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Full Length Research Article

PHYTOCOMPOUND SCREENING, CYTOTOXICITY AND ANTIFUNGAL ACTIVITY OF CYMBOPOGON CITRATUS EXTRACT AGAINST ONYCHOMYCOSIS PATHOGENS IN BENUE STATE, NIGERIA

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ABSTRACT
Onychomycosis, a prevalent fungal nail infection, poses a substantial public health challenge, particularly in tropical regions such as Nigeria. This study investigates the phytocompounds, cytotoxicity and antifungal activity of Cymbopogon citratus extracts, as a source of novel antifungal compounds for onychomycosis treatment. Gas Chromatography-Mass Spectrometry technique was employed for the phytocompounds screening, while minimum inhibitory concentration and minimum fungicidal concentrations were determined using standard method. The results revealed that a total of twenty-four (24) compounds were detected out of which 7 compounds were assessed from the essential oil, extracts of methanol had five (5), ethyl-acetate (8), and hexane (4) compounds respectively. Compound with the highest percentage height was Dodecanoic acid, 1,2,3-propane (6.48%). Hexane had higher MIC ranging from 0.232 µg/ml. whereas diacetate had MIC range of 0.03-2 µg/ml, however essential oil (EO) showed the lowest MIC range of 0.03 -1 µg/ml as compared to all other extracts and the control (Terbinafine) showing a MIC of 0.03-4 µg/ml. The antifungal potential of Cymbopogon citratus extracts, particularly essential oil, was demonstrated against various fungal isolates. Essential oil consistently exhibited the lowest MIC and MFC values, indicating potent antifungal properties. The study added valuable information to the growing body of evidence supporting the antimicrobial efficacy of C. citratus extracts.

Keywords: Phytocompounds screening, *Cymbopogon citratus,* Antifungal, Onychomycosis, Nigeria.

INTRODUCTION

Cymbopogon citratus also commonly referred to as lemongrass, belongs to the grass family poaceae (Vlahovic *et al*., 2016). Lemon grass is a fast-growing perennial aromatic grass native of South India and Sri Lanka and commonly cultivated in the tropical America, Asia and Africa including Nigeria (Adejuwon, 2012). The Greek words "*kymbe*" (boat) and "*pogon*" (beard), which relate to the flower spike arrangement, are the source of the name "*Cymbopogon*" (Shah *et al*., 2011). The important aspect of lemon grass is the antimicrobial action of essential oil in the vapour phase (Lopez *et al*., 2015). The rising use of various antibiotics and their unfavorable side effects, as well as the multidrug resistance of pathogenic microorganisms, has sparked a great interest in the development of novel herbal antimicrobial medications (Boukhatem *et al.,* 2014). Numerous varieties of lemon grass have been used as medicines due to their positive benefits on health, including their ability to stimulate digestion, their anti-inflammatory, anti-microbial, hypolidemic, anti-carcinogenic, and antimutagenic characteristics (Zhou *et al*., 2016).

Onychomycosis, a fungal infection of the nails, is a common condition worldwide (Westerberg *et al.,* 2013). The infection is caused by various fungi, including dermatophytes, yeasts, or non-dermatophyte molds (Westerberg *et al.,* 2013). This condition can significantly impact patients' emotional, social, and occupational functioning (Girois *et al*., 2016). Patients with onychomycosis may feel embarrassed in social and workplace situations, unwilling to expose their hands or feet due to the perception of being blighted or unclean (Westerberg *et al.,* 2013). The use of medicinal plants in the treatment of onychomycosis has gained attention in recent years due to concerns about the side effects of conventional antifungal drugs (Girois *et al*., 2016). One such plant is *Cymbopogon citratus*, locally known as lemongrass. Lemongrass has been used traditionally for various medicinal purposes, including its antifungal properties (Vlahovic, 2016). Previous studies have reported the antifungal activity of lemon grass against various fungal pathogens, including *Candida albicans*, *Aspergillus niger*, and *Trichophyton mentagrophytes* (Ekpenyong *et al*., 2015). Onychomycosis is a significant public health issue as it causes pain, discomfort, and disfigurement (Westerberg *et al.,* 2013). In Nigeria, the incidence of onychomycosis is high, with more than 1.5 million cases reported annually (Amichai *et al*., 2011). Irrational use of antifungal drugs is widespread, leading to the development of resistance and treatment failure (Khairy *et al*., 2021). To this end, this study seeks to determine the antifungal activity, cytotoxicity and phytocompounds screening of *Cymbopogon citratus* extract against onychomycosis pathogens in Benue State, Nigeria.

