https://dx.doi.org/10.4314/jpb.v16i2.7
Vol. 16 no. 2, pp. 139-144 (September 2019)
http://ajol.info/index.php/jpb

Journal of
PHARMACY AND BIORESOURCES

Comparative assessment of antibacterial activities of *Syzygium aromaticum* and *Cyperus articulatus* against *Staphylococcus aureus* and *Escherichia coli*

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Received 26th April 2019; Accepted 31st May 2019

Abstract
Antibacterial activities of Syzygium aromaticum and Cyperus articulatus was tested against staphylococcus aureus and Escherichia coli. The plant extract Syzygium aromaticum and Cyperus articulatus were extracted using soxhlet extraction technique and bacterial isolates were collected from Microbiology laboratory of Federal University Dutse. The inocula were standardized using 0.5 Mac-farland standard of turbidity. Mueller Hilton agar was used for sensitivity test and nutrient agar for culture and broth. Both antibacterial activity, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were evaluated using different concentration 100mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml respectively. The minimum inhibitory concentration of Syzygium aromaticum against gram positive bacteria (S. aureus) was 12.5 mg/ml (7mm) while that of E. coli (gram negative) was 25mg/ml (9.5mm) whereas the MIC of Cyperus articulatus was found to be 12.5 mg/ml (8mm) for E. coli and 6.25mg/lm (8mm) for S. aureus. For the mixture of S. aromaticum and C. articulatus, MIC determined was the same (12.5mg/ml) for both S. aureus and E. coli. MBC of Syzygium aromaticum determined against the bacterial isolates for S. aureus 50mg/ml and that of E. coli was 100mg/ml whereas for Cyperus articulatus, both S. aureus and E. coli was the same (50mg/ml) and for the mixture, MBC for S. aureus 25mg/ml and that of E. coli was 100mg/ml. Hence, both Syzygium aromaticum and Cyperus articulatus possess antibacterial activity against tested isolates responsible for many diseases.

Keywords: *Syzygium aromaticum; Cyperus articulates; Staphylococcus aureus; Escherichia coli.*

INTRODUCTION

Cyperus articulatus belongs to the family of *Cyperaceae*; they are annual rhizomatous occasionally tuberous, perennial with underground root, bearing scales, which grade into culms leaves. They are herbs, normal plants, switch-plants, with principal photosynthesizing function [1]. *Cyperus articulatus* represents a practical explored reservoir of potentially useful drugs or substances for the treatment of so many or wide range of disorder, this is because many of the species of this family have undergone phytochemicals, biological activity as well as ethno medicinal analysis to ascertain their potentiality in the treatment of human

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ISSN 0189-8442

disorders [2]. According to Mahailet [3], *C. articulatus* flavonoids, polyphenols, saponins, tannins, and terpenes. Severe specific compound isolated from this tropical grass include, alpha-corymbolol, alpha-cyperone, alpha-pinene, carophyllene oxide, corybolane, cyperotundone, and mustakone. *C. articulatus* has antimicrobial and DNA-binding effects or activity against *Staphylococcus aureus* in the broth culture as media [4]. Great antibacterial activity or properties of *Cyperus articulatus* decoction against *Escherichia coli*, weak activity against *Pseudomonas aureginosa* and inactive against *Salmonella gallinarium* was determined [5].

Syzygium aromaticum commonly known as clove, is a median size tree (8-12m) from the *Myrtaceae* family native from the Maluku islands in east Indonesia, and found to have stimulated the economic development of this Asiatic region [6]. As disclosed that [6, 7], clove oil consist essentially of acetyl Eugenol, beta-caryophyllene and vanillin, crategolic acid, tannins, gallotannic acid, methyl salicylate as well as several sesquiterpenes [8]. Due to its antibacterial, antifungal, antiviral and anticarcinogenic properties, *S aromaticum* in particular has attracted the attention of many researches in various institution across the globe [9].

EXPERIMENTAL

Collection and identification of plant samples. The plants material (Tuberous rhizome) of *Cyperus articulatus* and *Syzyguim aromaticum* (clove) were bought From Rimi market Kano State on February 2017. In addition, the taxonomic identification of the plant was confirmed in the Department of Biological Sciences by Dr. A. M. Auyo.

