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Original Research Article

Chemical Constituents and Antifungal Potential of Combined Essential Oils of the Leaves of *Coleus barbatus* and *Corymbia citriodora* Growing in Tanzania against Skin Fungal Pathogens

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ABSTRACT

New antifungal agents are required to address the challenges of treating skin fungal infections. *Coleus barbatus* and *Corymbia citriodora* are used traditionally to treat skin fungal infections. The antifungal efficacy of essential oils (EOs) of these plants against fungal pathogens has been reported, however, there is no report on the antifungal efficacy of their combination. This study aimed to determine the chemical compositions and antifungal activity of the combined EOs of the leaves of *C. barbatus* and *C. citriodora* from Tanzania. The EOs were extracted by steam distillation and compounds were analyzed by Gas Chromatography-Mass Spectrometry (GC-MS). Antifungal activity against *Aspergillus niger, Candida albicans, Candida tropicalis, Candida glabrata, Candida krusei, Trichophyton interdigitale, Trichophyton rubrum,* and *Microsporum gypseum* was assessed using agar-well diffusion method. Means of inhibition zone diameters of different treatments of EOs were compared by One-way ANOVA and Tukey's post-hoc test. Major components of *C. barbatus* EO were camphor (17.83%), 1,3,8-*p*-menthatriene (13.57%), thymol (10.43%), and camphene (10.18%). The major phytoconstituents of *C. citriodora* EO were (1R,2R,5S)-2-isopropenyl-5-methylcyclohexanol (*p*-menth-8-en-3-ol) (75.43%) and (1S,2R,5R)-2-(2-Hydroxypropan-2-yl)-5-methylcyclohexanol (5.92%). The *C. barbatus* EO was more active against *C. tropicalis, C. albicans* clinical isolates, and *M. gypseum* while *C. citriodora* EO was more active against *C. albicans* 23Q (NR-29341) and *T. rubrum*. The EOs of both plants exhibited similar activity against *A. niger* and *T. interdigitale*. Combinations of the EOs improved activity against dermatophytes and *Candida* species; therefore, further studies are recommended to investigate the antifungal efficacy of combined *C. barbatus* and *C. citriodora* EOs.

Keywords: Essential oils, Antifungal, *Coleus barbatus, Corymbia citriodora*, Tanzania.

Introduction

Superficial fungal skin, nail, and hair infections are widespread fungal diseases affecting about one billion people worldwide.[1] *Epidermophyton, Microsporum,* and *Trichophyton* dermatophytes are common skin fungal pathogens growing in the keratin of the hair, skin, and nails of the hosts.[2] They infect different body parts, including the groin, feet, scalp, toe, fingernails, trunk, arms, and legs.[3,4] Apart from the dermatophytes, skin fungal infections also result from the infection by non-dermatophyte fungi like *Candida species* (mainly *Candida albicans*), *Malassezia species, Aspergillus species, Fusarium species, Trichoderma species, Penicillium species,* and *Trichosporon species*.[5-7] Globally, the distribution of causative agents, prevalence, and the burden of superficial fungal infections is not uniform. The distribution is limited by geographical location, age, socioeconomic status, and environment.[8] Infection of the skin of the head (scalp) is the most common among African schoolchildren.[9] In Tanzania, a prevalence of skin fungal infections between 15 and 50% was reported for students aged 6-19 years.[10-12] Various antifungal agents of the imidazoles, triazoles, allylamines, and hydroxypyridones groups and others are available for topical and oral applications against skin fungal infections.[13] However, the narrow spectrum of activity, long-term treatment plans, and resistance to the existing antifungal drugs,[14-16] calls for the search for new antifungal compounds.

Historically, fungal skin infections have been traditionally treated with extracts from medicinal plants.[17] Some plant crude extracts, fractions, and isolated compounds have been documented to possess antifungal activity against skin fungal pathogens.[18] The essential oils (EOs) of *Coleus barbatus* and *Corymbia citriodora* were previously reported to exhibit growth inhibitory properties against dermatophytes and other fungi.[19, 20] In Tanzania, *C. barbatus* leaf extract is used by Traditional Health Practitioners to treat oral thrush, skin rashes, and ringworms.[21] However, no study has reported the chemical compositions of the EOs of *C. barbatus* and *C. citriodora* growing in Tanzania and the antifungal efficacy of the combined essential oils. Thus, this study reports the chemical compositions and the antifungal activity of individual and combined EOs of *C. barbatus and C. citriodora* collected in Tanzania.

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Materials and Methods

Plant materials

The leaves of *Coleus barbatus* (Andrews) Benth. ex G. Don, synonym: *Plectranthus barbatus* Andrews (family Lamiaceae) were collected in April 2023 from Mbala village in Chalinze District Council, Pwani region, Tanzania (Latitude: 0450295; Longitude: 9268294; Altitude: 87m). The leaves of *Corymbia citriodora* (Hook.) K.D. Hill & L.A.S. Johnson, synonyms *Eucalyptus citriodora* Hook.; *Eucalyptus maculate* var. citriodora (Hook.) F.M. Bailey; *Eucalyptus melissiodora* Lindl. were collected in May 2023 from North Ruvu Forest Reserve in Kongowe, Kibaha (Latitude: 0486932; Longitude 9257473; Altitude 115m), Tanzania. The voucher specimens of *C. barbatus* (SH 1506) and *C. citriodora* (SH 1509) were deposited in the Institute of Traditional Medicine Herbarium.