MATERIALS AND METHODS

Study Area

The study was carried out in selected health facilities (Federal Medical Centre, Makurdi, Benue State University Teaching Hospital, Makurdi, Bishop Murray Hospital, Makurdi, General Hospital Otukpo and General Hospital Gboko) in Benue State, Nigeria. Benue State lies within the lower River Benue trough in the middle belt region of Nigeria. Its geographic coordinates are longitude 7º 47' and 10º 0' East, Latitude 6º 25' and 8º 8' North (Agada and Nirupama, 2015). Based on the Koppen climate classification, Benue State lies within the AW climate and experiences two distinct seasons, the wet season and the dry season. The rainy season lasts from April to October with annual

rainfall in the range of 100-200mm. The dry season begins in November and ends in March. The temperature fluctuates between 21-37°C in the year (Adejuwon, 2012). Benue state comprises of people from all works of life mostly the working class who wear fashionably fitted shoes throughout the day and farmers who work on their farmlands bare footed, with sometimes using their hands to weed off unwanted grasses which pre-disposes them of harmful micro-organisms including infection with Onychomycosis.

Ethical Consideration for the Study

The study was approved by the ethics committee of the Benue State Ministry of Health and Federal Medical Centre, Makurdi respectively, with an issuance of letters of ethical clearance approval reference numbers FMH/FMC/HREC/108/VOL.1 and MOH/STA/204/VOL.1/242.

Collection/Preparation of Plant Extract of *Cymbopogon citratus*

The recently harvested stems and leaves of *Cymbopogon citratus* plant was transported to the laboratory and meticulously washed under running water to eliminate any adhering contaminants. The plant material was then partitioned into two portions. The initial portion underwent steam distillation to extract essential oil, employing a modified Clevenger-type apparatus. The second portion was chopped into pieces and air-dried in the laboratory for two weeks at room temperature. Subsequently, it was pounded using a sterile mortar and pestle to obtain the Crude (dried powdered plant material) which was stored in an airtight container before further processing.

Soxhlet Extraction of the Crude in Hexane

In a conventional Soxhlet extractor, 50g of the plant material was put in 240 mm Whatman filter paper and inserted into the thimble of the extractor. It was gradually filled up with 500 ml of hexane as solvent. The boiling temperate of the Soxhlet unit was maintained to 67-70 degree Celsius. The flask containing the extraction solvent was heated to reflux. When the solvent got to a particular level, a siphon pulled the thimble contents into the distillation flask, thus carrying the extracts into the bulk liquid. The process was continued for three days (15-20 cycles), and each was replicated followed by the evaporation using the water bath to allow the solvent to get evaporated and to obtain the concentrated sample which was used for the experimental process. Similarly, the essential oil of the plant was extracted using the method described by Zhou *et al.* (2016).

