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Short Communication

Chemical composition and antimicrobial properties of the rhizome essential oil of *Cyperus articulatus* L. grown in Karnataka, India

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Cyperus articulatus L. is widely distributed in various geographical regions of the world, and it has been used as a folk medicine for treating haemorrhoids, diarrhoea, and other diseases. The present study aimed to analyze the chemical constituents and antimicrobial activities of essential oil (EO) extracted from *C. articulatus* grown in the Karnataka region to explore its potential pharmaceutical usage. The EO from the rhizomes of *C. articulatus* was extracted by hydro-distillation and was tested for its antimicrobial activities against selected bacteria (*Staphylococcus aureus*, *Salmonella enterica serovar Abony*, and *Escherichia coli*) and fungi (*Candida albicans*, *Aspergillus flavus*, and *Aspergillus niger*). The EO yield was 1.24 g/100 g of dried rhizome powder. The EO recorded a significant inhibition against *S. aureus* and *A. flavus*. The GC-MS analysis of EO showed the predominance of important metabolites such as mustakone (20.2%), longifolenaldehyde (14.9%), cedroxyde (8.7%), α-copaene (4.7%), cyperene (2%), cyperotundone (2.6%), khusinol (2.3%), and corymbolone (1.1%) along with several other monoterpenoids and sesquiterpenoids. The study revealed the EO of *C. articulatus* as a promising source of antibacterial and antifungal metabolites which may lead to its application in managing bacterial and fungal infections and storage mould.

Keywords: Antibacterial, Antifungal, *Cyperus articulates* L., Khusinol, Mustakone, Storage mould.

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Introduction

The essential oils (EOs) from native plants especially members of the Cyperaceae family of various geographical regions are reported as an integral part of their local medicine. The EOs are effective against several diseases and disorders, and have been widely used for such applications since ancient times[1]. Among different *Cyperus* species, the EO from *Cyperus rotundus* L. has been extensively studied for its antioxidant, biomolecular protection, antibacterial/antimicrobial, etc. properties and has been in demand in pharmaceutical industries[1–3].

Cyperus articulatus is perennial grass-like species widely distributed in the tropical and sub-tropical zone of the world habitat and widely distributed in India and the Asian subcontinent. They are usually found in aquatic or marshy habitats[4–6]. The rhizome of the matured plant appears like a bulb (dark outside and dark brown to red inside followed by white peripheral region). It is commonly known as 'woire' by the Fulanis, 'kajiji' by the Hausas, 'ifin' by the Yorubas in African regions, and 'priprioca' in the Amazon region and Brazil[5]. As a traditional medicine, the plant rhizome is used to treat several ailments like epilepsy, malaria, and dysentery in various areas of the world[4].

The rhizome contains EO of intensely yellow colour, with a pleasant fragrance that has a pharmacological and economic interest in the perfume and cosmetics industries. In folk medicine, the EO has been used for treating haemorrhoids, diarrhoea and other diseases[7]. Thus, in recent years, there has been an increased interest in studies of bioactive constituents of oils extracted from *C. articulatus*. Conversely, pathogenic microbes are an emerging threat to human and animal health (multidrug-resistant bacteria), and food security (storage moulds). The use of synthetic chemicals (antibiotics or fungicides) is a common practice, which often have non-target effects and adversely affects the environment. Due to the extensive use of these chemicals, both target and non-target microbes are developing resistance with time. In this regard, several antimicrobial substances from natural origin are evaluated and reported as potential inhibitors of pathogenic microbes and storage moulds[8]. The objective of the present study was to assess the efficacy of EO of *C. articulatus* for their antimicrobial activities and correlate with their chemical composition.

Materials and Methods

The rhizomes of the matured *C. articulatus* plant were collected in May 2016 from the riverbank of the

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Supplementary table is available online only.

Cauvery in the Mysore and Mandya region of Karnataka, India. The identity of the plant was confirmed by the Botanical Survey of India, Kolkata (India) (Specimen No. IIT/HP-02). The rhizomes were cut into small pieces of 0.5 to 1 cm size and dried in an oven at 45 °C for two days. The dried rhizome was ground to a coarse powder using a mechanical grinder and used for EO extraction. Rhizome powder (200 g) was hydro-distilled for 3 h in a Clevenger-type apparatus, and the EO collected at the top of the water column was carefully collected. The collected oil was preserved in an airtight glass vial at 4 °C.

