

## Antidermatophytic activity and chemical composition of Nigerian *Citrus senensis* (L.) Osbeck essential oil against Multidrug-Resistant Pathogenic dermatophytes Isolated from tinea capitis Samples

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### ABSTRACT

Essential oil (EO) may confer a possible panacea to fight against multidrug-resistant dermatophytes. This study aims at determining the antidermatophytic activity of EO from the fruit peel of Nigerian *Citrus senensis* (L.) Osbeck against multidrug-resistant (MDR) tinea capitis dermatophytes and their possible chemical composition. The EO was reaped by hydro-distillation and the chemical composition was investigated using gas chromatography/ mass spectroscopy (GC-MS). The antidermatophytic activity was evaluated using the agar diffusion method against four dermatophyte strains (Two MDR dermatophytes, one reference strain, and one susceptible). The minimum inhibitory and fungicidal concentrations (MIC/MFC) were determined by agar dilution methods. The test results revealed EO to be active against all strains. The EO subjected to boiling temperature (100 °C) had an increased amount of  $\alpha$ -terpineol,  $\beta$ -Linalool, Carveol, Carvone, and other imaginative compounds such as 1-Octanol (Heptyl carbinols) with increased antidermatophytic activity. Our study suggests that this EO could be used clinically to treat or prevent dermatophytic infections associated with multidrug-resistant strains.

**Keywords:** Multidrug-resistant dermatophytes; *Citrus senensis* (L.) Osbeck; Essential oil; GC-MS; antidermatophytic activity.

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### 1. Introduction

Fungi on both human and animal skin (dermatophytes) are the cause of dermatophytosis, also referred to as tinea in humans and ringworm in the animal. Tinea capitis is the most common dermatophytosis

among children and adolescents [1]. These pathogens are mostly from animal (zoophilic) dermatophytes whose sources of infection are mostly house pets and less often from farm animals [2]. In Nigeria-probably due to immigration and large urban densities as compared to villages. Anthropophilic

dermatophytes are becoming more common and are transmitted directly or indirectly which are isolated in children [3]. Dermatophytes have become resistant to many available conventional antifungals including fluconazole, ketoconazole, griseofulvin, amphotericin B, and terbinafine [4]. Multidrug-resistant (MDR) dermatophytes have become a major challenge in healthcare worldwide, thus the need to search for new natural molecules that may proffer solutions to MDR dermatophyte strains [5].

Essential oils (EOs) are volatile and natural secondary metabolites produced by most aromatic plants [6]. EO is a complex mixture of terpenes (monoterpenes, sesquiterpenes, and their oxygenated derivatives, such as alcohols, aldehydes, esters, ethers, ketones, phenols, and oxides), and also phenolic and phenylpropanoid compounds derived from the acetate-mevalonic acid and shikimic acid pathways, respectively [7]. The presence of volatile compounds makes EO bioactive in the vapor phase along with the liquid phase makes them potential natural antimicrobial agents [8]. The antimicrobial activity of EOs could be achieved at higher concentrations of minimum inhibitory concentration [9].

*Citrus senensis* (orange or sweet orange) can be distinguished from other related species (sour orange, *C. aurantium*, and mandarin orange, *C. reticulata*,) as a small tree in the Rutaceae (citrus family) that originated in southern China, where it has been cultivated for millennia [10]. Oranges are grown mostly for commercial purposes worldwide in tropical, semi-tropical, and some warm temperate regions thus becoming the most widely planted fruit in Nigeria and the world in general. It has been reported to be used traditionally to treat ailments such as constipation, cramps, colic, diarrhea, bronchitis, tuberculosis, cough, ringworm worm, cold, obesity, menstrual disorder, angina, hypertension,

anxiety, depression, and stress [11].

To the best of our knowledge, there have been no reports on the antidermatophytic activity of *Citrus senensis* (L.) Osbeck EO against MDR-tinea capitis dermatophyte strains. Thus, the aims of this study were: (i) to determine the chemical composition of the crude and heated (at 100 °C) EO extracted from *Citrus senensis* (L.) Osbeck growing in Nigeria (ii) to investigate the potential antidermatophytic activity of the EO against MDR-tinea capitis dermatophyte strains and (iii) to investigate the effect of pH and temperature on antidermatophytic activity of the EO.

## 2. Material and Methods

### 2.1. Plant Material

*Citrus senensis* (L.) Osbeck. fruit samples were purchased and collected from Sokoto central market within the Sokoto metropolis in May 2017 Sokoto State, Nigeria. Identification and authentication of the fruits were done in the herbarium of the Department of Pharmacognosy and Ethnopharmacy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Sokoto State, Nigeria.

