Antibacterial Activities and Quantitative Phytochemical Screening of the Hexane and Ethanolic Oil Extracts of *Syzygium aromaticum* Against ESBL Producing Bacteria Isolates

S. Musbau
Department of Microbiology, Yobe State University, Damaturu, Nigeria
M. Yushau
Department of Microbiology, Bayero University Kano, Nigeria
W.T. Dalha
Department of Microbiology, Bayero University Kano, Nigeria

Abstract
The study was designed to determine antibacterial potentials of *Syzygium aromaticum* and phytochemical screening of the hexane and ethanolic oil extracts of *Syzygium aromaticum* (clove oil) against some multi-drug resistant bacteria (*Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Shigella boydii* and *Klebsiella oxytoca*) isolated from urine and stool samples was carried out using standard methods. Phytochemical screening revealed the presence of alkaloids, saponins, tannins, phenol, flavonoids and sterols in both oil extracts. Similarly, the percentage yield of ethanol oil extract was 34.4 with characteristic pale-brown colour and hexane percentage yield was 16.4 with characteristic pale-brown colour. The bioassay studies showed that the ethanolic oil extract exhibited an activity against *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Shigella boydii* and *Klebsiella oxytoca* with the zone of inhibition which ranged between (10mm - 24.33mm, 9mm - 20mm, 2.33mm - 19mm, 8.33mm – 21.67mm and 8.67mm - 20.33mm) respectively for each bacterium at a concentration percent/disc... On the other hand, the hexane oil extract were found to be more active against the isolates with the following zone of inhibition which ranged between (10.27mm - 22.60mm, 13.53mm - 27.50mm, 9.34mm - 21mm, 9.10mm - 20,33mm and 4.23mm - 21.57mm) for *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Shigella boydii* and *Klebsiella oxytoca* respectively. The results of the study prove that the ethanolic and hexane oil extracts of *Syzygium aromaticum* have antibacterial potentials against the tested clinical bacterial isolates, thus confirming the use of the plant for medicinal purposes.

Introduction
Antimicrobial resistance is an emerging problem worldwide. Each year, 33,000 people die from an infection due to bacterial resistance to antibiotics in Europe. The burden of infections with bacterial resistance to antibiotics on the European population is comparable to that of influenza, tuberculosis and HIV/AIDS combined [1]. It has been estimated that by 2050, 10 million lives a year and a cumulative 100 trillion USD economic output are at risk worldwide due to the rise of drug resistant infections if we do not find proactive solutions to slow down drug resistance [2]. Resistance of Gram-positive bacteria is generally stable or even decreasing in Europe, whereas resistance to Gram-negative bacteria (such as *Enterobacteraceae*) has an increasing trend in several European and African countries [3]. Extended spectrum beta lactamase (ESBL) were the first beta-lactamase enzymes produced by some bacteria that provide resistance to beta lactam antibiotics like penicillins, cephalosporins, and carbapenems although, carbapenems are relatively resistant to beta-lactamase. Beta-lactamase provides antibiotic resistance by breaking the antibiotics structure [4]. Extended Spectrum beta-Lactamase were
first described in the 1980s and have been detected in Klebsiellas and later in Escherichia coli, Pseudomonas aeruginosa, Serratia marcescens and other Gram negative bacilli. Extended Spectrum beta-Lactamase are enzymes conferring broad resistance to penicillins, Aztreonam and Cephalosporins. These enzymes are able to hydrolyze 3rd and 4th generation Cephalosporins and Monobactams. They are an increasingly important cause of transferable multidrug resistance in Gram negative bacteria throughout the world [4].

Clove buds belong to the Myrtaceae family, indigenous to India, Indonesia, Zanzibar, Mauritius and Sri Lanka; Cloves are available throughout the year due to different harvest seasons in different countries [5]. The clove tree can live up to 100 years and above. The tree prefers to grow in well-drained soil with sufficient soil moisture. Clove trees require heavy sunlight with high atmospheric temperature (25 to 35°C), well-distributed rainfall above 150 cm and high humidity above 70% [6]. The fruit nearly contains one or two seeds known as ‘mother of clove’. The cultivated trees are rarely allowed to reach the fruit stage. These are harvested when they develop dark red ellipsoid berries. Clove essential oil (CEO) was reported to possess antibacterial, antifungal, insecticidal, and antioxidant properties [7].