Gas Chromatography-Mass Spectrometry Analysis of *Cymbopogon citratus* Extract

Gas Chromatography-Mass Spectrometry (GC-MS) analysis was done on a HP 7890 gas chromatograph (Agilent Technologies, Buenos Aires, Argentina) coupled to a HP 5978 quadrupole mass spectrometer (Agilent Technologies) outfitted with a PerkinElmer Elite-5MS slim sections (5% phenyl methyl siloxane, length 80m, internal width 0.55mm, film thickness 0.55µm). Helium was utilized as the transporter gas at a flow pace of 1mL/minute. GC-MS interphase, particle source and particular mass identifier temperatures were kept up with at 580°, 580° and 150°C, individually; ionization energy: 70eV. The broiler temperature program was equivalent to for the GC-FID examination. The parts rate was taken from capillary GC follows with the FID (Gouse *et al.,* 2017; Hasson and Mustafa, 2019). The identification of the singular parts depended on (I) PC coordinating with the Wiley 575 and National Institute of Standards and Technology (NIST) libraries gave PC controlling GC-MS framework; (ii) comparison with spectra accessible in our files and writing information (Hayes and Markovic, 2012; Heydorn *et al.,* 2013; Kong *et al.,* 2016) and (iii) correlation of their GC math lists (AI) on a HP-5 segment. The AIs for the essential oil parts were determined utilizing a homologous series of n-alkanes C8-C18 (Muazzam *et al.,* 2020; Nyarko *et al.,* 2022).

Collection of Clinical Specimens

All patients had their nails clinically examined. Written informed consent with a structured questionnaire form on socio-demographic information and lifestyle was obtained from the patients before the collection of samples. The fingernails and/or toenails were disinfected by applying 70% ethyl alcohol before the sample collection to avoid contamination (Cheesbrough, 2012). Nail scrapings and trimmings were done using sterile surgical blades to scrap and trim a small portion of the affected nail plate and put in a sterile polythene envelope and sealed properly, it was labeled properly and then transported to the JOSTUM Microbiology Laboratory for Mycological analysis.

Identification and Characterization of Fungi Isolates

Isolated fungi were identified by comparing photographic Atlas for the Microbiology laboratory based on physiological and morphology properties (Cheesbrough, 2000). Colonies developed on Sabouraud Dextrose Agar (SDA) were classified according to their shape, colour, size and their margin. Plates with no visible growth after 21 days were considered negative, visible colonies were stored in Tween 80 broth and afterwards on SDA broth for molecular identification.

Synthetic Antifungal as Control

The control in this study was Terbinafine, a synthetic antifungal. Its medical significance was derived through interviews with medical professionals and pharmacists in the selected Benue State hospitals. Terbinafine was selected for this research due to its widespread use as an effective treatment for fungal infections and its ready availability.

Preparation of Synthetic Antifungal

The Terbinafine tablets (250 mg) were purchased from a pharmacy, transported to the laboratory, and then dissolved in 500ml of distilled water within a conical flask.

Concentration (in mg/ml) = Amount of Terbinafine (in mg) / Volume of Solution (in ml)

Given that you dissolved 250 mg of terbinafine in 500 ml of water:

Concentration = 250 mg / 500 ml

Concentration = 0.5 mg/ml

So, the concentration of terbinafine in the solution is 0.5 mg/ml.

RESULTS

Seven compounds were detected from the essential oil of the plant extract with Benzene, 1,2,3-trimethyl- having the highest percentage height (3.82%) followed by Dodecane (2.30%), 2-Cyclohexene-1-carboxaldehy (1.99%), Mesitylene (1.88%), Others includes Benzene, 1-ethyl-3-methyl- (1.81%), Benzene, (1-methylethyl)- (1.28%), and Benzene, propyl- (0.99%) respectively.

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Table 1: GC-MS Analysis of *Cymbopogon citratus* Essential Oil

RT(s)	Area Peak (%)	%Average Height	Compound
3.522	0.09	0.99	Benzene, propyl-
3.618	0.70	1.81	Benzene, 1-ethyl-3-methyl-
3.862	0.14	1.28	Benzene, (1-methylethyl)-
4.088	1.04	1.88	Mesitylene
4.141	1 .53	3.82	Benzene, 1, 2, 3-trimethyl-
6.839	0.32	1.99	2-Cyclohexene-1-carboxaldehy
7.144	0.37	2.30	Dodecane

Key:
RT: Retention time
%: Percentage

Table 2 below showed the detection of eight compounds from Ethyl acetate extract of C. citratus plant. Dodecanoic acid, 1,2,3-propane had the highest percentage height (6.48%), followed by Cyclooctasiloxane, hexadecane (3.48%), n-Hexadecanoic acid (2.70%), 2,6-Octadienal, 3,7-dimethyl- (2.63%), Heneicosane (2.40%), Tetradecane (1.65%). The compound that showed low percentage height was Heptadecane with (1.44%) respectively.