Three bacterial isolates *viz*., *Staphylococcus aureus* (ATCC 6538), *Salmonella enterica serovar Abony* (MTCC 3858) and *Escherichia coli* (MTCC 1687) and three fungal isolates *viz*., *Candida albicans* (ATCC 10231), *Aspergillus flavus* (AFG1) (isolated from groundnut seeds), and *Aspergillus niger* (AN1) (isolated from groundnut seeds), obtained and maintained at microbial culture collection of Environmental Biotechnology Lab, CRDT, IIT Delhi. The antimicrobial assays were performed following the disc diffusion method, where the EO was used in two dilutions (20 and 10 mg/mL) dissolved in hexane.

Antimicrobial assay

All the bacteria were grown on nutrient agar for 36 h and used for the antimicrobial assay. Fresh bacterial culture was streaked on nutrient agar as a uniform lawn. A 6 mm sterile empty disc (Hi-media) was impregnated with EO (50 µL) and kept in laminar airflow for 30 min to evaporate hexane. Then the discs were placed equidistantly on the nutrient agar. The control discs were prepared by impregnating hexane. The plates were incubated for 36 h at 35±1 °C. Towards the end of the incubation period, the plates were observed for the zone of inhibition and represented in mm[9].

Similarly, a potato dextrose agar (PDA) medium was used for antifungal studies. The conidial suspension ($1x10^8$ conidia/mL) was prepared from fresh cultures, and 100 µL was spread plated on PDA. As explained earlier, control and EO impregnated discs were prepared and placed on PDA. The incubation was carried out at 28±1 °C for 4-5 days. The zone of inhibition was represented in mm.

Statistical analysis

The data obtained were subjected to One way analysis of variance (ANOVA) using SPSS Inc. 17.0.

The mean values were compared for significant differences (at $P \leq 0.05$) using Tukey's honest significant differences (HSD) test.

GC-MS and GC-FID analysis

The analysis was performed using Shimadzu QP-2010 Plus with Thermal Desorption System TD 20 EI/CI MSD mass spectrometer (Duisburg, Germany) with a triple-axes detector following reported methods with little modification[10]. An HP-SMS column with a length of 30 m and i.d of 0.25 nm was used with a film thickness of 0.25 microns. The injection volume was 40 µL, and the column flow rate was 1.21 mL/min. The purge flow was kept at 3 mL/min, and the split ratio was 100:1. Injection temperature was maintained at 260 °C, and the detector was set at 280 °C. The oven temperature increasing rate was programmed at 3 °C/min from 50 to 280 °C and was finally held for 15 min at this temperature. The compounds were detected through the connected library (NIST20 EI mass spectral library and FFNSC2 GC-MS library) of GC-MS system. The qualitative data were measured by electronic integration of FID area per cent without correction factors. The MS operating system was maintained by keeping the ion source temperature at 230 °C and ionization voltage at 70 eV. The mass scan range was from m/z 40 to 550. The percentage composition was determined through GC-FID analysis using the same column and temperature program as mentioned above.

Results

The rhizome extracted EO was dark yellow in colour, and the yield was 1.24 g/100 g of dried rhizome powder. The EO (20 mg/mL) showed potent antibacterial activity against *S. aureus* (3 mm zone of inhibition). However, no inhibition was observed against *E. coli* and *S. enterica sv Abony* (Fig. 1). The zone of inhibition recorded by EO against tested bacteria and fungus are listed in Table 1. The antifungal activity showed that *A. flavus* is highly sensitive (12 mm zone of inhibition) to EO (20 mg/mL) followed by *A. niger* and *C. albicans*.