Antibacterial activity of clove essential oil has been reported against Staphylococcus aureus and Listeria monocytogenes, pasteurized milk, antimicrobial resistance against Penicillium sp., Aspergillus flavus and Staphylococcus aureus found on dried fish. Zengin et al. reported the antimicrobial activity of clove oil against three gram-positive bacteria (Listeria innocua, Carnobacterium divergens and Staphylococcus aureus) and four gram-negative bacteria (Salmonella typhimurium, Escherichia coli, Serratia liquefaciens and Shewanella putrefaciens) by broth microdilution method [8]. The result showed that clove essential oil inhibited the growth of all bacteria while Shewanella and Listeria were found to be resistant to oil. Clove oil was also found to be effective against food borne gram negative bacteria (Staphylococcus aureus (MHA) (Oxoid, UK). A sterile glass rod was used to spread the organism on the petri dish. Three holes, each measuring 6.0 mm in diameter were made in each of the solid agar plates using a sterile Cork borer (6 mm). A stock solution of the extract were prepared by dissolving 2ml of the extract into 8ml of Dimethyl sulfoxide (DMSO) to obtain a concentration of 2000mg. A stock solution were further diluted to obtain different concentrations of 20%, 40%, 60%, 80%, and 100%. Using a Pasteur pipette, different concentration of plant extracts were transferred into the holes made on petridish. Positive control (Amikacin 30 mg) and DMSO were used as positive and negative/solvent control respectively; two plates were used for each

**Materials and Methods**

**Collection, Identification and Preparation of the Plant Materials**

Syzygium aromaticum were purchased from Damaturu market. Samples of the spices were identified in the Herbarium section of the Department of Plant Biology, Bayero University Kano with accession numbers BUKHAN 342. The spices were thoroughly washed with sterile distilled water and then allowed to air-dry for a few days. It was then ground into powder form using pestle and mortar, sieved through 250μm mesh to obtain fine powder which was stored at room temperature in sealed containers until required for use.

**Extraction of oil from the spices**

Syzygium aromaticum were extracted using Soxhlet extraction process as described by [9]. Five Hundred Milliliters (500ml) of ethanol and hexane were poured into a round bottom flask which is equipped with a Soxhlet apparatus and condenser. Six pieces of anti-bumping granules were added and 50g of the powdered sample were inserted in the center of the extractor. The extraction was carried out at 61.2°C, 78.4°C and 69.0°C for ethanol and hexane respectively. When the solvent boils, the vapor rises through the vertical tube into the condenser at the top. The liquid condensers dips into the filter paper thimble in the center which contains the solid sample that was extracted. The extracts sip through pores of the thimble and fill the siphon table, where it flows back down into the round bottom flask quick fit. These were allowed to continue for 3-4 hours. It was removed from the tube, dry in the oven, cooled in the desiccators and then weighed again so as to determine the amount extracted. Further extraction was repeated at intervals. At the end of the extraction process the resulting filtrate was left in an evaporating dish so that the solvent would evaporate.

**Evaluation of antimicrobial activity**

The agar diffusion methods as described by [10] and [11] were adopted for the study. Using McFarland standard (1.5 x 10⁸ CFU/ml) a suspension of the test organisms was inoculated on Mueller- Hinton agar (MHA) (Oxoid, UK). A sterile glass rod was used to spread the organism on the petri dish. Three holes, each measuring 6.0 mm in diameter were made in each of the solid agar plates using a sterile Cork borer (6 mm). A stock solution of the extract were prepared by dissolving 2ml of the extract into 8ml of Dimethyl sulfoxide (DMSO) to obtain a concentration of 2000mg, the stock solution were further diluted to obtain different concentrations of 20%, 40%, 60%, 80%, and 100%. Using a Pasteur pipette, different concentration of plant extracts were transferred into the holes made on petridish. Positive control (Amikacin 30 mg) and DMSO were used as positive and negative/solvent control respectively; two plates were used for each.
concentration of the extracts. The plant extracts were thereafter allowed to stand for one hour for pre-diffusion of the extracts [11] and were subsequently incubated at 37°C for 24 h. After incubation, plates were observed for formation of a clear zone around the hole which corresponds to the antimicrobial activity of the tested compounds. The zone of inhibition (ZOI) was observed and measured in mm.