Preparation of plant samples. The (tuberous rhizome) of *Cyperus articulatus* and *Syzyguim aromaticum* (clove) were properly dried, after drying, the plants was ground to powdered using mortar and pestle, it was then sieved and packaged in an air tight container and labeled.

Soxhlet extraction method. Fifty grams (50 g) of both *Cyperus articulatus* and *Syzyguim aromaticum* (clove) powder were weighed using a weighing balance and poured into 250ml of ethanol solvent in round button flask, which was attached to a Soxhlet extractor and condenser on an isomantle. The side arm is lagged with wool. The solvent was heated using the isomantle and begin to evaporate, moving through the apparatus to the condenser. The condenser then drips into the reservoir containing the thimble. Once the level of solvent reached the siphon, it pours back into the flask and the cycle repeated again. The process was run for 16 hours. The equipment was monitored due to the mix of running water and electrical appliance.

Test organisms. The test organisms used for the antibacterial bioassay were (*Escherichia coli* and *Staphylococcus aureus)* clinical isolates obtained from Microbiology and Biotechnology laboratory of Federal university, Dutse.

Confirmatory test for the organisms. The following tests were carried out; Gram's staining, Indole, Methyl-red, Voges-proskaeur, Citrate utilization for *Escherichia coli* and Coagulase, Catalase for *Staphylococcus aureus* respectively.

Preparation of culture media. Nutrient agar (Oxoid Ltd., London) was used for the preparation of the inoculum and determination of Minimum Inhibitory Concentration (MIC) as well as while Mueller Hinton agar (Oxoid Ltd., London) was used for the sensitivity for antibacterial activity. All the media were prepared according to manufacturers' instruction.

Standardization of inoculum. Two bacterial isolate namely; *Escherichia coli and Staphylococcus aureus*, were sub cultured on the nutrient agar slants using a sterile wire

loop and incubated for 24 hours at 37ºC and this served as the store. These were in turn sub- cultured to nutrient broth and incubated again for 24 hours. Growth of such bacteria in the broth was indicated by turbidity. The broth cultures were further diluted in normal saline (NaCl). McFarland's turbidity standard scale number 0.5 was prepared by dissolving 0.5 g of Barium chloride in distilled water to obtain 50ml solution, 1 ml of concentrated H_2SO_4 was dissolved in 99 ml distilled water to make it 100 ml out of which 0.6 ml was measured and discarded. From already prepared dilute 50 ml Barium chloride, 0.6 ml was measured and then added into 99.4 ml diluted H_2SO_4 to give 100 ml turbid solution, which was, compared with the turbid suspension of the test microbes (Bacteria). Normal saline was used to make a turbid suspension of the microbes. Incubated at 37ºC for 6 hours, dilution of the microbes was done continuously using the normal saline until turbidity match that of the McFarland's scale by comparison. The crude extracts were then obtained following filtration and evaporation to dryness using a rotary evaporator at 400ºC, and the extracts were stored in a freezer until needed.

The bioassay procedure: The antibacterial activity of ethanolic extract was evaluated using well diffusion method. The antibacterial assay was evaluated using different concentrations (100 mg/ml, 50 mg/ml, 25 mg/ml 12.5 mg/ml and 6.25 mg/ml) of the extract made by dissolving the corresponding weight of the powder in distilled water. Nutrient agar plates were seeded with 0.1 ml of the standard inoculums of the test microbes (*E. coli or S. aureus*) separately; the inocula were spread evenly by the use of sterile swab stick over the surface of the agar media. A standard sterile cork borer was used to make a well at the centre of each inoculated plates and the extract was then introduced into each well on the media. The inoculated plates were then incubated in non-inverted position at 37ºC for 24 hours after which the media were observed for zones of inhibition. The diameter of growth inhibition zones were measured with a transparent ruler and recorded in mm.