The GC-MS chromatogram of the EO from *C. articulatus* detected a wide distribution of monoterpenoids, sesquiterpenoids, and several aldehydes and ketone derivatives. Terpenoids and other compounds of different classes were detected in the GC-MS (Fig. 2 & 3). The major compounds having high peak areas (more than 1%) are listed in Table 2. Mustakone (20.2%) and longifolenaldehyde

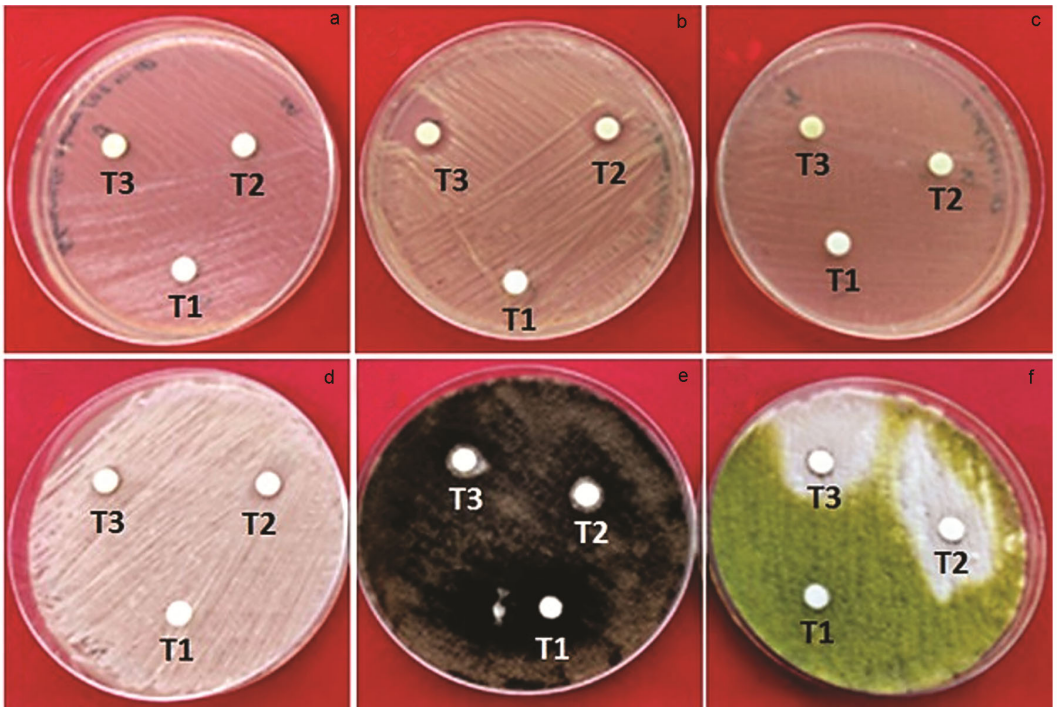

Fig. 1 — The antimicrobial activity showed by essential oil extract of *Cyperus articulatus* rhizome against studied species *Salmonella enterica serovar Abony*, *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, *Aspergillus niger* and *Aspergillus flavus*.

(14.9%) were present in relatively higher concentrations compared to others. The compounds cedroxyde (8.7%), cyperene (2%), copaene (4.7%), cyperotundone (2.6%), viridiflorol (2.8%) and khusinol (2.3%) were detected in optimum concentrations. Some lead representative compounds identified through GC-MS analysis are shown in Fig. 3. The commonly occurred EO metabolites such as corymbolone, cis-thujopsadiene, myrtenol, pinocarvone, trans-verbenol, khusinol, α-calacorene, β-calacorene, longipinanol and khusimone were present in lesser concentrations along with several other terpenoids (see Supplementary Table 1 for the detailed list of compounds).

Table 1 — Growth inhibition of bacteria and fungus in presence and absence of essential oil of *Cyperus articulatus* rhizome

Test microbes	Concentration of EO	
	20 mg/mL	10 mg/mL
	Inhibition (mm)	
Bacteria		
Salmonella enterica serovar Abony	0.0±0.0[a]	0.0±0.0[a]
Staphylococcus aureus	3.0±0.1[b]	2.0±0.1[b]
Escherichia coli	0.0±0.0[a]	0.0±0.0[a]
Fungus		
Candida albicans	1.0±0.0[a]	0.5±0.0[a]
Aspergillus niger	1.0±0.0[a]	0.5±0.0[a]
Aspergillus flavus	12.0±0.3[b]	10.0±0.2[b]

The experimental results were expressed as mean±SD (n = 3). Mean values in the same column with different superscripted letters were significantly different at $P \leq 0.05$