Consumables

Sabouraud dextrose agar (Liofilchem, Abruzzi, Italy), clotrimazole 10 µg/disc (Biomaxima S.A, Lublin, Poland), glass Pasteur pipette, normal saline (0.9% NaCl), Tween 20, and pipette tips were purchased from local vendors in Tanzania.

Fungal strains

Laboratory strains and clinical isolates of fungi were used. Clinical isolates of *Candida albicans* 23Q (NR-29341) from China were donated by BEI Resources (USA). Also, *Candida albicans*, *Candida glabrata*, *Candida krusei*, and *Candida tropicalis* clinical isolates were obtained from the Department of Microbiology and Immunology in the School of Diagnostic Medicine of Muhimbili University of Health and Allied Sciences (MUHAS), Tanzania. *Microsporum gypseum* (Ref: 0893P derived from ATCC® 24104™), *Trichophyton interdigitale* (Ref: 0442P derived from ATCC® 9533™), and *Trichophyton rubrum* (Ref: 0444P derived from ATCC® 28188™) laboratory strain dermatophytes were purchased from Microbiologics® (Minnesota, USA). *Aspergillus niger* clinical isolate was obtained from the School of Pharmacy, MUHAS.

Extraction of essential oil

The EOs were extracted from the fresh leaves by steam distillation method,[22] using an essential oil distiller (EC 30, Shanghai Better Industry Co. Ltd, Shanghai, China). Fresh leaves (2.0 kg) of *C. barbatus* or *C. citriodora* in a mini stainless-steel basket holder were placed in a distillation unit with a water boiler. The steam from the boiling water heated at 95-100 °C was allowed to pass through the leaves to extract EOs. The steam and EOs vapours were then circulated to a condenser (set at 3°C) for liquefaction, before being directed to the Clevenger tube where the separation occurred. The EOs which floated on the water were collected from the tap. This process was repeated until 23.8 kg of *C. barbatus* and 13.6 kg of *C. citriodora* leaves were exhaustively extracted. Residual water in the EOs was removed using anhydrous sodium sulphate. The pure EOs were stored at 4°C. The yield of EOs was calculated using equation 1 below.[23]

$$Y = \frac{ME}{ML} x100\% \ = \text{Equation 1}$$

Where Y is the percentage yield of the EOs, ME is the volume of the EOs obtained in milliliters, and ML is the mass (in grams) of the fresh leaves extracted.

Analysis of the chemical compositions of the EOs

Chemical compositions of *C. barbatus* and *C. citriodora* EOs were analyzed by GC-MS analysis at Shivaji University, Kolhapur, India. One part of the EOs was diluted in twenty parts of diethyl ether (1:20) and 1.0 µL of the diluted EOs was loaded into the GC-MS (model TQ8050 plus with HS20, Shimadzu, Japan) machine. The temperature of the injector was calibrated at 240°C and that of the column started at 70°C for 2 minutes and then increased gradually to 280°C at a rate of 6°C per minute. The column was maintained at 280°C for 2 minutes. The detector and the ion source temperatures were kept at 300°C and 200°C, respectively. Helium carrier gas was maintained at a flow rate of 1 mL/min**.** The composition and dimensions of a capillary column, the setup of the mass-spectrometry conditions, data acquisition, and interpretation of the spectra were described previously.[24]

Assessment of antifungal activity

The antifungal efficacy of individual and combined EOs from *C. barbatus* and *C. citriodora* was assessed using the agar-well diffusion method.[25] Both, dermatophytes and non-dermatophytes fungi were cultured in the Sabouraud dextrose agar (SDA) medium prepared by dissolving 65 g powder in 1 L of distilled water and then autoclaved at 121°C for 15 minutes. *Candida species* were cultured at 37°C for 24 h, followed by preparation of fungal suspensions (0.5 Mac Farhland turbidity). Dermatophytes were cultured at 27°C for 3-7 days. Sterile cotton swabs moistened with 0.1% Tween 20 were used to touch the colonies of the dermatophytes gently and transfer spores to a tube containing normal saline (0.9% NaCl). The fungal suspensions in normal saline were vortexed at 2000 rpm for 5 minutes.[26]

Different ratios were prepared by combining different volumes of *C. barbatus* and *C. citriodora* EOs and vortexing for 5 minutes. Wells (6 mm) were made in SDA plates using sterile glass Pasteur pipettes. Fungal suspensions were added on the agar plates using sterile cotton swabs and then 5 µL of individual EOs or their combination were added in duplicate wells. Clotrimazole antifungal standard discs (10 µg/disc) were included as the positive control. The plates with EOs were left in the biosafety cabinet for 30 minutes to allow the EOs to diffuse then kept in the incubator at 27ºC for 5-7 days (dermatophytes) and 37ºC for 1-2 days (Candida species). Antifungal activities were determined by measuring inhibition zones diameter (IZD) around the agar well or disc.[27]

Statistical analysis

Data were summarized and analyzed using GraphPad Prism software (GraphPad Software LLC, Version 9.0.0 (2020). Means of IZD in different treated wells and discs were compared using One-way ANOVA and Tukey's multiple comparison test.

