Antibacterial Activity of Rhizomes Essential Oils of Two Types of *Cyperus articulatus* Growing in Nigeria


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**Abstract:** The present study seeks to evaluate and compare the medicinal relevance of the red and black types of *Cyperus articulates* L (Cyperaceae) essential oils against some clinically identified strains of bacteria. Pulverized rhizomes (500 g) of red and black types of *C. articulates* were separately hydrodistilled to obtain rhizome essential oils. The two samples afforded 0.62 and 0.60% v/w yield respectively. *In vitro* antibacterial activities of the two oils were separately evaluated against ten bacteria. The essential oil of the red type was active against *Bacillus megaterium* (MIC 0.2 µg/ml) and *Streptococcus pyogenes* (MIC 1.0 µg/ml). *Proteus mirabilis, Klebsiella pneumonia* and *Serratia marcescens* were equally sensitive to the oil, with MICs of 1.0, 2.0 and 2.0 µg/mL respectively. For the oil of black type, *Bacillus megaterium, Streptococcus pyogene, Staphylococcus epidermidis* and *Bacillus cereus* showed inhibition at MICs of 0.1, 0.2, 0.2 and 0.2 µg/mL respectively. *Escherichia coli, Proteus mirabilis, Klebsiella pneumonia* and *Serratia marcescens* were equally sensitive to the oil of the black type with MICs of 0.1, 0.5, 2.0 and 2.0 µg/mL respectively. Susceptible organisms were more sensitive to the oil of black type than the oil of the red type. Interestingly, the Gram negative bacteria were more susceptible to both oils than the Gram positive bacteria. *Staphylococcus aureus and Pseudomonas aeruginosa* have shown sensitivity to the two oils, whereas, this sensitivity was not detected through the disc diffusion method. The results suggest that the essential oils of the red and black types of *C. articulates* can serve as a good cut for the formation of some antimicrobial drugs.

**Key words:** Antimicrobial activity • Black cyperus • *Cyperus articulatus* • Essential oil • Red cyperus

**INTRODUCTION**

Constituents of essential oils have been reported to be responsible for antibacterial activities in some plants [1]. Phenol and its derivatives, terpenols, terpenals, terpenone and terpenic oxide have significant level of bio-activities. Thymol, a phenolic constituent of thyme oil, has been reported to have significant level of antibacterial activities against *Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, Staphylococcus pneumonia* and Haemophilus influenza [2]. The same workers reported that cinnamde, an aldehyde constituent of cinnamon bark oil had inhibitory actions comparable to that of thymol. Similar activities were equally demonstrated by terpenols found in the essential oil cinnamon bark extracts. Significantly, the activities of the constituents were close to the parent oils [2].

Antibacterial properties of carophyllene oxide, camphor and 1,8-cineole identified in essential oils of *Achillea fraasii* Sch. Bip. (Asteraceae) and *Achillea taygetea* Boiss & Heldr. (Asteraceae) have been reported by Magiatis et al. [1]. They stated that, carophyllene oxide is the most efficient constituent on the tested organisms followed by camphor and 1,8-cineole. Ulubelen et al. [3] previously reported some antibacterial properties of carophyllene oxide. The same properties have been reported for camphor and 1,8-cineole by Alijannis et al. [4] and Prudent et al. [5] respectively. Despite the fact that some monoterpenes and sesquiterpenes were found to lower the antimicrobial activity of essential oils by Chalchat et al. [6], some still posses antibacterial activity. Camphene for instance, is found to be effective against *Staphylococcus aureus* and *Escherichia coli*. α-pinene has significantly reduced growth of *Erwinia amylovira,*
while β-pinene is found to be inhibitory to the bacteria even at higher bacteria population [7].

Essential oils from Cyperus species are generally constituted by sesquiterpenoids and traces of monoterpenoids. The main hydrocarbon is always caryophyllene [8]. But many other sesquiterpenes of the carophyllene, eudesmane, patchoulane and rotundane types are also present as hydrocarbon and oxygenated compounds. Kubosone and isokubusone have been isolated from *Cyperus rotundus* [9]. Sonwa and König [10] isolated (−)-norrotundene, (−)-isorotundene, cypera-2, 4 (15)-diene and (+)-cyperadone from *Cyperus rotundus*. α-Humulene, humulene epoxide II, β-carophyllene and carophyllene oxide have been isolated from *Cyperus tuberosus* [11]. Corymbolone was isolated from *Cyperus corymbosus* (syn *Cyperus articulatus*) [12].