**Phytochemical screening of the spices extracts (quantitative determination)**

**Test for flavonoids**
The stock solution (1 mL) was taken in a test tube and added a few drops of dilute NaOH solution. An intense yellow colour appeared in the test tube. It became colourless when adding a few drops of dilute acid that indicated the presence of flavonoids [10].

**Test for alkaloid**
The extract (1.0 ml) was shaken with 5.0 ml of 2% HCl on a steam bath and filtered. To 1 ml of the filtrate, Wagner reagent (iodine in potassium iodine solution) was added. A reddish brown precipitate confirms its presence of alkaloid. [11]

**Test for saponin**
The stock solution (1 mL) was taken in a test tube and diluted with 20 mL of distilled water. It was shaken by hand for 15 min. A foam layer was obtained on the top of the test tube. This foam layer indicated the presence of saponins [10].

**Test for tannins**
The stock solution (3 mL) was taken in a test tube and diluted with chloroform and added acetic anhydride (1 mL). Finally, sulphuric acid (1 mL) was added carefully by the side of the test tube to the solution. A green colour was formed which showed the presence of tannins [10].

**Test for phenol**
The extract of 1.0 ml was added with 1.0ml of 10% ferride chloride. The formation of greenish brown or black precipitate or colour is taken as positive for phenolic test [11].

**Test for sterol**
The extract (1 ml) was dissolved in 2.0 ml of chloroform in a test tube and then 1ml of concentrated H2SO4 was added. Formation of reddish brown colour at the interphase confirms the presence of sterol [11].

**Results**

**Physical and Phytochemical Characteristics of the oil extracts**
The results of the physical characteristics of the oil extracts showed yields of 16.4% and 34.4% of the hexane and ethanolic oil extracts were obtained respectively. The hexane and ethanolic extraction were pale brown and oily. (Table1).

The results of the bioassay studies showed that the ethanolic oil extract was active at all concentration (20%, 40%, 60%, 80%, 100% /disc) but the activity were more pronounced on *Klebsiella oxytoca* at 20%, while at 40%, 60%, 80% and 100% activity was more pronounced on *Escherichia coli* (Table 3). Similarly when using hexane extraction all the concentrations (20%,40%, 60%, 80% and 100%) were more active against *Proteus vulgaris* (Table 4) even with control (amikacin).
Discussion
The activities of the hexane and ethanolic oil extracts of *Syzygium aromaticum* against the ESBL producing bacterial isolates in this study could be attributed to the presence of the bioactive ingredients such as saponins, alkaloids and tannins in the oil extracts. These compounds have been reported to have exhibited antibacterial potentials [12,13,14]. According to the present study, preparing an oil extract with organic solvent such as hexane and ethanol has been shown to have exhibited better antibacterial activity. This is in accordance with the results earlier reported by [15] who reported ethanol as the best solvent that dissolves multivariable types of compounds. The effectiveness of the organic solvent might be attributed to the nature of biologically-active components; their activities could be enhanced in the presence of organic solvents. Similarly, the strength of the extraction capacity of the ethanol and hexane could have allowed more of the bioactive constituents responsible for the antibacterial activities. From the study, ethanolic oil extract was found to be more active against the *Escherichia coli* and *Shigella boydii* while the hexane oil extract was found to be more active against the *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Klebsiella oxytoca* (Table 3 and 4). This variability could be related to the ability of the different solvents to dissolve some of the chemical constituents in the oil extracts.

Conclusions and Recommendations
The results obtained in this study prove that *Syzygium aromaticum* has a strong effect against the ESBL producing bacterial isolates; this plant can be used for the treatment of infections. The range of bacteria inhibited suggests that the plant could be used in the treatment of respiratory and urinary tract as well as wound infections. The high vulnerability of *Escherichia coli* and *Pseudomonas aeruginosa* to the oil extracts tested in this study is worthy of note as this bacteria is resistant to most of the available disinfectants and antimicrobials [16,17]. It is therefore recommended that further research needs to be carried out using other solvents to ensure the antibacterial potentials of the oil extracts. Similarly, there is need to purify and characterize phytochemical constituents responsible for the observed bioactivities with a view to supplementing conventional drugs especially in developing countries like Nigeria and also determine toxicological properties of *Syzygium aromaticum*.

## References