Results and Discussion

Chemical compositions of C. barbatus and C. citriodora EOs

The GC-MS results showed variations in the quantity and chemical compositions of the *C. barbatus* and *C. citriodora* EOs growing in Tanzania. The extraction process afforded 18.4 mL of *C. barbatus* and 40.0 mL of *C. citriodora* EOs corresponding to 0.08% (v/w) and 0.29% (v/w) yields, respectively. The GC-MS chromatogram of *C. barbatus* EOs gave 34 peaks corresponding to 34 compounds (Figure 1) while the chromatogram of *C. citriodora* EOs had 42 peaks corresponding to 42 compounds (Figure 2). The identity, retention time, and relative abundance of the chemical constituents are provided in Table 1. The major compounds of *C. barbatus* (Figure 3A) EOs were (1S)-1,7,7-Trimethyl-bicyclo [2.2.1]heptan-2-one (camphor) (17.83%) followed by 1,3,8-p-Menthatriene (13.57%), thymol (10.43%), (1R)-2,2-dimethyl-3-methylene-bicyclo[2.2.1]heptane (camphene) (10.18%), 2,5,5-Trimethyl-1,3,6-heptatriene (9.69%), caryophyllene (7.43%), fenchone (4.43%), and 1,5,9,9-Tetramethyl-1,4,7-cycloundecatriene (4.36%) constituting about 78% of the total oils. Other components of *C. barbatus* EOs were present in less than 3% each. The main constituents contributing about 90% of *C. citriodora* (Figure 3B) EOs were (1R,2R,5S)-2-Isopropenyl-5-methylcyclohexanol (p-menth-8-en-3-ol) (75.43%), 2-(2-Hydroxypropan-2-yl)-5-methylcyclohexanol (5.92%), citronellol (3.95%), eucalyptol (2.52%), and 5-(1-Bromo-1-methyl-ethyl)-2-methyl-cyclohexanol (2.08%). The remaining compounds contributed less than 2% each. In addition, eight (8) compounds including *β*-myrcene, 3-carene, (+)-4-carene, linalool, (1R,2R,5S)-2-Isopropenyl-5-methylcyclohexanol (*p*-menth-8-en-3-ol), caryophyllene, *β*-copaene, and caryophyllene oxide were present in the EOs of the leaves of both plants (Table 1). Chemical profiles, percentage compositions, and biological activities of EOs are known to be affected by various factors including collection sites, plant species, plant parts, growth stage, time and season of harvest, as well as agricultural practices.[28, 29] *C. barbatus* synonym *Plectranthus barbatus* is a shrub growing in South America, Asia, and Africa,[30] while