Discussion

In the present study, EO's chemical composition and antimicrobial activity from *C. articulatus* were analyzed. Constituents such as cyperene, rotundene, α-cyperone, khusinol, mustakone etc. are very common in all plants of Cyperaceae family[11–13]. EO from *C. articulatus* grown in Nigeria was studied to possess monoterpenes such as pinocarvone, trans-pinocarveol, trans-verbenol, α-pinene, β-pinene, myrtenal and myrtenol. The presence of sesquiterpene compounds such as α-corymbolol, mandassidione, mustakone was also reported in that study. da Silva *et al.* reported terpenes, mustakone, α-cyperone, corymbolone and caryophyllene oxide as the major constituents EO of *C. articulatus* rhizome[14]. The present study showed differences in the composition of oil compared to those previously reported in the literature on *C. articulatus*. Different soil, climatic, and environmental conditions can also affect EO's quantity and relative compositions.

The EO of *C. articulatus* rhizome was reported as significant in inhibiting the growth of *S. aureus* and *P. aeruginosa*[15,16]. Similarly, Oladosu *et al.* evaluated

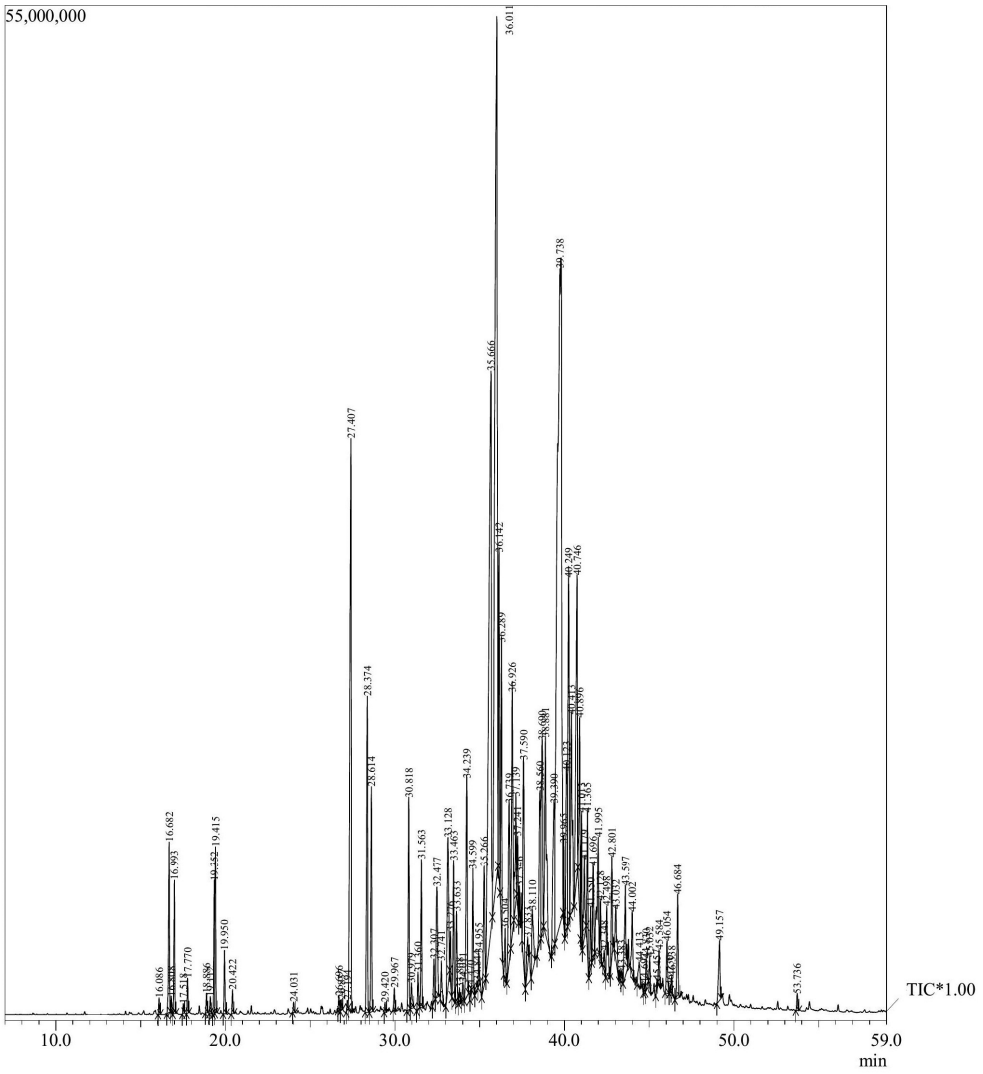

Fig. 2 — GC-MS chromatogram of essential oil of *Cyperus articulates*.

