhammad Ali, Department of Microbiology, Kano University of Science and Technology, Wudil Kano, Nigeria, Email: alimuhd4real@gmail.com

Research Article

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Abstract

The study was aimed to investigate the phytochemical constituents and antibacterial activity of *Syzygium aromaticum* extracts against some food borne pathogen. Aqueous and methanol extracts from *Syzygium aromaticum* buds were prepared, screened for phytochemical analysis and tested for antibacterial activity against 6 pathogenic bacteria (*Klebsiella pneumoneae, Salmonella typhi, Shigella spp, Pseudomonas aeruginosa, Escherichia coli,* and *Staphylococcus aureus*). Phytochemical screening of the extracts showed that *Syzygium aromaticum* bud extracts contain Alkaloid, Anthraquinone, saponin, tannin, phenol, steroid, and flavonoid, terpenoid while glycoside and reducing sugar were absent. On the hand, alkaloids was found to be the most abundant phytochemical (9.6%) followed by tannin, saponin and flavonoids. Statistical analysis of the result showed that methanol extract demonstrated highest antibacterial activity with average zone of inhibition of 14.18±2.47 mm among the isolates than aqueous extracts (12.33±1.82 mm). Based on the susceptibility of the organisms to the extracts, *E. coli* was found to be the highest susceptible organisms with average zone of inhibition of 14.79±1.97 mm, followed *Salmonella typhi* (13.84±2.52 mm), *S. aureus* (13.7±1.65 mm), *Shigella* (13.44±1.12 mm), *Pseudomonas* (12.27 ±0.98mm) while least average zone of inhibition is shown by *Klebsiella* (11.55±1.60 mm). The MIC and MBC of the extracts ranges from 3.125 to 50 mg/ml There is no significant different on the susceptibility of the organisms against the extracts at $p< 0.05$. The results of present study have provided the justification for therapeutic potential of ginger and also used as dietary supplement for food flavoring and preservation.

Keywords: Syzygium aromaticum; Pathogenic bacteria; Antibacterial activity; Phytochemicals

Introduction

Spices such as clove, ginger, mint, thyme and cinnamon, have been employed for centuries as food preservatives and as medicinal plants mainly due to its antioxidant and antimicrobial activities [1]. Nowadays, many reports confirm the antibacterial, antifungal, antiviral and anticarcinogenic properties of spice plants [1].

Clove (*Syzygium aromaticum*) belong to the family Myrtaceae, it is widely cultivated in Spice Islands, Indonesia, Pemba and Zanzibar, though earlier production of the plant was in China. It is used in the seasoning of food [2]. Its antimicrobial potential was established when its essential oil extracts killed many Gram positive and Gram negative organisms including some fungi [3]. The antimicrobial activity of *Syzygium aromaticum* is attributable to eugenol, oleic acids and lipids found in its essential oils [4].

Syzygium aromaticum in particular has attracted the attention due to the potent antioxidant and antimicrobial activities standing out among the other spices [5]. It is one of the most valuable spices that have been as food preservative and for many medicinal purposes. *Syzygium aromaticum* is an important medicinal plant due to the wide range of pharmacological effects consolidated from traditional use for centuries. The antimicrobial activities of clove have been proved against several bacterial and fungal strains. Sofia, et al. tested the antimicrobial activity of different Indian spice plants as mint, cinnamon, mustard, ginger, garlic and clove, the only sampled that showed complete bactericidal effect against all the food-borne pathogens tested *Escherichia coli, Staphylococcus aureus* and *Bacillus cereus* was the aqueous extract of *Syzygium aromaticum* at 3% [6]. At the concentration of 1% *Syzygium aromaticum* extract also showed good inhibitory action. The antibacterial activity of clove essential oil was tested against *E. coli* O157:H7, the result showed different grades of inhibition of these essential oils [7]. The aim of the research is determine the bioactive constituents and antibacterial activity of aqueous and methanol extract of *Syzygium aromaticum* against food borne pathogens.

Materials and Methods

Sample Collection and Identification of Plant Materials

Clove (*Syzygium aromaticum*) buds were used in this study and were purchased from Rimi market in Kano city, Nigeria. Identification and authentication of the plant material was done at compounding laboratory in the Department of Pharmaceutical Technology, School of Technology Kano with the following voucher number SOT/PCT/01/081. Voucher specimen has been deposited there for future reference (Figure 1).

Figure 1: Syzygium aromaticum buds.

Test Organisms

Six (6) bacterial strains responsible for food spoilage including *Klebsiella pneumoneae, Salmonella typhi, Shigella sp, Pseudomonas aeruginosa, Escherichia coli,* and *Staphylococcus aureus* were obtained from Laboratory of Science Lab Technology, School of Technology Kano. The bacteria were isolated from spoiled food and diagnosed to the species level by using different laboratory procedures including; Gram's stain, cultural characterization and Biochemical tests include (Indole, Methyl red, Vougues Proskeaur, Catalase, Citrate utilization and coagulase tests). The isolates were maintained on Nutrient agar slants.