Determination of Minimum Inhibitory Concentration (MIC). Minimum inhibitory concentration of the extracts was Determined carried out on *Escherichia coli* and *Staphylococcus aureus* and was done using broth dilution method. Nutrient broth was weighed and dispensed into labeled, arranged test tubes, the initial test tube contained 10 ml and other four contained 5 ml of nutrient broth respectively. These were sterilized at 121ºC for 15 minutes; the broth was allowed to cool. Two-fold serial dilutions of the extract in the broth were made to obtain the concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml respectively. The highest concentration was obtained by dissolving 0.2g of the extract in 10ml of the nutrient broth. Having obtained the different concentrations of the extracts in the broth, 0.1 ml of the standard inoculum of the test microbes in the normal saline were inoculated into the different concentration of diluted extract in the broth, and then incubated at 37ºC for 24 hours. The lowest concentration of the extract in the broth that inhibited the growth of the microbes was recorded as the Minimum Inhibitory Concentration (MIC).

Determination of Minimum Bactericidal Concentration (MBC).

Minimum Bactericidal Concentration (MBC) of the extracts was also carried out to determine whether the tested microbes were killed or only their growth was inhibited. Nutrient agar was prepared according to the manufacturer's instructions, autoclaved at 121ºC for 15 minutes cooled at 40ºC and poured into sterile petri dishes, the plates were allowed to cool and solidify. The contents of the MIC in the serial dilution that show no growth (turbidity) were sub cultured

onto the solidified medium, the plates were incubated at 37^{o}C for 24 hours after which the plates were observed for bacterial growth. The MBC was the plate with lowest concentration without colony growth.

RESULTS

Yield of the extracts. The physical properties of *Cyperus articulatus* and *Syzygium aromaticum* are shown in Table 1. with yields of 4.6 g and 5.8 g, respectively. *Cyperus articulatus* had a Gummy texture with a Dark brown color, while *Syzygium aromaticum* had a Gummy and oily texture with a Brown color.

Table 1: Physical properties *Cyperus articulatus* of and *Syzygium aromaticum.*

Properties	*Cyperus articulatus*	*Syzygium aromaticum*
Yield (g)	4.6 g	5.8 g
Colour	Brown	Dark brown
Texture	Gummy	Gummy and oily

Table 2: The biochemical (confirmatory) test for *Escherichia coli* and *Staphylococcus aureus.*

Test organism	Microscopic examination	Biochemical characteristics						
		G.R.	I	M	V	C	Cat	Coa
E. coli	green metallic sheen, small spread and slightly raised colonies on EMB	-	+	+	-	-		
S. aureus	Small, yellow & mucoid colonies	+					+	+

Key: GR: Gram's reaction, EMB: Eosin methylene blue, I: Indole, M: Methyl red, V: Voges-proskaus, C: Citrate utilization test, Cao: coagulase, Cat: Catalase.

Table 3: Mean zone of inhibition (mm) of the *S. aromaticum* concentration (mg/ml) against *E.coli* and *S. aureus*

conc. of *S. aromaticum* (mg/ml)→	100	50	25	12.5	6.25
E. coli	14	11	8.5	-	-
S. aureus	15.5	12	9.5	7	-

- = no zone of inhibition

Table 4: Mean zone of inhibition (mm) of the *Cyperus articulatus* against *E. coli* and *S. aureus.*

conc. of *C. articulatus* (mg/ml)→	100	50	25	12.5	6.25
E. coli	15	12	8	-	-
S. aureus	20	16	11.5	8	-

- = no zone of inhibition

Table 5: Mean zone of inhibition (mm) of the mixtures of *C. articulatus and S. aromaticum* (mg/ml) against *E.coli* and *S.aureus*

conc. of *S. aromaticum & C. articulatus* (mg/ml)→	100	50	25	12.5	6.25
E. coli	18	14	10	8	-
S. aureus	21	15	12	9	8

- = no zone of inhibition

Table 6:- Minimum Inhibitory Concentration (MIC) of *S. aromaticum, C. articulatus* and their mixtures

Bacterial isolates (mg/ml)	+ve C	-ve C	100	50	25	12.5	6.25
E. coli (*S. aromaticum*)	+	-	-	-	MIC	+	+
S. aureus (*S. aromaticum*)	+	-	-	-	-	MIC	+
E. coli (*C. articulatus*)	+	-	-	-	MIC	+	+
S. aureus (*C. articulatus*)	+	-	-	-	MIC	+	+
E. coli (mixtures)	+	-	-	-	-	MIC	+
S. aureus (mixtures)	+	-	-	-	-	MIC	+