the antibacterial properties of *C. articulatus* rhizome EO (red and black type) against some clinically important bacterial strains, including *Bacillus megaterium*, *Bacillus cereus*, *Streptococcus pyogenes*, *Staphylococcus epidermidis*, *Proteus mirabilis*, *Klebsiella pneumonia*, and *Serratia marcescens*. Their study revealed the differential sensitivity of these bacteria against red and black type rhizome EO, and Gram-negative bacteria were more susceptible to both the oils compared to the Gram-positive bacteria[17]. In this study, similar results were observed as only *S. aureus* was found sensitive to EO. Based on previous studies, the antibacterial activities of the oil can be correlated with the chemical composition of the EO (Table 2). Further, it revealed the possibilities of using EO from *C. articulatus* rhizome as a natural antimicrobial agent to manage multiple drug resistance (MDR) bacterial infection in humans and animals.

However, the potential of EO from *C. articulatus* for their antifungal activity was not explored earlier. *Aspergillus flavus* and *Aspergillus niger* are ubiquitous, generally causing a significant loss in grain storage. In addition, they are opportunistic fungi found to infect immune compromised patients in hospitals. Also, aflatoxin produced by *A. flavus* is classified as carcinogenic[18]. The EO of *C. articulatus* in this study significantly inhibited the growth of aflatoxigenic *A. flavus* at lower concentration. The efficacy of the EO against aflatoxigenic strain *A. flavus* indicated its possible applications in managing storage moulds and aflatoxin associated