Table 2: GC-MS Analysis of *Cymbopogon citratus* Ethyl acetate Extract

RT(s)	Area Peak (%)	%Average Height	Compound
8.15	9.74	2.63	2, 6-Octadienal, 3, 7-dimethyl-
9.976	0.46	1.65	Tetradecane
12.494	0.54	1.44	Heptadecane
12.597	0.51	3.48	Cyclooctasiloxane, hexadecame
14.814	0.49	1.80	Heneicosane
17.137	5.00	2.70	n-Hexadecanoic acid
17.543	13.79	6.48	Dodecanoic acid, 1, 2, 3-propane
17.685	0.34	2.46	Heneicosane

Key:
RT: Retention time
%: Percentage

Compounds analyzed from methanol extract of *Cymbopogon citratus* revealed that, the compound Betulin had the highest percentage (4.62%), Oleic Acid (4.14%), 9,12-Octadecadienoic acid (Z,Z) (3.91%), Dotriacontane (2.31%), while the compound, Octadecanoic acid had the lowest percentage height (0.69%) respectively as shown in Table 3 below.

Table 3: GC-MS Analysis of *Cymbopogon citratus* Methanol Extract

RT(s)	Area Peak (%)	%Average Height	Compound
19.345	4.92	3.91	9, 12-Octadecadienoic acid (Z, Z)
19.425	21.80	4.14	Oleic Acid
19.702	12.12	0.69	Octadecanoic acid
24.137	18.67	4.62	Betulin
24.596	0.56	2.31	Dotriacontane

Key:
RT: Retention time
%: Percentage

Figure 1: Zone of inhibition of *C. citratus* Extracts and Terbinafine on isolate from fingernail.
Key: (1: Hex-Meth 50/50, 2: Diacetate, 3: Essential Oil, 4: 100% Ethyl, 5: Soxhlet Extract in Hexane, 6: Terbinafine, 7: Methanol-ethyl 50/50, 8: 100% methanol)

Minimum Inhibitory Concentration and Minimum Fungi Concentration of the Plant Extracts, Essential Oil and Control
The Minimum inhibitory concentration ranges that inhibited 50% of the isolates (MIC_{50}) and 90% of the isolates (MIC_{90}) including the mean MIC values of each *C. citratus* extract and terbenafine are shown in Table 4. Fungi isolates were *Trychophyton rubrum* (N: 159), *Epidermophyton floccosum* (N: 8), *T. interdigitale* (N: 11), *Candida* (Yeast) (N: 19) and *Aspergillus* species (N: 30), tested against seven (7) *Cymbapogon citratus* extracts and Terbinafine as control.

Majority of the *T. rubrum* had higher MIC values for Hex-Meth 50/50% which ranged from 0.258 µg/ml. while 100% Ethyl was observed to be inhibiting *T. rubrum* effectively even at lower MIC which ranged from 0.068µg/ml. 100% methanol also showed similar MIC range of 0.06-4 µg/ml. ethyl-meth 50/50% also had

higher MIC range of 0.25-16 µg/ml, soxlet extract in Hexane had higher MIC ranging from 0.2532 µg/ml. whereas diacetate had MIC range of 0.03-2 µg/ml, however essential oil (EO) showed the lowest MIC range of 0.03 -1 µg/ml as compared to all other extracts and the control (Terbinafine) showing a MIC of 0.03-4 µg/ml.

Minimum inhibitory concentration range of *E. floccosum* for Hex-Meth 50/50 was at 1-4µg/ml. 100% ethyl was 0.03-1µg/ml. 100% methanol showed a MIC values for *E. floccosum* which was 0.125-0.5µg/ml, ethyl-methanol 50/50 showed MIC range between 0.52µg/ml. MIC range of soxlet extraction in hexane was 1-4 µg/ml which was higher than all other extracts including terbinafine (control). More so Diacetate recorded a MIC of 0.06-1µg/ml for *E. fluccosum* while EO recorded the lowest MIC of 0-03-025µg/ml as compare to other extracts including Terbinafine (control) and terbinafine was able to inhibit *E. floccosum* at 0.06-0.5 µg/ml.