Preparation of Extracts

Aqueous and methanol extracts of *Syzygium aromaticum* were prepared separately. The fresh buds of *Syzygium aromaticum* were washed and air dried for two weeks. After drying, the buds were grounded to fine powder using sterile pestle and mortar under laboratory condition. Fifty grams (50 g) powder of the plant material was soaked in 500 ml of distilled water and methanol respectively. The flasks were kept at room temperature for 3 days with intermittent shaking after which filtration was done using What man filter paper. The methanol extracts was evaporated at 50°C using rotary evaporator while the aqueous extract was evaporated at 70°C in water bath. All dried extract samples were dissolved in 10% DMSO separately to the final concentration of 200 mg/ml as a stock concentration. The extract solutions were stored at 4°C before use.

Qualitative Phytochemical Screening

The phytochemical screening of the plant materials for various phytochemical constituents such as terpenoids, flavonoids, alkaloids, reducing sugars, steroid, glycoside, phenol, Anthraquinones, saponin and tannin was

conducted using standard methods as described by Sofowora and Trease and Evans [8,9].

Quantitative Phytochemical Analysis

Different methods were employed in evaluating the quantity of phytochemical constituents of the plant material used. Spectrophotometry method was used to determine Terpenoids, tannins, steroids, anthraquinones, and glycosides. Folin-Ciocalteu procedure was used to determine phenol content. Flavonoids, alkaloids and saponin were determined by the methods described by Adeniyi, et al. [10].

Antibacterial Susceptibility Test

The sensitivity of each extracts was determined using the agar well diffusion method as described by Ahmed and Beg with modifications [11]. The prepared bacterial suspension equivalent to 0.5 McFarland Standard (1.5 x 10^6 CFU) was inoculated into sterile Mueller- Hinton agar medium in a sterile Petri-dish. A sterile 6 mm diameter sterile cork borer was used to bore 5 wells into the agar medium. The wells were then filled up with approximately 0.1ml of the extract solution at a concentration of 25, 50, 75 and 100 mg/ml taking care to prevent spillage onto the surface of the agar medium. The plates were allowed to stand on the laboratory bench for 1 hour to allow proper diffusion of the extract into the medium after which the plates were incubated at 37^0C for 24 hours, and thereafter the plates were observed for zones of inhibition and measured. Ciprofloxacin 50 mg/ml from Micro Lab limited was used as a positive control.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the extracts was determined using broth dilution technique. Two fold serial dilutions of the extracts were prepared by adding 2ml of 100mg/ml of the extract into a test tube containing 2ml of Nutrient broth, thus producing solution containing 50mg/ml of the extract. The process continue serially up to test tube No. 5, hence producing the following concentrations; 50, 25, 12.5, 6.25 3.125 mg/ml. Test tube No. 6 do not contain extracts and serve as negative control. Exactly 0.5 ml of 0.5 McFarland equivalent standards of test organisms were introduced into the test tubes and incubated at 37°C for 24 hours. After incubation the test tubes were observed for growth by checking for turbidity [11].

Determination of Minimum Bactericidal Concentration (MBC)

From each tube that did not show visible growth in the MIC, 0.1ml was aseptically transferred into extract free Mueller Hilton agar plates. The plates were incubated at 37°C for 24 hours. The MBC was recorded as the lowest concentration of the extract that had less than 99% growth on the agar plates [11].

Statistical Analysis

The data of average zone of inhibition produced by the isolates against the antibiotics used was analyzed using One-Way ANOVAs and the statistical program SPSS 21.0 (Statistical Package for the Social Sciences). The results were presented as the means ± standard deviation. Significance level for the differences was set at $p<0.05$.

Results

Phytochemical Screening

The qualitative and quantitative phytochemical screening of *Syzygium aromaticum* extract is presented in (Table 1). The result indicated the presence of Alkaloid, terpenoids, flavonoids, steroid, phenol, Anthraquinones, saponin and tannin while reducing sugars and glycoside are absent. Quantitatively, Alkaloid was found to be the abundant constituent making about 9.6%, followed by Tannin and saponin constituting 4.8% and 3.7% respectively.