*Cyperus articulatus* is used in traditional medicine for the treatment of several ailments like, epilepsy, malaria, migraine and dysentery in different parts of the world [13-14]. These properties have been established by various workers. Nonvolatile extracts of the rhizome of *Cyperus articulatus* are known to possess anticonvulsant, antimalaria and antimicrobial properties [15-16]. The properties were linked with the phytochemicals in the extracts from the rhizomes.

Olawore *et al.* [17] have characterized rhizomes essential oils of red and black types of *Cyperus articulatus* growing in Nigeria. Like other Cyperus species, both oils are richer in sesquiterpenoids than monoterpenoids. However, the two oils showed substantial qualitative and quantitative differences. Oil of the red type was characterized by the abundance of cyperotundone, pipertone, α-maoline, germacrone, 1-epicubenol, α-pinene and caryophyllene epoxide. For the oil of the black type, the main constituents were; cedrol, guaia-5-en-11-ol, cyperotundone, sabinene, α-pinene, trans-pinocarveol, cis-carveol, trans-carveol and α-cardinol.

Significantly, the oil of the black type was richer in terpenols than the oil of the red type. Meanwhile, oil of the red type had abundance of sesquiterpenes than the oil of the black type. The variations are attributable to phenotypic difference in the rhizomes. It is on the basis of these variations that we investigated the bio-activities of the essential oil on ten selected bacteria and subsequently established both qualitative and quantitative variations in the activities of them. The selected bacterial were; *Bacillus megaterium*, *Bacillus cereus*, *Streptococcus pyogenes*, *Staphylococcus epidermis*, *S. aureus*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumonia*, *Serratia marcescens* and *Pseudomonas aeruginosa*.

**MATERIALS AND METHODS**

**Plant Materials:** The dried rhizomes of the red and black types of *C. articulatus* were obtained in Ilorin, Kwara State, Nigeria. Identification was carried out by Mr. F. Usang at the Herbarium of the Forestry Research Institute of Nigeria (FRIN), Ibadan, where voucher specimens were deposited.

**Oil Isolation:** Pulverized rhizomes of the red and black types of *C. articulatus* (500 g) were separately hydrodistilled for 3 h in a Clevenger-type apparatus according to the British Pharmacopoeia specification [18].

**Antibacterial Activity**

**Source of Bacteria Strains:** The bacteria were obtained from the collection of microorganisms at the Department of Microbiology, Faculty of Science, University of Ilorin, Nigeria. Ten bacteria species comprising of five Gram positive and five Gram negative bacteria were used. The Gram positive bacteria included: *B. megaterium*, *B. cereus*, *S. pyogenes*, *S. epidermis* and *S. aureus*. The Gram negative bacteria were; *Escherichia coli*, *P. mirabilis*, *K. pneumonia*, *S. marcescens* and *P. aeruginosa*. The microorganisms’ cultures were maintained on nutrient agar (NA) medium for 18 h at 37±1°C.

**Screening for Antibacterial Activity:** Diffusion method using Whatmann filter paper disc was used for the antibacterial assay of the oils. Organism suspension was prepared with sterile physiological saline [19] uniformly mixed with sterile liquid nutrient agar in Petri dishes. Inoculum’s density was compared with Tube 0.5 of MacFarland’s standard solution (equivalent to 1.5 x 10⁶ units of colonies formed per milliliter). Essential oils (2 ml) of red and black types of *C. articulatus* were separately dissolved in 2 ml of Tween 80 to give a stock solution of 1mL oil/mL of Tween 80. From this solution, a serial dilution was prepared. Sterile filter paper discs (8 mm) were impregnated in varying concentrations (1, 0.5 and 0.25 mL of oil/mL of tween 80) of the oils extract prepared separately and placed on the surface of NA medium. The positive controls were prepared as described for the test samples at the same time. Each test was made in three replications. The plates were incubated at 37°C for 24 h. They were subsequently observed for zones of inhibition. The inhibition zones were measured with metric ruler and compared with the inhibition zone produced by 100 μg/ml of gentamicin.
Table 1: Antimicrobial activity of rhizome essential oils of red and black types of *C. articulatus*

<table>
<thead>
<tr>
<th>Gram-positive bacteria</th>
<th>Diameter of the inhibition zone (mm) at different concentration (mL of oil/mL of Tween 80)</th>
<th>MIC (µg/mL) (red type)</th>
<th>MIC (µg/mL) (black type)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus megaterium</em></td>
<td>6 10 4 9 1 8 15 0.2</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>- 9 - 5 - 4 13 12 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em></td>
<td>8 7 - 5 - 4 12 10 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>- 7 - 5 - 4 10 14 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>- - - - - - 14 14 12 14</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Gram-negative bacteria</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>- 15 - 14 - 12 30 8 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>5 5 3 3 1 2 25 1.0</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td><em>Klebsiella pneumonia</em></td>
<td>9 9 7 6 - - 15 2.0</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td><em>Serratia marcescens</em></td>
<td>4 6 2 4 - 3 12 2.0</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>- - - - - - 10 10 12</td>
<td></td>
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</tr>
</tbody>
</table>