For *T. interdigitale* Hex-meth 50/50% was able to inhibit it visible growth at a MIC range of 0.06-4 µg/ml. 100% ethyl inhibited visible growth of *T. interdigitale* 0.5-4 µg/ml. while 100% methanol inhibited growth at 0.125-2µg/ml. The result also showed that ethyl-methanol 50/50% inhibited *T. interdigitale* at a much higher MIC value of 1-16 µg/ml. Similarly, soxlet extract in hexane showed a MIC of 0.5-8µg/ml. Diacetate, inhibited visible growth of *T. interdigitale* at MIC range of 0.06-1µg/ml. EO showed a lower MIC range of 0.03-0.5 µg/ml against *T. interdigitale* while Terbinafine (Control) was capable of inhibiting the visible growth of *T. interdigitale* 0.125-0.5µg/ml. Again hex-meth 50/50 was able to inhibit visible colonies of *Candida* (yeast) at 0.03 -16 µg/ml, 100% ethyl showed a MIC of 0.5-8µg/ml against *Candida* (yeast) while 100% methanol was effective against *Candida* at 0.25-2µg/ml MIC. The study also revealed that ethyl-methanol 50/50% recorded 0.05-16 µg/ml while soxlet extract in hexane was able to inhibit visible growth of *Candida* at 0.125-32µg/ml which was the highest MIC against *Candida* as compare to other extract and terbinafine. The study further showed the MIC of 0.06-1µg/ml against *Candida*, while EO inhibited *Candida* at 0.03-1µg/ml which was the lowest as compare to other extract and terbenafine. The control (terbenafine) recorded the MIC of 0.125 -4µg/ml against *Candida* species.

The MIC tested for hex-meth 50/50% against *Aspergillus* species showed a high rang of 1-8 µg/ml, 100% ethyl was able to inhibit the growth of *Aspergillus* species at 0.25-4µg/ml while 100% meth showed a MIC of 0.06 -4 µg/ml against *Aspergillus s*p. In ethyl-methanol 50/50, *Aspergillus* sp. was inhibited at 0.5-32 µg/ml which was very high as compare to hex-meth 50/50%, 100% ethyl and 100% methanol but was low as compared to soxlet extract in hexane which showed a MIC of 1-32µg/ml against *Aspergillus* sp. Diacetate was effective against *Aspergillus* sp. at 0.06-2µg/ml while EO showed the lowest MIC of 0.03-1µg/ml against *Aspergillus* sp. The control Terbenafine was able to inhibit visible growth of *Aspergillus* sp. at 0.06-2µg/ml.

Other five (5) *C. citratus* extracts namely Hex-Methanol 50/50%, 100% Ethyl, 100% methanol, Ethyl-meth 50/50% and Soxlet extract in Hexane have high MFC against the isolates ranging from 0.25-32, and the $MFC_{50}$was at 0.5-8µg/ml and $MFC_{90}$was at 2-16µg/ml.

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Table 4: Minimum Inhibitory Concentration of Fungi Isolates for *C. citratus* Extracts and Control