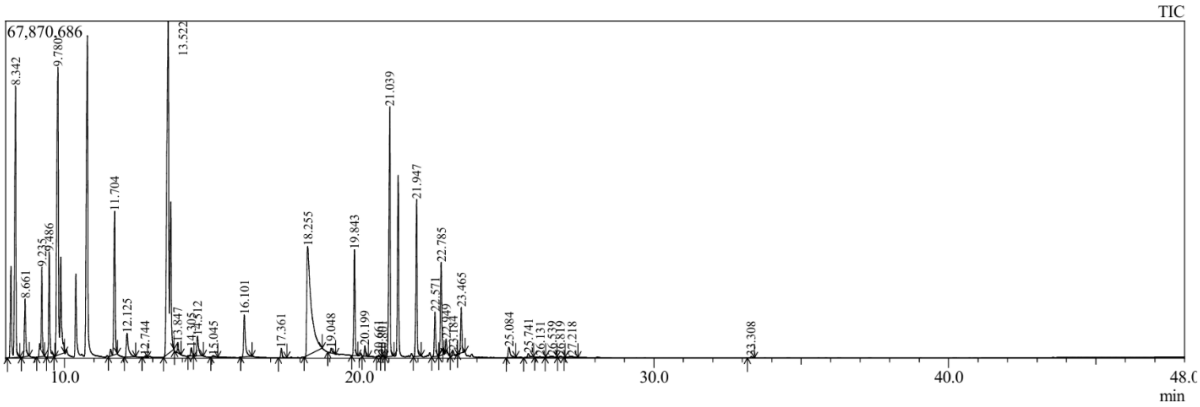

Figure 1: GC chromatogram of *C. barbatus* leaves essential oils

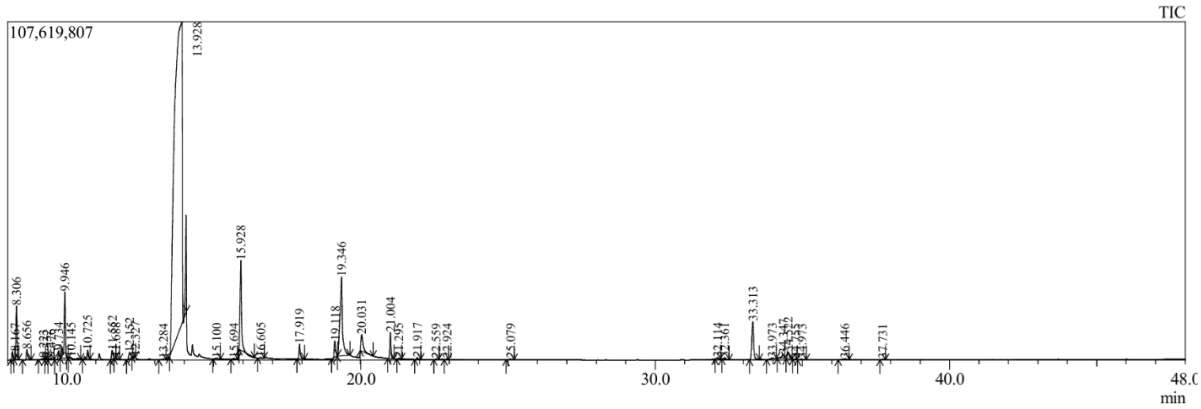

Figure 2: GC chromatogram of *C. citriodora* leaves essential oils

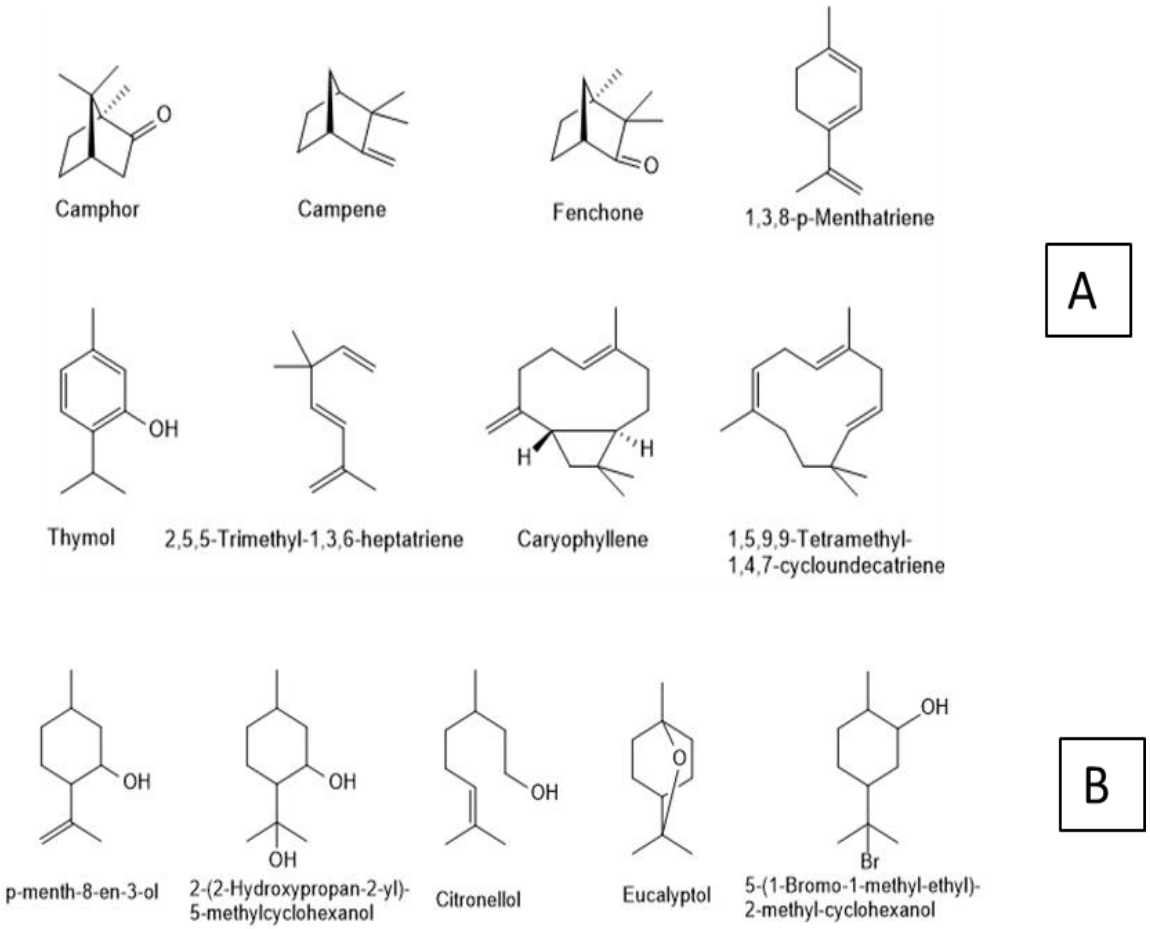

Figure 3: Chemical structures of major compounds in *C. barbatus* (A) and *C. citriodora* (B) essential oils

Table 1: GC-MS spectral data for *C. barbatus* and *C. citriodora* essential oils

