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Full Length Research Article

STUDY ON ANTIBACTERIAL ACTIVITY OF CLOVE (*SYZYGIUM AROMATICUM*) CRUDE EXTRACT AGAINST *STAPHYLOCOCCUS AUREUS, ESCHERICHIA COLI, SALMONELLA* Sp. AND *PSEUDOMONAS* Sp.

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ABSTRACT
This study was designed to determine the phytochemical constituents and antibacterial activity of aqueous and ethanolic extract of *Syzygium aromaticum* (clove) seed at varying concentrations; against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* sp and *Pseudomonas* sp. Preliminary phytochemical screening of the *S. aromaticum* extracts was done using standard analytical methods. The aqueous and ethanol extracts of *S. aromaticum* were evaluated for antimicrobial activities against the isolates using agar well diffusion and broth dilution assay. The results of the phytochemical components revealed the presence of Alkaloid, Flavanoid, Tannin, Saponin, Glycosides, Terpenoid and Phenol in the extracts. Ethanol extract of *S. aromaticum* displayed antibacterial activity against all the tested organisms with highest activity (27mm at 100mg/mL concentration) on *Salmonella*. The aqueous extract of clove was found to be less active, though, it was active against all the organisms tested, with highest activity on *Salmonella* sp (24mm at 100mg/mL concentration). Both the aqueous and ethanolic extracts showed MIC at 6.25 mg/mL on all the tested isolates with the exception of aqueous extract against *E. coli* that showed MIC at 12.5 mg/mL. MBC was only observed on ethanolic extract against *Salmonella* sp. and *S. aureus* both at 6.25 mg/mL. The results provide a scientific basis for the centuries-old traditional usage of *S. aromaticum*.

Keywords: *Syzygium aromaticum,* Antibacterial activity, Phytochemicals, Agar well diffusion, Broth dilution assay.

INTRODUCTION

Natural products from different sources are used to preserve food from spoilage and pathogenic microorganisms. Plant are the main source of product and contain many essential oil that have preservation effect against different microorganisms (Arshad & Batool, 2017; Saeed *et al.,* 2019).

Several researches have been conducted to find out the antimicrobial potential of natural products, especially the plant sources like fruits, vegetables, herbs, and spices because they are enriched with compounds having antimicrobial activity. Nowadays, there are more than 1350 plants with antimicrobial activities and more than 30,000 antimicrobial components have been extracted from plants (Cocolin *et al*., 2004). As compared to chemical or synthetic additives herbal additives are preferred as these are safer, flavor enhancer and without any side effects (Brull and Coote, 1999). Plant secondary metabolites contain many antimicrobial agents, so they have a greater inhibitory effect against Gram-positive and Gram-negative bacteria (Prakasha *et al*., 2013; Roundsa *et al*., 2013; Burt 2014).

Clove belongs to a tree *Eugenia caryophyllata* (*Syzygium aromaticum*) is used as a spice in almost all the world's fare. It has a very major role in spice trade and is highly appreciated for their therapeutic properties (Atkinson, 2016). *S. aromaticum* are an excellent source of manganese, dietary fiber, vitamin C, vitamin K, and Ω-3 fatty acids and calcium (Atkinson, 2016). The most important constituent of *S. aromaticum* is the phenylpropene eugenol due to which it has strong characteristic aroma (Atkinson, 2016). They are usually Known for their antiseptic, i.e. bactericidal, virucidal and fungicidal, and medicinal properties and their fragrance, they are used in embalmment, preservation of foods and as antimicrobial, analgesic, sedative, anti-inflammatory, spasmolytic and locally anesthetic remedies (Shaaban, 2020). The oil of *S. aromaticum* has been used traditionally in a variety of health conditions including indigestion, generalized stress, parasitic infestations, cough, toothaches, headache, and blood impurities (Fagere & Al Magbou, 2016). It has also been used for nausea and vomiting, while in tropical Asia; it has been given to treat such diverse infections as malaria, cholera and tuberculosis (Mintah, 2019). The study is very important because Drug resistance, emerging and re-emerging microbial infections that shows resistance to various antibiotics have led to search of new antibiotics source and plants have emerged as a credible candidate for new antimicrobials. This study will help to identify new active agents if they exist in the plant that can be used against microorganisms. Thus, the aim of this study was to determine the antibacterial activity, phytochemical screening of *S. aromaticum* plant extracts on *Staphylococcus aureus, Escherichia coli, Salmonella* sp. and *Pseudomonas* spp.