Isolates	Number of Isolates	MIC Range Tested in ug/mL										
Hex-Meth 50/50		0.03	0.06	0.125	0.25	0.05	1	2	4	8	16	32
T. rubrum	159	0	0	0	67	29	10	45	2	6	0	0
E. floccosum	8	0	0	0	0	0	4	3	1	0	0	0
T. interdigitale	11	0	1	0	1	1	2	3	3	0	0	0
Candida (Yeast)	19	1	0	0	0	0	6	3	4	3	2	0
Aspergillus Spp.	30	0	0	0	0	0	1	3	3	23	0	0
100% Ethyl												
T. rubrum	159	0	3	17	30	44	56	3	4	2	0	0
E. floccosum	8	1	0	0	0	4	3	0	0	0	0	0
T. interdigitale	11	0	0	0	0	2	6	0	3	0	0	0
Candida (Yeast)	19	0	0	0	0	5	4	2	3	5	0	0
Aspergillus Spp.	30	0	0	0	12	3	5	7	3	0	0	0
100% Meth												
T. rubrum	159	0	16	37	50	30	10	9	7	0	0	0
E. floccosum	8	0	0	3	3	2	0	0	0	0	0	0
T. interdigitale	11	0	0	2	1	2	1	5	0	0	0	0
Candida (Yeast)	19	0	0	0	3	13	2	1	0	0	0	0
Aspergillus Spp.	30	0	1	0	3	10	7	6	3	0	0	0
Ethyl-meth 50/50												
T. rubrum	159	0	0	0	40	50	18	12	20	1	18	0
E. floccosum	8	0	0	0	0	2	3	3	0	0	0	0
T. interdigitale	11	0	0	0	0	0	4	4	1	1	1	0
Candida (Yeast)	19	0	0	0	0	2	1	4	5	6	1	0
Aspergillus Spp.	30	0	0	0	0	2	3	5	5	6	8	1
Soxhlet Extract in Hexane												
T. rubrum	159	0	0	0	67	40	10	4	6	6	20	6
E. floccosum	8	0	0	0	0	0	4	2	2	0	0	0
T. interdigitale	11	0	0	0	0	4	2	3	1	1	0	0
Candida (Yeast)	19	0	0	2	2	1	0	0	3	4	4	3
Aspergillus Spp.	30	0	3	0	0	0	2	2	3	6	7	7
Diacetate												
T. rubrum	159	39	33	45	13	12	12	5	0	0	0	0
E. floccosum	8	0	3	1	1	2	1	0	0	0	0	0
T. interdigitale	11	0	2	3	2	2	2	0	0	0	0	0
Candida (Yeast)	19	0	2	8	5	3	1	0	0	0	0	0
Aspergillus Spp.	30	0	3	4	15	6	2	0	0	0	0	0
EO												
T. rubrum	159	69	56	10	7	9	8	0	0	0	0	0
E. floccosum	8	2	3	2	1	0	0	0	0	0	0	0
T. interdigitale	11	3	2	3	1	2	0	0	0	0	0	0
Candida (Yeast)	19	4	4	4	2	2	3	0	0	0	0	0
Aspergillus Spp.	30	7	10	2	7	3	1	0	0	0	0	0
Terbenafine												
T. rubrum	159	12	22	13	23	45	12	29	3	0	0	0
E. floccosum	8	0	2	3	2	1	0	0	0	0	0	0
T. interdigitale	11	0	0	3	4	4	0	0	0	0	0	0
Candida (Yeast)	19	0	0	1	2	3	5	6	2	0	0	0
Aspergillus Spp.	30	0	3	5	7	11	3	1	0	0	0	0

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The result in table 5 below showed the minimum fungicidal concentration of seven (7) *C. citratus* extracts and terbinafine. Among the antifungal, essential oil (EO) had the lowest fungicidal concentration against all the 5 isolates, ranging from 0.03-1 µg/ml. The result further showed that EO had MFC_{50} against *T. rubrum, E. floccosum, and Aspergillus* species at 0.06 µg/ml and 50% of *T. interdigitale* and *Candida* (Yeast) at 0.25µg/ml and 0.125µg/ml respectively. At MFC_{90} Essential oil (EO) was able to kill *T. rubrum, T. interdigitale* and *Aspergillus* species at 0.5 dilution factor with *E. Floccosum* and *Candida* (yeast) at 0.125 and 1 µg/ml respectively. Similarly Diacetate was effective against the five isolates at MFC range of 0.03-1µg/ml and at MFC_{50} it was able to kill *T. rubrum, Candida* and *Aspergillus* sp. at 0.25µg/ml this was higher as compared to EO. Diacetate also showed a MFC_{90} at 1 µg/ml against *T. rubrum, E. Floccosum, T. interdigitale* and 0.5 µg/ml on *Candida (*yeast) and *Aspergillus* species.