S/N	Compound	Retention Time	Relative abundance (%)	
			C. barbatus	*C. citriodora*
1	3-Isopropyl-1-methyl-cyclopentane	5.27	-	0.05
2	3-methylpropyl 2-methylpropanoate	6.50	-	0.09
3	5-Isopropyl-2-methylbicyclo[3.1.0]hex-2-ene	6.82	-	0.12
4	2-Methyl-5-isopropylbicyclo[3.1.0]hex-2-ene	6.83	2.05	-
5	*α*-Pinene	7.03	-	0.83
6	2,5,5-Trimethyl-1,3,6-heptatriene	7.07	9.69	-
7	Camphene	7.50	-	0.42
8	1-Isopropyl 4-methylenebicyclo[3.1.0]hexane	8.17	-	0.26
9	(1S)-6,6-Dimethyl-2-methylenebicyclo[3.1.1]heptane	8.31	-	1.71
10	(1R)-2,2-dimethyl-3-methylene-bicyclo[2.2.1]heptane (Camphene)	8.34	10.18	-
11	*β*-Myrcene	8.66	1.55	0.40
12	3-Carene	9.23	2.38	0.06
13	3-Methylbuty 2-methylpropanoate	9.33	-	0.03
14	(+)-4-Carene	9.48	2.72	0.08
15	*o*-Cymene	9.73	-	0.26
16	1,3,8-p-Menthatriene	9.78	13.57	-
17	Eucalyptol	9.95	-	2.52
18	Guanidine carbonate	10.14	-	-
19	*γ*-Terpinene	10.72	-	0.45
20	(+)-4-Carene	11.55	-	0.31
21	Fenchone	11.69	-	0.04
	Fenchone	11.70	4.43	-
22	Linalool	12.1	1.06	0.40
	Fenchol	12.74	0.04	-
23	2,6-Dimethyl-5-hepten-1-ol	13.28	-	0.02
24	(1S)-1,7,7-Trimethyl-bicyclo[2.2.1]heptan-2-one (Camphor)	13.52	17.83	-
25	(1R,2R,5S)-2-Isopropenyl-5-methylcyclohexanol (p-menth-8-en-3-ol)	13.9	0.26	75.43
26	endo-Borneol	14.30	0.25	-
27	(R)-1-Isopropyl-4-methyl-3-cyclohexen-1-ol	14.51	0.75	-
28	*α*-Terpineol	15.04	0.07	-
29	L-*α*-Terpineol	15.10	-	0.04
30	3-Undecanol	15.69	-	0.06
31	Citronellol	15.93	-	3.95
32	2-Isopropyl-1-Methoxy-4-methylbenzene	16.10	1.51	-
33	(S)-(-)- Methyl citronellate	16.60	-	0.01
34	Bornyl acetate	17.36	0.25	
35	8,8-Dimethoxy-2,6-dimethyl-2-0ctanol	17.92	-	0.64
36	Thymol	18.25	10.43	-
37	*α*-Cubebene	19.05	0.16	-
38	3,7-Dimethyl-6-octenyl ethanoate	19.12	-	0.66
39	(1S,2R,5R)-2-(2-Hydroxypropan-2-yl)-5-methylcyclohexanol	19.35	-	5.92
40	*cis*-1,3-trans-1,4-p-menthane-3,8-diol	20.03	-	1.08
41	1-Ethenyl-2,4-diisopropenyl-1-methylcyclohexane	20.20	0.25	-

42	1a,2,3,3a,4,5,6,7b-Octahydro-1,1,3a,7-tetramethyl-1H-cyclopropa[a]naphthalene	20.66	0.04	-
43	*cis*-*α*-Bergamotene	20.80	0.04	-
44	Caryophyllene	21.0	7.43	0.97
45	*cis*-*α*-Bergamotene	21.295	-	0.03
46	1,5,9,9-Tetramethyl-1Z,4Z,7Z-cycloundecatriene	21.917	-	0.05
47	Z,Z,Z-1,5,9,9-Tetramethyl-1,4,7-cycloundecatriene	21.947	4.36	-
48	*β*-Copaene	22.6	1.35	0.02
49	7-Isopropenyl-4a-methyl-1-methylenedecahydronaphthalene	22.78	2.54	
50	(1S,2E,6E,10R)-3,7,11,11-Tetramethylbicyclo[8.1.0]undeca-2,6-diene	22.92	-	0.02
51	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,8a-octahydronaphthalene	22.95	0.17	-
52	*β*-Bisabolene	23.18	0.03	-
53	1-Isopropenyl-,4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene	23.46	1.18	-
54	Caryophyllene oxide	25.08	0.43	0.06
55	(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	25.74	0.13	-
56	*τ*-Cadinol	26.54	0.08	-
57	*α*-Cadinol	26.82	0.05	-
58	Isopulegol acetate	32.114	-	0.07
59	Nerol pentafluoropropionate	32.361	-	0.08
60	5-(1-Bromo-1-methyl-ethyl)-2-methyl-cyclohexanol	33.308	0.05	-
61	5-(1-Bromo-1-methyl-ethyl)-2-methyl-cyclohexanol	33.313	-	2.08
62	Nerol pentafluoropropionate	34.347	-	0.26
63	3-Decanylbicyclo[4,4,0]decane	34.522	-	0.41
64	*p*-Menth-8-en-3-ol, acetate	34.755	-	0.02
65	(E)-3-Methyl-5-((1R,4aR,8aR)-5,5,8a-trimethyl-2-methylenedecahydronaphthalen-1-yl)pent-2-en-1-ol	34.973	-	0.04
66	Citronellal	36.446	-	0.14
67	4-(2,6,6-Trimethyl-cyclohex-1-enyl)-butan-2-ol	37.731	-	0.02

Key: GC-MS = Gas Chromatography-Mass Spectrometry

C. citriodora is distributed in Australia, Africa, America, Europe, and Asia.[31] This study reports 34 compounds from the EOs of *C. barbatus* leaves with the major compounds being camphor, 1,3,8-p-menthatriene, thymol, and camphene (Table 1). Do Santos et al. (2015)[32] published the same number of compounds in the EOs of *C. barbatus* leaves collected in São Paulo State in Brazil but the main chemical components were (Z)-caryophyllene, germacrene D, and viridiflorol. Other compounds reported in large quantities were *α*-pinene, erernophilen, myrcene, and humulenone in the EOs of leaves of *C. barbatus* from northeast Brazil.[33] Anethol, *β*-caryophyllene, D-germacrene, 1-octen-3-ol were the main constituents in the leaves of *C. barbatus* from Italy.[20] A total of 102 compounds were characterized in the aerial parts (stem and Leaves) of *C. barbatus* from Portugal with the major components being α-pinene.[34] The *α*-pinene was reported as the major component in the EOs of *C. barbatus* collected in Brazil and Portugal, however, this compound was absent in the *C. barbatus* reported in this study (Table 1).