*a* inhibition zone diameter in millimeters around disc (diameter of the disc, 8 mm, excluded)

*b* µL of oil/mL of medium

' red type

' black type

+ positive control

- no detectable activity

**RESULTS AND DISCUSSION**

The oil was obtained by hydrodistillation of the rhizomes of *C. articulatus* with an average yield of 0.62% (w/w) for red type and 0.60% (w/w) for the black type. The phenotypical, climatic or soil conditions, period of the harvest, age of the plant, drying of biomass prior to extraction, storage of oil, presence of weed and wilt diseases in the rhizomes may partly be responsible for this difference in the yield. The antimicrobial assay of the extracted oils using the paper disc diffusion method showed that the two types of oils exhibited a moderate antimicrobial effect on some tested organisms. *B. megaterium* and *S. pyogenes* were inhibited by the oil of the red type both in the agar dilution and in the paper disc methods with MIC of 0.2 and 1.0 µg/mL respectively. The oil of the red type showed reticent inhibitory activity against *B. cereus*, *S. epidermidis* and *S. aureus* in the agar dilution method but this activity was not observed in the paper disc method. Three of the Gram-negative bacteria; *P. mirabilis*, *K. pneumonia* and *S. marcescens* were sensitive to the oil of the red type. The oil had moderate activity against *E. coli* and *P. aeruginosa* in the agar dilution method though this does not show in the paper disc method.

Oil of the black type showed inhibitory actions on four of the Gram-positive bacteria; *B. megaterium*, *S. pyogenes*, *S. epidermidis* and *B. cereus*. The oil showed significant levels of activities against *B. megaterium*, *B. cereus* and *S. epidermidis*. Slight activity was observed on *S. aureus* during agar dilution method but failed to show in paper disc method. The following Gram-negative bacteria; (*E. coli*, *P. mirabilis*, *K. pneumonia* and *S. marcescens*) were sensitive to the oil of black type. Slight level of antibacterial activities was shown on *P. aeruginosa* during agar dilution method.

The comparison of the inhibitory actions of the two oils revealed that the organisms are more susceptible to the oil of black type than the oil of the red type. For the Gram-positive bacteria, oil of the black type showed higher inhibitory actions on *B. megaterium* than the oil of...
red type. Similar activity was shown by the oil of the black type on *S. pyogene* at 1 mL of oil/mL of tween 80 concentration. Four of the Gram-negative bacteria tested were sensitive to the oil of the black type as against three of the organisms that were sensitive to the oil of the red type.

The determination of MIC using agar dilution method showed MIC for *S. aureus* (14, 12 µg/mL) and *P. aeruginosa* (10, 12 µg/mL) whereas these sensitivity were not detected during the paper disc diffusion method. Suffice to say that the concentration on the paper disc was not sufficient to inhibit the growth of these bacteria. Majority of the MIC results on the black type falls within 0.2 µg/mL whereas the red type is closer to 2 µg/mL. Essential oils constituent varies quantitatively and qualitatively between different or the same plant species. Olawore *et al.* [17] have reported variation in the constituents of rhizome essential oils of red and black type of *C. articulates*. This variation could be a function of their antimicrobial properties. The antimicrobial activity differences may, therefore, be due to the presence of strong antimicrobial active components with high percentage in the black against the red type.

**CONCLUSIONS**

From the analyses above, Gram-negative bacteria were observed to be more susceptible to the rhizome essential oils of red and black types of *C. articulates* than Gram-positive bacteria. These findings disagreed with earlier report in the literature indicating that Gram-positive bacteria are more susceptible to essential oils Cyperus species than Gram-negative bacteria [20-21], but the results agreed with that of Magiatis *et al.* [1], in which *A. taggeta* and *A. frasii* essential oils showed higher antibacterial activities on Gram-negative bacteria than Gram-positive bacteria. Abundance of terpenols in the oil of black type than the red type has been reported earlier [17]. Interestingly, oil rich in terpenols are known to be broader spectrum oils used in numerous cases of bacteria infections. To this end, higher inhibitory activities of oil of the black type can be linked with higher content of terpenol in the oil. Hence, the oil of the black would have wide spectrum of applications in the treatment of pathogenic diseases caused by the sensitive bacteria than the oil of the red type. However, the results suggest that the essential oils of the red and black types of *C. articulates* can serve as a good cut for the formation of some antimicrobial drugs.

**REFERENCES**