S/N	Phytochemical	Qualitative analysis	Quantitative analysis (%)
1	Alkaloids	+	9.60±0.12
2	Flavonoid	+	3.00±0.09
3	Glycosides	-	0.00±0.00
4	Reducing sugar	-	0.00±0.00
5	Saponin	+	3.70±0.00
6	Steroids	+	1.80±0.04
7	Phenols	+	0.10±0.01
8	Terpenoid	+	1.70±0.01
9	Anthraquinones	+	1.20±0.03
10	Tannin	+	4.80±0.00

Table 1: Qualitative and quantitative phytochemical screening of *Syzygium aromaticum* extract

Antibacterial Activity of Aqueous Extract

The antibacterial activity of aqueous *Syzygium aromaticum* extract is presented in (Table 2). The results showed that zones of inhibition recorded by the isolates depend on the type of bacterial isolates and concentration of the extracts. Highest zone of inhibition is demonstrated by *E. coli* (17.4 mm) at 100 mg /ml. The zone of inhibition of the control (Ciprofloxacin 50 mg/ml) ranges from to 19-22 mm.

Isolates	Concentration (mg /ml)/zone of inhibition (mm)				
	25	50	75	100	Control
Klebsiella pneumoneae	9.2±0.00	9.8±0.00	10.5±0.11	12.1±0.13	22
Salmonella typhi	11.3±0.15	12.4±0.13	13.7±0.22	16.5±0.26	21
Shigella sp	9.7±0.17	11.2±0.20	13.8±0.09	13.6±0.31	22
Pseudomonas aeruginosa	8.3±0.00	10.2±0.26	12.4±0.14	12.6±0.21	20
Escherichia coli	12.3±0.20	13.5±0.12	14.7±0.17	17.4±0.36	22
Staphylococcus aureus	9.3±0.15	11.8±0.20	14.3±0.23	15.0±0.12	19

Table 2: Antibacterial activity of *Syzygium aromaticum* aqueous extract

Antibacterial Activity of Methanol Extract

The antibacterial activity of methanol extract is presented in (Table 3). The results showed that zones of inhibition recorded by the isolates depend on the type of bacterial isolates and concentration of the extracts. Highest zone of inhibition is demonstrated by *Shigella sp* (19.7 mm) at 100 mg /ml. The zone of inhibition of the control (Ciprofloxacin 50 mg/ml) ranges from to 19-22 mm.

Isolates	Concentration (mg /ml)/zone of inhibition (mm)				
	25	50	75	100	Control
Klebsiella pneumoneae	10.8±0.20	11.4±0.12	13.2±0.17	15.4±0.17	22
Salmonella typhi	12.8±0.12	13.5±0.17	14.4±0.25	16.2±0.20	21
Shigella sp	11.7±0.32	12.8±0.25	15.1±0.32	19.7±0.37	22
Pseudomonas aeruginosa	12.0±0.12	12.2±0.36	13.9±0.15	16.0±0.23	20
Escherichia coli	13.3±0.32	14.2±0.20	15.8±0.12	16.9±0.32	22
Staphylococcus aureus	12.3±0.17	12.5±0.32	16.1±0.20	18.3±0.47	19

Table 3: Antibacterial activity of *Syzygium aromaticum* methanol extract

MIC and MBC of the Extract

Minimum inhibitory concentration of aqueous and methanol extract of *Syzygium aromaticum* is represented in (Table 4). The result showed dilutions of various concentrations of aqueous and methanol extracts can inhibit and/or kill the isolates. Lower MIC (3.125 mg/ml) was shown by methanol extract than aqueous extract. MBC of methanol extract ranges between 12.5 - 50mg/ml while the MBC of *Shigella sp* and *Pseudomonas aeruginosa* was not found in aqueous extract.

Isolates	Aqueous extract		Methanol extract	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
Klebsiella pneumoneae	12.5	50	6.25	25
Salmonella typhi	6.25	12.5	6.25	25
Shigella sp	12.5	NF	6.25	50
Pseudomonas aeruginosa	12.5	NF	12.5	50
Escherichia coli	6.25	25	3.125	12.5
Staphylococcus aureus	12.5	50	6.25	25

Key: NF = Not found

Table 4: Minimum inhibitory concentration (MIC) and MBC of the extracts.

Discussion

The results of the present study suggested that several phytochemicals are present in *Syzygium aromaticum* bud extracts. The presence of the phytochemicals can be correlated with the fact that solvent extracts showed antibacterial activity against the bacterial strains. Phytochemicals give plants their colour, flavour, smell and are part of a plant`s natural defense system and protect them against herbivorous insects and vertebrates, fungi, pathogens, and parasites [12]. The phytochemicals alkaloid, terpenoids, flavonoids, steroid, phenol, Anthraquinones, saponin and tannin were present in *Syzygium aromaticum* extracts according to this study. The results are in accordance with the findings of other authors who have studied this plant [13].