MIC = Minimum Inhibitory Concentration; +ve C = Positive Control; -ve C = Negative Control
- = No growth; + = Growth (turbidity);

Table 7:- Minimum Bactericidal Concentration (MBC) of *C. articulatus, S. aromaticum* and their mixture

Bacterial isolates (mg/ml)	+ve C	-ve C	100	50	25	12.5	6.25
E. coli (*S. aromaticum*)	+	-	MBC	+	+	+	+
S. aureus (*S. aromaticum*)	+	-	-	-	MBC	+	+
E. coli (*C. articulatus*)	+	-	-	MBC	+	+	+
S. aureus (*C. articulatus*)	+	-	-	-MBC	+	+	+
E. coli (mixtures)	+	-	MBC	+	+	+	+
S. aureus (mixtures)	+	-	-	-	MBC	+	+

MBC = Minimum Bactericidal Concentration; +ve C = Positive Control; -ve C = Negative Control
- = No growth; + = Growth (turbidity);

DISCUSSION

It could be deduced from table 3 that both plant extracts (*S. aromaticum* and *Cyperus articulatus*) have antibacterial activities against *E. coli* and *S. aureus* at 100 mg/ml, 50 mg/ml and 25 mg/ml and 12.5 mg/ml (for *S. aureus* only*)* whereas both test organisms have resistance at lowest concentration of 6.25 mg/ml. Antibacterial activity of *S. aromaticum* dried flower buds against the Gram positive *S. aureus* and Gram negative organisms (*E. coli*) and was found to be really effective [10,11]. It has been discovered *S. aromaticum* exhibited pronounced and erratic degree of growth inhibition against *S. aureus* and *E. coli* [12]. On comparison, *S. aureus* was more sensitive to both antibacterial agents (plant extracts) than *E. coli.* Generally, Gram-positive bacteria were more active to *S. aromaticum* extract than the Gram-negative bacteria. This could be due to the fact that the cell wall of Gram-positive bacteria is less complex and lack the natural sieve effect against large molecule due to the small pores in their cell envelope. The Minimum Inhibitory Concentration (MIC) of *S. aromaticum* was 12.5 mg/ml against *S. aureus* while was 25 mg/ml against *E coli,* as shown in table 3 above which indicated *S. aureus* was more sensitive than *E. coli*. Similarly, the minimum bactericidal activity (MBC) of the *S. aromaticum* was found at the concentration of 25 mg/ml against *S. aureus, which* was the lowest concentration that killed *S. aureus*, whereas the extract was found to be only bacteriostatic against *E. coli* even at higher concentration this finding is in agreement with the previous report [13].

It has been observed that both test organisms were sensitive to also to *C. articulatus* where the *S. aureus* exhibits more sensitivity in all concentrations except at 6.25 mg/ml (0mm zone of inhibition) for both. The antibacterial activity of ethanolic extracts agreed with some findings [4,5]. Minimum Inhibitory Concentration (MIC) of *C. articulatus* was found to be effective against *E.coli* and *S. aureus* at the lowest concentration (25 mg/ml). Similarly, the minimum bactericidal activity (MBC) of the *C. articulatus* extract was found at the concentration on the *E.coli* and *S. aureus* where 50 mg/ml which was the lowest concentration that kills the bacteria.

The mixture of the extract has the highest activity on *S. aureus* with the zone diameter ranging from 21 mm to 8 mm at the concentration of 100 mg/ml and 6.25 mg/ml, respectively whereas, 18 mm to 0mm was observed in *E. coli* ranges at the concentration of 100 mg/ml to 6.25 mg/ml, as shown in table 6. Similarly, the minimum bactericidal concentration (MBC) of the *C. articulatus* extract was 100 mg/ml for *E. coli* and that of *S. aureus* was 25 mg/ml presented in table 7. This may be due to increase in concentration of phytochemicals after mixing the two ethanolic extracts and differences in their cell wall. The observed antibacterial activity of the extracts against the test organisms could be attributed to the presence of different

secondary metabolites detected in the plants extract [14].

Conclusion. The ethanolic extracts of *Cyperus articulatus* and *Syzygium aromaticum* has great antibacterial activities against various strain of bacterium ranges from gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*). However, the mixture of the two extracts exerts more inhibitory activities on the bacterial isolates than the separate extracts.

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