Table 5: Minimum Fungicidal Concentration (µg/ml)

Fungi isolates	Hex-Meth 50/50	100% Ethyl	100% meth	Ethyl-meth 50/50	Soxlet extract in Hexane	Diacetate	EO	Terbinafine
T. rubrum (159)								
Ranges	0.5-8	0.5-8	0.25-4	0.5-16	1-32	0.03-1	0.03-1	0.125-4
MFC_{50}	1	0.5	1	4	8	0.25	0.06	2
MFC_{90}	4	4	8	8	16	1	0.5	4
E. floccosum (8)								
Ranges	1-4	1-4	0.25-2	0.5-8	1-16	0.06-1	0.03-0.25	0.125-0.5
MFC_{50}	1	1	1	4	4	0.5	0.06	0.25
MFC_{90}	2	4	2	8	8	1	0.125	0.5
T. interdigitale (11)								
Ranges	0.5-4	0.5-4	0.25-2	1-16	1-16	0.06-1	0.03-0.5	0.25-0.5
MFC_{50}	2	1	1	2	4	0.5	0.25	0.25
MFC_{90}	4	4	2	8	8	1	0.5	0.5
Candida (Yeast) (19)								
Ranges	0.5-16	0.5-8	0.25-2	1-16	1-32	0.125-1	0.03-1	0.5-4
MFC_{50}	2	2	1	4	8	0.25	0.125	1
MFC_{90}	8	8	2	8	16	0.5	1	4
Aspergillus spp. (30)								
Ranges	1-8	0.5-4	0.25-4	1-32	1-32	0.125-1	0.03-1	0.25-2
MFC_{50}	8	2	1	4	8	0.25	0.06	1
MFC_{90}	8	4	4	16	16	0.5	0.5	2

DISCUSSION

In this study, GC-MS analyses revealed the total presence of 24 compounds out of which 7 compounds were compounds present in essential oil of *Cymbopogon citratus*. The remaining compounds were distributed among extracts of methanol (5), Ethyl-acetate (8) and hexane (4) respectively. It is well-known that grass plants produce terpenoidal hydrocarbons and EOs that can be grouped as medicinal, industrial, and perfumery, depending on their chemical composition. Dodecanoic acid, 1,2,3-propane had the highest percentage average height of 6.48% with an area peak of 13.79% at a retention time of 17.543s, while Benzene, propyl- had the least percentage average height 0.99%, with an area peak of 0.09% at 3.522s respectively. This result is similar to the findings of Edwin *et al.* (2012) who reported in their study that a total of nine components, with different retention times, were eluted from the GC column of *Cymbopogon citratus* essential oil. Some of the compounds screened in this study have been confirmed to be present in essential oil of *Cymbopogon citratus* extracts as reported in previous studies. Mahmoud *et al.* (2017) reported the presence of citral (34.8%), neral (30.72%), β-myrcene (11.28%), geraniol (5.54%) 1,3,4-trimethyl -3- cyclohexene-1-carboxaldehyde (2.20%), and citronellol (1.34%). Geranyl acetate (0.57%), Bicyclo [3.1.1] heptane-2- Carboxaldehyde-6,6-dimethyl(0.23%) and D-lemonene as major components of essential oils of *C. citratus* extract using GC-MS. The findings of this study also agrees with the report of Muazzam *et al.* (2022) who in their study confirmed the presence of Octadecanoic acid, Hexadecanoic acid, 2-Cyclohexane-1- carboxaldehyde among other compounds to be present in the essential oil of *C. citratus* extract using GC-MS.

Similarly, in a more recent study, it was reported that the water distillation extract of Lemon grass stems contains various types of essential oil chemical compounds, such as citronellal, citronellol and graniol, where based on the results of the analysis that have been analyzed from the GC results, 13 peaks were detected as chemical compounds of essential oil groups after being analyzed into the form of essential oil grouping based on GC and MS information, 3 peaks of chemical compounds were obtained which had the highest percentage area where the essential oil compounds 2,6-Octadional acid, 3,7-dimethyl-, (z)- citronellal group had the highest peak percentage area of 14. 83% retention

time 7.86 min (Rezki *et al.,* 2023). However, the absence of some of the compounds analyzed as compared to other previous studies may be attributed to differences in species of the plants, regional/topographical variations, part of the plant used and methods of plant extraction among several others.