MATERIAL AND METHODS

Sample Collection, Identification and Preparation

The plant samples of *S. aromaticum* were obtained from Dutsinma market, Katsina State, Nigeria. It was then identified and confirmed by a botanist in the Department of Biological Science in Federal University Dutsin-Ma. The dried cloves were washed with distilled water to remove all dust and contaminations on the surfaces of the plant samples. The plant samples were properly dried and

pulverized into fine powder using mortar and pestle, it was then sieved and packaged in an air tight container and labeled (Mainasara *et al.,* 2017).

Extraction of Plant Material

Twenty-five grams (25g) of the fine powdered *S. aromaticum* was dissolved in two hundred and fifty mL (250mL) of sterile distilled water to give a dilution ratio of 1:10 (1g/10mL), and another grams 25g of the powder was dissolved in 250mL of 95% ethanol to arrive at the same dilution ratio of 1:10 and then kept for 3 days with constant shaking at regular intervals (Fatope, 2001). The solutions were filtered using Whatman (No 1) filter paper. The crude extracts (aqueous and ethanolic) of *S. aromaticum* were then obtained following filtration and evaporation to dryness using a water bath and then stored at 4°C in a freezer until needed for further experiment (Sanusi *et al.,* 2019).

Phytochemical Screening of the Extracts

The aqueous and the ethanolic extract were investigated for the presence of bioactive phytochemical constituents as demonstrated by Abdu and Dimas (2016).

Collection of test isolates

The tested organisms including *Escherichia coli, Staphylococcus aureus, Salmonella* sp. and *Pseudomonas* sp. were collected from Microbiology laboratory, Department of Microbiology, Federal University Dutsin-Ma. After collection, they were then reconfirmed by culturing in their various specific solidified agar media plates and incubated at 37°C for 24 h. Thereafter, the colonies were studied macroscopically for their colonial characters and microscopically (gram stain), followed by biochemical reaction for further identification and confirmation (Cheesbrough, 2005).

Standardization of Inoculum

Using a sterile inoculating wire loop, colonies from the overnight culture of the test organisms was transferred into a tube containing about 2.0mL normal saline (0.9%) and the volume was adjusted to achieve a turbidity which equaled that of 0.5 McFarland's standard (Sanusi *et al.,* 2022).

Preparation of Extract Working Concentrations

One gram of the crude extract was dissolved in 10mL of 2% Dimethyl Sulfoxide (DMSO) in a test tube to get 100mg/mL which the highest stock concentration used. This was then followed the by serial dilution with distilled water to give the desired concentrations (50mg/mL, 25mg/mL, 12.5mg/mL and 6. 25mg/mL). Likewise, the aqueous dilution was also prepared using the same technique.

Determination of antibacterial Activity

The agar well diffusion technique previously demonstrated by Sanusi *et al.* (2022) was adopted to test for the antibacterial activity of the *S. aromaticum* extracts. The adjusted bacterial suspensions were inoculated using a sterile swab onto the prepared and solidified Mueller-Hinton agar, and the allowed to stand for 15 minutes. Thereafter, six wells of 4 mm each were bored using a sterile cork borer. 0.2mL of the ethanol extract of varying concentration (100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL and 6.25 mg/mL) were added to five wells. Ciprofloxacin was added to the other well as a positive control. The prepared petri-dishes were incubated at 37°C overnight. The efficacy of the crude extracts was assessed by measuring the diameters of inhibition zones (mm). Same procedure was followed for the aqueous concentrations.

Minimum Inhibitory Concentration (MBC) and Determination of Minimum Bactericidal Concentration (MBC)

To determine the minimum inhibitory concentrations (MIC) of the *S. aromaticum* extract, National Committee for Clinical Laboratory standards procedure as described by Lar *et al.* (2001) was followed. Briefly, seven tubes each containing 5 mLs of Muller-Hinton broth (labeled 1-7) were used for the MIC determination of the ethanolic extract. 1 mL of each of the crude extract concentrations (100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL and 6.25 mg/mL) were introduced into the seven tubes (1-7), and were then mixed thoroughly. To six test tubes, 0.1ml of broth cultures of the test organism was added with the sixth serving as positive control (broth and culture) while the seventh as negative control (broth only). Thereafter, all the inoculated tubes were incubated overnight at 37°C after which they were observed for bacterial growth. The MIC of the test crude extract was defined as the lowest concentration of the extract capable of inhibiting the growth of the test organism. The same procedure was carried out for the aqueous extract concentrations

RESULTS

The results of phytochemical analysis of both the aqueous and ethanolic extract of *S. aromaticum* shows presence of alkaloid, tannin, saponin, flavonoid, phenol, steroid, glycoside, and terpenoid in both extracts with the exception of flavonoid which is absent in aqueous extract (Table 1)

Table 1: Phytochemical Constituents in both Aqueous and Ethanolic Extracts of *S. aromaticum*.