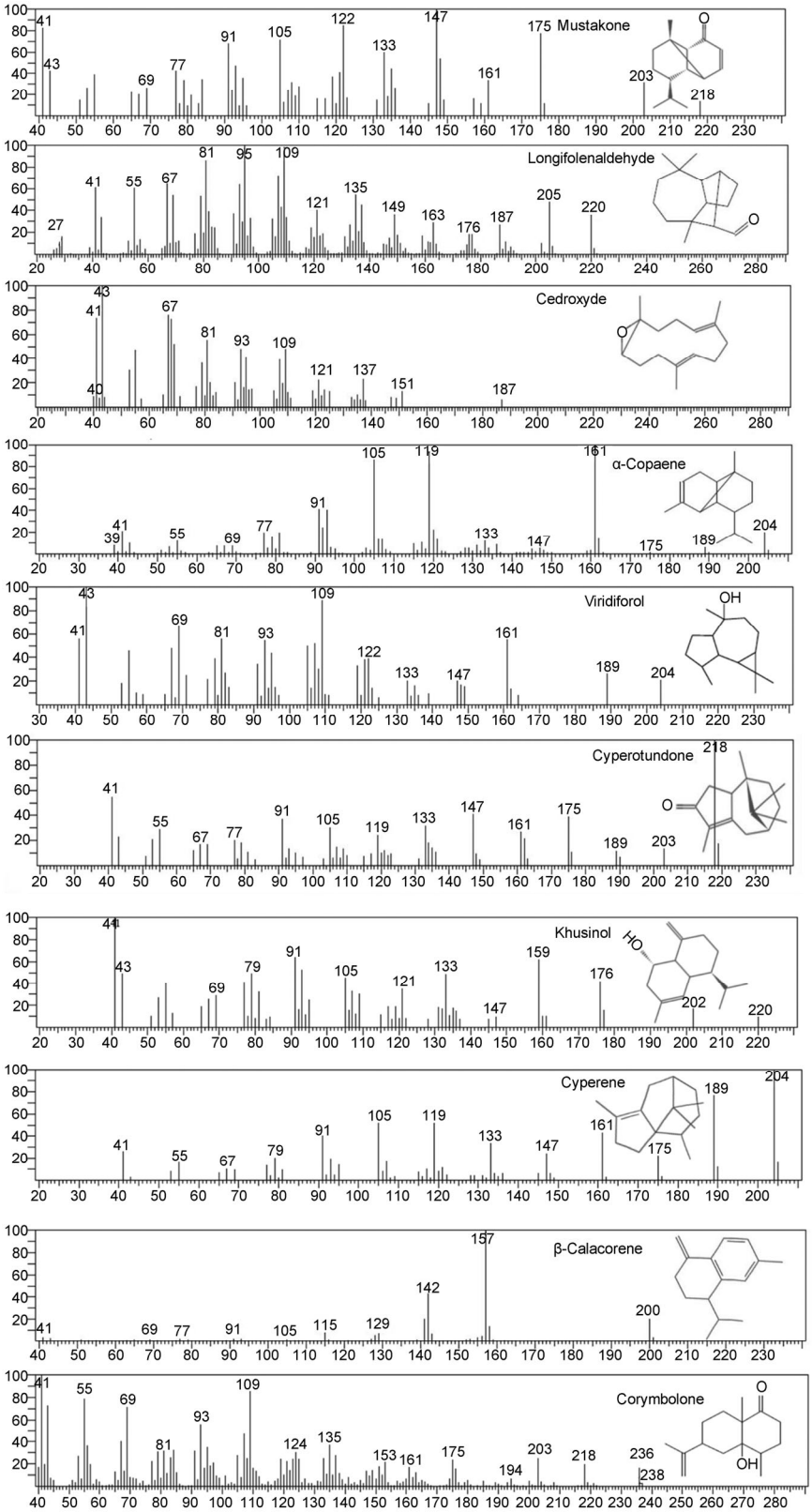

Fig. 3 — GC-MS fragmentation pattern of some representative compounds detected in the essential oil of *Cyperus articulatus* rhizome.

Table 2 — Major chemical constituents* and their composition (%) in the essential oil of *C. articulatus* rhizome

R-time	Compound name	Composition (%)	R index
27.40	α-Copaene	4.7	1221
28.37	Cyperene	2.0	1407
28.61	cis-Thujopsadiene	1.3	1467
30.81	Rotundene	1.3	1456
33.12	Khusinol	2.3	1677
34.23	β-Calacorene	1.7	1564
35.66	Cedroxyde	8.7	1716
36.01	Longifolenaldehyde	14.9	1581
36.14	β-copaen-4-α-ol	2.2	1591
36.28	Nootkatol	1.6	1715
36.92	Humulene epoxide II	2.0	1613
37.59	Viridiflorol	2.8	1594
38.69	Pogostol	1.7	1650
39.39	α-Calacorene	1.6	1544
39.73	Mustakone	20.2	1681
40.24	Cyperotundone	2.6	1693
40.41	Amorpha-4,9-dien-2-ol	1.8	1702
40.89	3,5,6,7,8,8α-Hexahydro-4,8α-dimethyl-6-(1-methylethenyl)-2(1H)-naphthalenone	1.2	1673
41.99	8-oxo-9H-Cycloisolongifolene	1.1	1368
42.49	Longipinanol	1.1	1572
46.68	Corymbolone	1.1	1785

*Only major compounds having more than 1% peak area are listed. Complete list is provided in Supplementary Table 1.

complications. The EO may be studied further as an alternative source to synthetic preservatives and chemical pesticides[8,19].

At present, humans and animals' microbial infections and storage moulds are managed using antibiotics and chemical fungicides, respectively. Though EOs from various sources recorded antimicrobial activity under laboratory conditions[19-22], their large-scale applications are hindered by their cost and availability. *C. articulatus* in this context can be a potential source of the EO having desired properties as its availability is abundant, and it is a high biomass producer. *C. articulatus* can also be a suitable alternative source of EO of their related species like *C. rotundus*.

Conclusion

The present study showed differences in the composition of oil compared to those previously reported in the literature on *C. articulatus*. The antimicrobial activity of the *C. articulatus* EO showed that this species could be a suitable alternative source of EO to other species like *C. rotundus*. The inhibiting potential of EO against *S. aureus* and *A. flavus* revealed its application in managing bacterial and fungal infections and storage mould. A broad spectrum of important metabolites in the *C. articulatus* EO indicated its pharmacological importance. It envisaged further investigation of the synergy mechanisms of different components with the biomolecules in microorganisms.

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Conflict of interest

The authors declare that there is no conflict of interest.

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