Furthermore, this study reported a yield of 0.29% EOs, 42 compounds, and citronellal and citronellol contents less than 4% in the leaves of *C. citriodora* from Tanzania. Citronellal and citronellol were documented as main constituents of *C. citriodora* leaves studied in other places. These findings support the variations of chemical profiles of the EOs of *C. citriodora* reported previously. Ghaffar *et al*. (2015),[35] reported 1.82% yield and 31 chemical constituents in the EOs of *C. citriodora* leaves collected in Pakistan while Tolba *et al*. (2015),[19] reported a yield of 2.26% and 22 compounds in the EOs of the leaves of *C. citriodora* collected in Algeria.

Antifungal activity against non-dermatophyte and dermatophyte fungi

The *C. barbatus* and *C. citriodora* EOs individually or in combination exhibited antifungal activity against non-dermatophyte fungi (Figure 4). *C. barbatus* EOs alone showed high antifungal activity against *C. albicans*, *C. glabrata*, and *C. tropicalis* clinical isolates. The activity was statistically significant ($p < 0.05$) compared to the activity of *C. citriodora* EOs alone. *C. citriodora* alone was more active against *C. albicans* 23Q-NR2934 strains ($p < 0.05$) compared to the *C. barbatus* EOs tested alone. The EOs from both plants showed high antifungal activity (IZD > 20 mm) against *A. niger* with no statistically significant difference ($p > 0.05$). The high antifungal activity of *C. citriodora* EOs against *A. niger* reported in this study corroborates the findings published previously using EOs of *C. citriodora* leaves from Pakistan.[35] The *C. krusei* clinical isolates were resistant to *C. barbatus* and *C. citriodora* individual EOs and some combinations except 1:1 (IZD = 28 mm), 2:1 (IZD = 19.5 mm), and 3:1 (IZD = 21 mm) ratios. The high antifungal activity of the 1:1 combination against *C. krusei* clinical isolates was comparable to that of the positive control (clotrimazole 10 µg/disc) drug ($p > 0.05$). Among the combinations, only 2:1 and 3:1 ratio showed activity with inhibition zone diameter greater than 18 mm in at least three Candida species.

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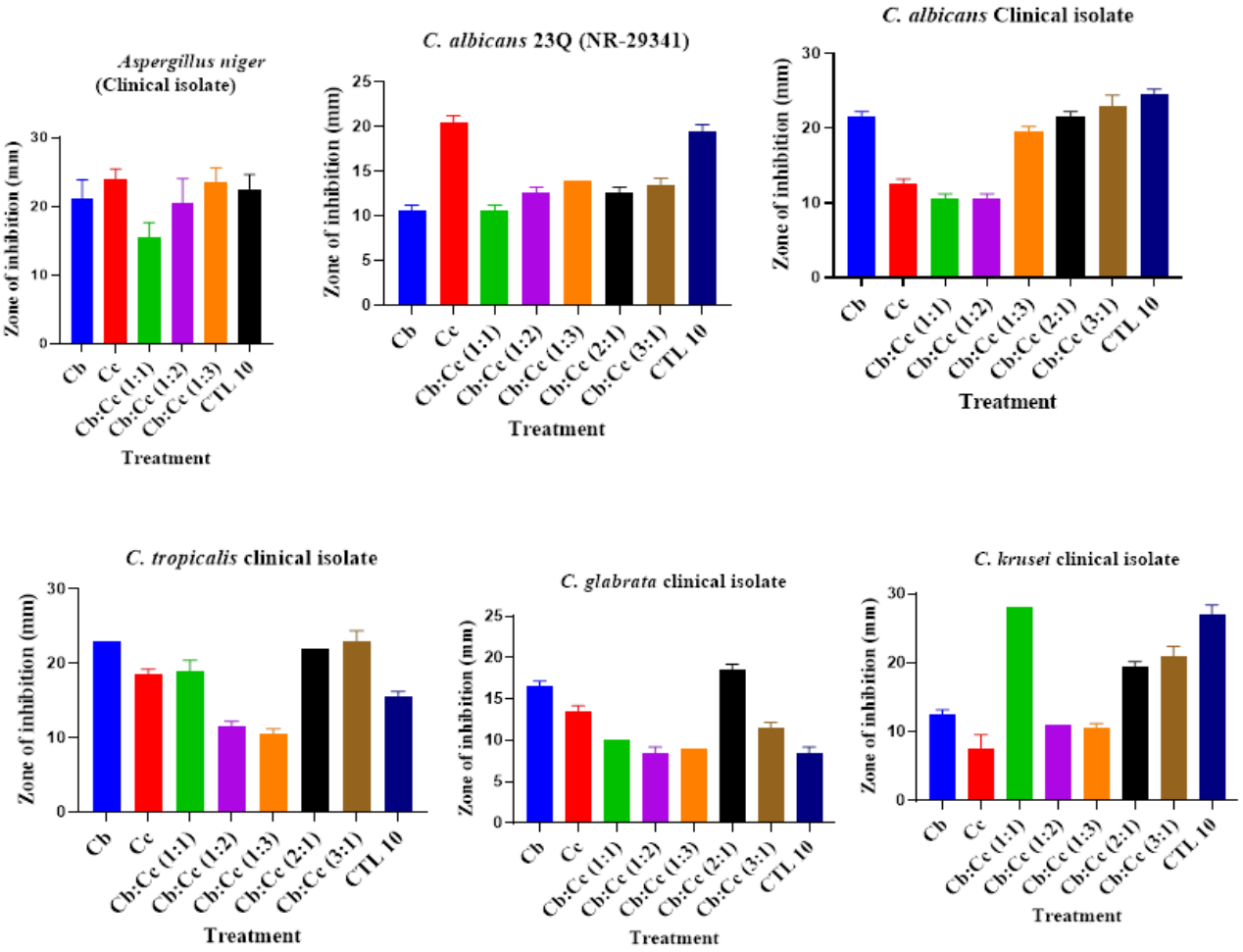