According to this study, Alkaloid is present in the extracts. Alkaloids comprising a large group of nitrogenous compounds are widely used as cancer chemotherapeutic agents, anaesthetics and Central Nervous Stimulants [14]. Alkaloids are known to play some metabolic roles and control development in living system [15]. It also interferes with cell division, hence the presence of alkaloids in clove could account for their use as antimicrobial agents. Aboaba, et al. had reported that the antimicrobial properties of substances are desirable tools in food spoilage and food safety [16]. This suggests that the *Syzygium aromaticum* extracts which have been confirmed to contain alkaloids may also be useful as preservatives in food. Terpenoids have been found to be useful in the prevention and therapy of several diseases, including cancer. Terpenoids are also known to possess antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, ant hyperglycemic, anti-inflammatory and immunomodulatory properties [17]. Flavonoids are also present in the extract as a potent water-soluble antioxidant and free radical scavenger, which prevent oxidative cell damage and also have strong anticancer activity [18,19]. It also helps in managing diabetes induced oxidative stress. Steroids are importance in pharmacy as they possess compounds like sex hormones and can be used for drug production [20]. Tannin and saponin were present in the extract. Saponins protect against hypercholesterolemia and antibiotics properties [21]. In addition, it has been found that saponins have antitumor, antioxidant and anti-mutagenic activities and can lower the risk of human cancers by inhibiting the growth of cancer cells [22,23]. The growth of many fungi, yeast, bacteria and viruses was inhibited by tannins [24]. The phytochemical content of the extract of *S. aromaticum* revealed that the Alkaloids was found to be the most abundant phytochemical (9.6%) followed by tannin, saponin and flavonoids.

The results of antibacterial activity of *Syzygium aromaticum* extracts against food borne pathogens are given in (Tables 2 and 3) which shows that the methanol extracts is more effective against the tested isolates than aqueous extracts. *E. coli* and *Salmonella* were also more susceptible to the extracts in comparison with the rest 14.97 and 13.84 mm respectively. The result of antimicrobial activity of *Syzygium aromaticum* in this study was inconformity with the study conducted by many researchers [6,7]. The extract of *Syzygium aromaticum* showed highest zone of inhibition (11.67±1.53mm) against *Salmonella spp*. and lowest zone of inhibition (8.0±1.73mm) against *Escherichia coli*. *Syzygium aromaticum* extract also showed lower zone of inhibition (8.67±2.52mm) against *Staphylococcus aureus* compared to the Gram-negative bacteria. Based on the susceptibility of the organisms to the extracts, *E. coli* was found to be the highest susceptible organisms with average zone of inhibition of 14.97±1.97 mm, followed *Salmonella typhi* (13.84±2.52 mm), *S. aureus* (13.7±1.65 mm), *Shigella* (13.44±1.12 mm), *Pseudomonas* (12.27 ±0.98mm) while least average zone of inhibition is shown by *Klebsiella* (11.55±1.16 mm).

The antibacterial activities of the extracts are expected due to the presence of compounds such as alkaloid, flavonoids and tannin. The results obtained in this study corroborate with the report of Nzeako, et al. which found that clove extract possessed a broad spectrum of antimicrobial activity exhibited for both bacteria and fungi [25]. The clove oils possess antimicrobial activity against *S. aureus*, *E. coli*, *P. aeruginosa* as well as against *S. pyogenes*, *Corynebacterium*, *Salmonella*, *Bacteroides* and *C. albicans* at various dilutions of the extracts. The result of this justified that of Sofia *et al*. [6] who tested the antimicrobial activity of different Indian spice plants as mint, cinnamon, mustard, ginger, garlic and clove, the result showed complete bactericidal effect against all the food-borne pathogens tested *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus* was the aqueous extract of clove at 3%. At the concentration of 1% clove extract also showed good inhibitory action.

The result of MIC and MBC of the extracts showed that dilutions of various concentrations of aqueous and methanol extracts of *Syzygium aromaticum* can inhibit and/or kill the isolates. Lower MIC (3.125 mg/ml) was shown by methanol extract than aqueous extract. MBC of methanol extract ranges between 12.5 - 50mg/ml while the MBC of *Shigella sp* and *Pseudomonas aeruginosa* was not found in aqueous extract. This is in line with the study of Gislene, *et al*., who found that the plant extract contained agents that killed *Salmonella*, *C. albicans*, *P. aeruginosa* and *E. coli* [3].

Conclusion

In conclusion, this study revealed that *Syzygium aromaticum* extracts possess medicinal properties and antibacterial activity that inhibit bacterial growth. The results of the present study show that *Syzygium aromaticum* ethanol extracts are more effective against all tested bacterial strains than aqueous extracts. *E. coli* and *Shigella* were also more susceptible to the extracts while *Klebsiella* was the least susceptible. The antibacterial activities of the extracts are expected perhaps due to the present of bioactive compounds like Alkaloid, Terpenoid, Saponin, Tannin, flavonoids and Anthraquinones which were dissolved in the solvents. The results of present study have provided the justification for therapeutic potential of *Syzygium aromaticum* and also used as dietary supplement for food preservation in addition pharmacological values.

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