Phytochemical constituent	SAA	SAE
Alkaloid	+	+
Flavanoid	–	+
Tannin	+	+
Saponin	+	+
Glycosides	+	+
Terpenoid	+	+
Phenol	+	+

KEYS: + (Present),- (Absent).
SAA = *Syzygium aromaticum* Aqueous
SAE= *Syzygium aromaticum* Ethanolic

Table 2 and 3 showed the results of antibacterial evaluation of *S. aromaticum* aqueous and ethanolic extract at varying concentration (100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL and 6.25 mg/mL) against bacterial isolates of *E. coli*, *Salmonella* sp., *S. aureus* and *Pseudomonas* sp. The result of the aqueous extract showed that, *Salmonella* sp. have the highest zones of inhibition of 24 mm, followed by *S. aureus* and *Pseudomonas* sp. both with the diameter of inhibition zone of 21mm all at 100 mg/mL concentration. Likewise, the ethanolic extract exhibited the highest inhibition against *Salmonella* sp. with 27mm diameter of inhibition zone followed by *Pseudomonas* sp. with 27mm diameter of inhibition zone. No antibacterial activity was observed at 6.25 mg/mL for extract on any of the tested bacteria. Ciprofloxacin was used as the

positive control antibiotic inhibiting *S. aureus* with 30mm and then, *E. coli*, *Salmonella* sp. and *Pseudomonas* sp both with 25mm.

Table 2: Diameter of inhibition zone of aqueous extract of Clove against the test isolates

Organisms	100 mg/mL	50 mg/Ml	25 mg/mL	12. 5 mg/mL	6. 25 mg/mL	CPX
E. coli	20mm	15mm	10mm	_	-	25mm
Salmonella sp.	24mm	18mm	14mm	10mm	-	25mm
S. aureus	21mm	17mm	13mm	8mm	-	30mm
Pseudomonas sp.	21mm	17mm	13mm	8mm	-	25mm

KEYS
- = No Zone of Inhibition
CPX= Ciprofloxacin

Table 3: Diameter of inhibition zone of ethanolic extract of Clove against the test isolates

Organisms	100 mg/Ml	50 mg/mL	25 mg/mL	12. 5 mg/mL	6. 25 mg/mL	CPX
E. coli	21mm	19mm	16mm	9mm	-	25mm
Salmonella sp.	27mm	21mm	17mm	12mm	-	25mm
S. aureus	22mm	20mm	17mm	10mm	-	30mm
Pseudomonas sp.	24mm	21mm	18mm	10mm	-	25mm

KEYS
- = No Zone of Inhibition
CPX= Ciprofloxacin

The results of MIC of the aqueous and ethanolic extracts of *S. aromaticum* is presented in Table 4. Both the aqueous and ethanolic extracts showed MIC at 6.25 mg/mL on all the tested isolates with the exception of aqueous extract against *E. coli* that showed MIC at 12.5 mg/mL.

Table 4: Minimum Inhibitory Concentration (MIC) of *S. aromaticum* against the isolates

Organism	SAA (mg/mL)	SAE (mg/mL)
E.coli	12.5	6.25
Salmonella sp.	6.25	6.25
S. aureus	6.25	6.25
Pseudomonas sp.	6.25	6.25

KEYS= **SAA=***Syzygium aromaticum* Aqueous
SAE= *Syzygium aromaticum* Ethanolic

The MBC results presented on Table 5 showed that MBC was only observed on ethanolic extract against *Salmonella* sp. and *S. aureus* both at 6.25 mg/mL.

Table 5: Minimum Bactericidal Concentration (MBC) of *S. aromaticum* against the isolates

Organism	SAA (mg/mL)	SAE (mg/mL)
E. coli	-	-
Salmonella sp.	-	6.25
S. aureus	-	6.25
Pseudomonas sp.	-	-

KEYS
SAA=*Syzygium aromaticum* Aqueous
SAE= *Syzygium aromaticum* Ethanol

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