These findings corroborate with prior studies highlighting the antimicrobial potential of *C. citratus* extract by Boukhatem *et al.,* (2014). Essential oils from *C. citratus* have demonstrated antibacterial and antifungal properties due to their high citral content (Boukhatem *et al.,* 2014). Additionally, diacetate derivatives of citral have exhibited enhanced antimicrobial activity against various pathogens (Boukhatem *et al.,* 2014). The observed variations in antimicrobial efficacy among different extract groups underscored the importance of compound composition and formulation. Essential oil, being a concentrated form, might possess higher antimicrobial activity compared to other fractions due to its higher concentration of active compounds. However, further studies elucidating the specific compounds responsible for antimicrobial activity within *C. citratus* extracts are warranted.

These findings have implications for the development of natural antimicrobial agents, especially considering the rising concerns over antibiotic resistance. Harnessing plant-derived compounds like those from *C. citratus* could offer sustainable and effective alternatives to conventional antimicrobial agents. The study investigated the antifungal potential of various *Cymbopogon citratus* extracts against fungal isolates, including *Trichophyton rubrum, Epidermophyton floccosum, Trichophyton interdigitale, Candida species*, and *Aspergillus* sp. The Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) were determined for each extract, revealing distinct antifungal activities.

For *T. rubrum,* 100% ethyl and 100% methanol extracts displayed potent antifungal effects with lower MIC values (0.06-8 µg/ml). Essential oil (EO) exhibited the lowest MIC (0.03-1 µg/ml), suggesting its superior inhibitory action compared to other extracts and control, aligning with previous studies by Boukhatem *et al* (2014) showcasing the antifungal properties of *C. citratus* essential oil. Diacetate also demonstrated notable antifungal activity against *T. rubrum.* Similarly, against *E. floccosum*, EO showcased the lowest MIC (0.03-0.25 µg/ml), emphasizing its efficacy compared to other extracts, including the control terbinafine. Diacetate exhibited potent activity as well, corroborating previous research by Barbosa *et al* (2010) highlighting the antimicrobial potential of diacetate derivatives.

The antifungal activity against *T. interdigitale* was evident, with EO presenting the lowest MIC (0.03-0.5 µg/ml). Hex-meth 50/50 and 100% ethyl extracts also demonstrated notable inhibitory effects. Terbinafine, a common antifungal medication, exhibited comparable activity against *T. interdigitale,* reinforcing the potential of *C. citratus* extracts as natural alternatives. Against *Candida species*, EO exhibited the lowest MIC (0.03-1 µg/ml), surpassing the activity of other extracts and terbinafine. Notably, hexane extract and diacetate demonstrated potent antifungal effects, suggesting the significance of solvent choice in extracting bioactive compounds.

For *Aspergillus* species, EO again displayed superior antifungal activity with the lowest MIC (0.03-1 µg/ml). Diacetate and other extracts exhibited varying degrees of inhibitory effects against *Aspergillus* sp. showcasing the complexity of *C. citratus* components in addressing different fungal strains. The MFC results reinforced EO's efficacy, as it exhibited the lowest fungicidal concentration against all isolates (0.03-1 µg/ml). Diacetate, while effective, had higher MFC values. The study underscored the variability in fungicidal activity among different extracts, emphasizing the need for comprehensive investigations to harness the full potential of *C. citratus* against diverse fungal pathogens.

Conclusion

Phytocompounds of *C. citratus* extracts were analyzed in this study. These compounds are known to form part of the antifungal agents present in the plant extracts. The antifungal potential of *Cymbopogon citratus* extracts, particularly essential oil, was demonstrated against various fungal isolates responsible for onychomycosis. Essential oil consistently exhibited the lowest MIC and MFC values, indicating potent antifungal properties. The study added valuable information to the growing body of evidence supporting the antimicrobial efficacy of *C. citratus* extracts.

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