Figure 4: Antifungal activity of individual and combined essential oils of *C. barbatus* and *C. citriodora* against *A. niger*, *C. albicans* 23Q (NR-29341), *C. albicans* clinical isolates, *C. tropicalis* clinical isolates, *C. glabrata* clinical isolates, and *C. krusei* clinical isolates. Individual and different combinations of essential oils were tested at 5 µL/well. Agar plates with oils or standard drug (clotrimazole) were incubated at 37ºC for 24 to 48 h. Zone of inhibition includes well size (6 mm); **Cb** = *Coleus barbatus* essential oils; **Cc** = *Corymbia citriodora* essential oils; **CTL 10** = Clotrimazole 10 µg/disc. Each value represents a mean of inhibition zone diameters of EOs tested in duplicate agar wells and means were compared using One-way ANOVA followed by Tukey's multiple comparison test.

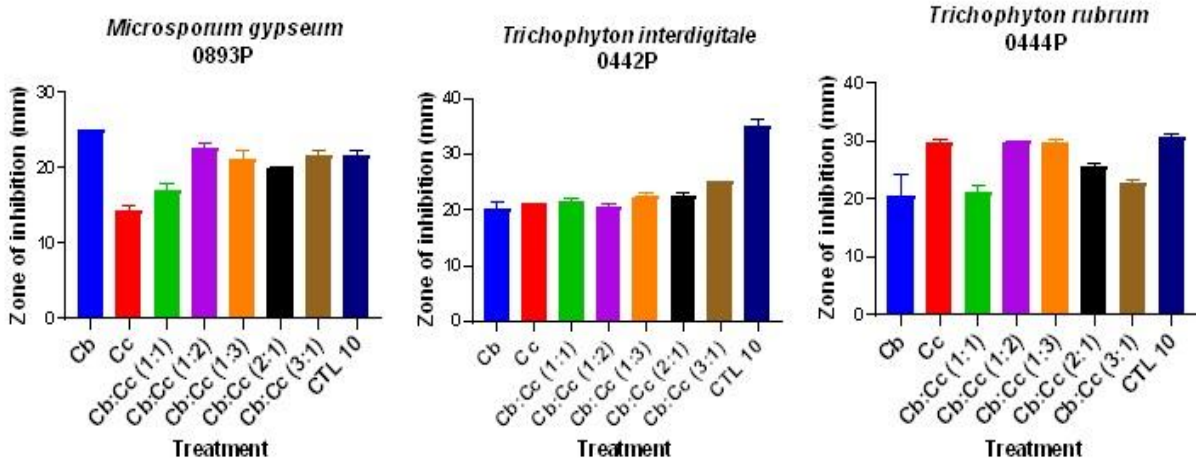

Figure 5: Antifungal activity of *C. barbatus* and *C. citriodora* essential oils against *M. gypseum*, *T. interdigitale*, and *T. rubrum* dermatophytes. Individual and different combinations of EOs were tested at 5 µL/well. Agar plates with EOs or standard drug (clotrimazole) were incubated at 27 ºC for 5 to 7 days. Zone of inhibition includes well size (6 mm) or clotrimazole disc size (6 mm); **Cb** = *Coleus barbatus* essential oils; **Cc** = *Corymbia citriodora* essential oils; **CTL 10** = Clotrimazole 10 µg/disc. Each value represents the mean of inhibition zone diameter of EOs tested in duplicate agar wells and means were compared using One-way ANOVA followed by Tukey's multiple comparison test ($p < 0.05$)

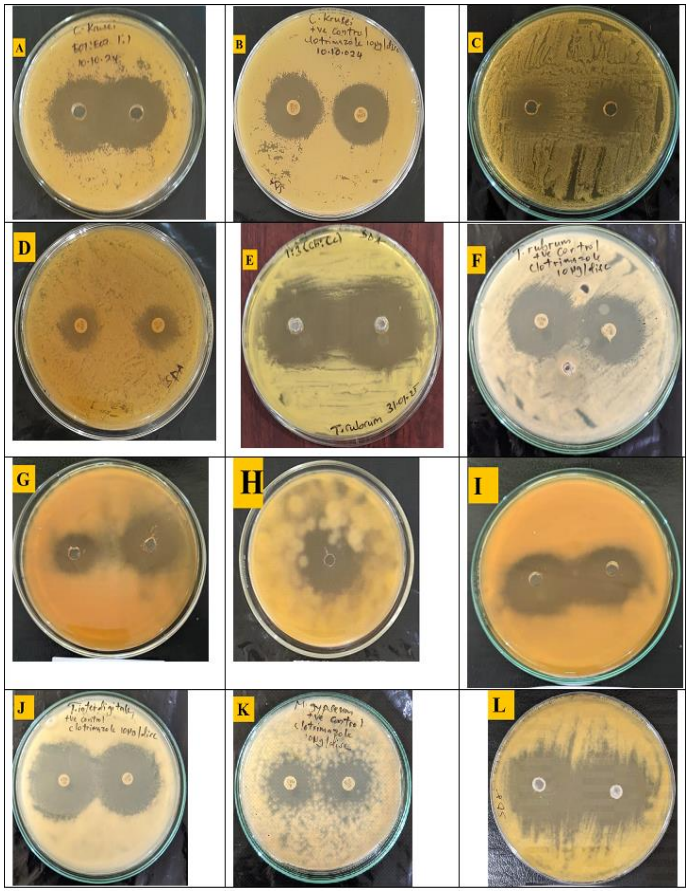

Figure 6: Antifungal activity of positive control (Clotrimazole 10 µg/disc) or 5 µL/well of individual or 5 µL/well combined essential oils of *C. barbatus* and *C. citriodora*. **A** = Cb:Cc (1:1) against *C. krusei* clinical isolates; **B** = Clotrimazole 10 µg/disc against *C. krusei* clinical isolates; **C** = Cb:Cc (2:1) against *C. glabrata* clinical isolates; **D** = Clotrimazole 10 µg/disc against *C. glabrata* clinical isolates; **E** = Cb:Cc (1:3) against *T. rubrum*; **F** = Clotrimazole 10 µg/disc against *T. rubrum;* **G** = Cb oil against *T. interdigitale*; **H** = Cc oil against *T. interdigitale;* **I** = Cb:Cc (3:1) against *T. interdigitale;* **J** = Clotrimazole 10 µg/disc against *T. interdigitale;* **K** = Clotrimazole 10 µg/disc against *M. gypseum*; **L** = Cb:Cc (3:1) against *M. gypseum*; Zone of inhibition includes well size (6 mm); **Cb** = *Coleus barbatus* essential oils; **Cc** = *Corymbia citriodora* essential oils

C. barbatus EOs tested alone were more active than *C. citriodora* EOs against *M. gypseum* ($p<0.001$) while *C. citriodora* EOs alone were more active ($p<0.05$) compared to *C. barbatus* EOs alone against *T. rubrum* (Figure 5). The activity of *C. barbatus* EOs alone was significantly higher than that of the clotrimazole against *M. gypseum* ($p<0.05$). Increasing concentrations of either *C. barbatus* or *C. citriodora* in the combinations showed an increase in the antifungal activity against *M. gypseum* compared to the activity of *C. citriodora* EOs alone. On the other hand, increasing the concentration of *C. citriodora* EOs in the combination increased the antifungal activity against *T. rubrum* while increasing the concentration of *C. barbatus* EOs decreased the activity against the *T. rubrum.* Furthermore, *C. barbatus* and *C. citriodora* EOs tested alone or in combination showed similar antifungal activity against *T. interdigitale* (Figure 5). The activity of *C. citriodora* EOs against *T. mentagrophytes* and *T. rubrum* was reported previously to be concentration-dependent.[19] Generally, the combination of EOs showed improvement in the antifungal activity against some strains of the dermatophytes and non-dermatophytes fungi tested (Figures 4, 5, and 6). Our study found some variations in the type and quantity of constituents of the EOs of *C. barbatus* and *C. citriodora* leaves. Some of the compounds were absent in *C. barbatus* oils but present in *C. citriodora* oils. This suggests that combining EOs with different compounds can improve antifungal activity by providing compounds with different mechanisms of action. Camphor and thymol are major constituents of EOs of *C. barbatus* leaves but were absent in *C. citriodora* EOs (Table 1). Thymol was reported to possess antifungal efficacy against *T. mentagrophytes, M. gypseum, T. violaceum, and M. canis*.[36] In addition, Ramsewak *et al*. (2003),[37] revealed the antifungal efficacy of thymol against *C. krusei, C. albicans*, and *T. rubrum* and that combination of menthol, camphor, thymol, and *C. citriodora* EOs inhibited significantly the growth of *C. albicans, T. rubrum, T. mentagrophytes, Microsporum canis*, and *Epidermophyton flocosum* fungi responsible for skin fungal infections.

Conclusion

The results showed variations in the major components and antifungal properties of the EOs of *C. barbatus* and *C. citriodora* leaves. The *C. barbatus* EOs were more active against *M. gypseum* while *C. citriodora* EOs were more active against *T. rubrum*. However, EOs from both plants were equally active against *T. interdigitale*. Combinations have improved the antifungal activity against dermatophytes and candida species, therefore, further studies are recommended to investigate the synergistic properties of *C. barbatus* and *C. citriodora* EOs.

Conflict of Interest

The author reports no conflicts of interest in this work.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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