### 2.2. Essential Oil Extraction

The fresh fruits were peeled and fresh barks (peel) and subjected to hydrodistillation for five (5) hours using a Clevenger-type apparatus. The obtained EO was subsequently dried over anhydrous sodium sulfate (BDH) [12] and collected in amber glass vials and stored at 4 °C until used for analysis.

### 2.3. GC/MS Analysis

The GC-MS analysis of the crude and heated oil were performed using a Perkin Elmer GC-MS (Perkin Elmer Clarus 680 GC-Clarus SQ 8T MS, Waltham, U.S.A) equipped with Elite-5 MS 30 m × 0.25 mm × 25 µm capillary column (5% diphenyl, 95% dimethylpolysiloxane). For GC-MS detection, an electron ionization system with

an ionization energy of 70 eV was used. Ultrapure helium gas was used as a carrier gas at a constant flow rate of 1 mL/min. The ion source, mass transfer line, and injector temperature were set at 250 °C. The oven temperature was 60 °C at a rate of 3 °C/min and then held in isothermal condition for 10 min and finally raised to 250 °C at 10 °C/min. Diluted samples (1/100, v/v in ethanol) of 1 µL were manually injected in the split mode of 120. Mass spectral scan range was at 45–700 m/z, with a solvent delay of 2 min. The components of the extracts were identified based on the comparison of their GC relative retention time and mass spectra with those of NIST MS Search Library Software version 2.0.

#### 2.4. Fungal Strains

The dermatophyte strains were previously isolated from tinea capitis samples and identified [13]. Two of the MDR-dermatophyte strains: *Trichophyton tonsurans*, *Ctenomyces serratus* (resistant to amphotericin B, ketoconazole, fluconazole, griseofulvin, and terbinafine), and *Trichophyton simii* strains (resistant to amphotericin, ketoconazole, and terbinafine) which possibly harbors squalene epoxidase (SQLE) gene encoded for terbinafine resistance. A reference strain *Trichophyton rubrum* derived from ATCC® 28188™\*, SKU: 0444P, and a susceptible dermatophyte strain (*Trichophyton eriotrephon*) were also used for the antidermatophytic tests. These MDR strains were obtained as previously described by Ungokore *et al.*, (2021).

#### 2.5. Antidermatophytic Activity

The agar diffusion method was used to investigate the potential of antidermatophytic activity of the EO against two tinea capitis MDR, one reference, and one susceptibility dermatophytes strains. The fungal strains were freshly subcultured on Sabouraud dextrose agar (SDA) (containing chloramphenicol) and

incubated at 25 °C for five days. The harvested conidial were suspended in normal saline containing 0.05% Tween 80. The inocula were then prepared spectrophotometrically and further diluted in normal saline to obtain a final inoculum concentration of  $1 \times 10^5$  CFU/mL. EO (100 µL) was pipetted into the bored hole on solidified SDA at two other dilutions (1/16, 1/8, and 1/4). Terbinafine (32 µg) and 10% v/v Dimethyl sulfoxide (DMSO) were used as positive and negative control respectively. Plates were incubated for 5-7 days at 30 °C and the diameter of the inhibition zones was measured. Sensitivity was classified as: not sensitive (diameter < 8 mm), sensitive (diameter of 9-14 mm), very sensitive (diameter of 15-19 mm) and extremely sensitive (diameter > 20 mm).

##### 2.5.1 Determination of Minimum inhibitory concentration (MIC) and Minimum fungicidal concentration (MFC)

The MIC and MFC were determined using the agar dilution method as modified by Ungokore *et al.* EO was diluted with 10% of DMSO in sterile distilled water and then serially diluted to get the range of 50, 25, 12.5, 6.25, and 3.125% v/v (1/2, 1/4, 1/8, 1/16 and 1/32). About 10 mL of the graded concentrations of the EO was mixed with 10 mL of double-strength SDA supplemented with 0.05% v/v Tween-80 and poured aseptically into sterile plates. The plates were allowed to set. About 10 µL of the standardized fungal isolates containing  $1 \times 10^5$  CFU/mL were inoculated on the equidistantly placed sterile filter paper disc. The plates were allowed to stand for one hour to allow interaction of the organism and test agent and then incubated at 30 °C for 48 h. The lowest concentration of the EO that inhibited the visible growth of the test organisms was taken as the MIC. The filter paper discs showing no visible growth from the determination of minimum inhibitory concentration were aseptically removed with the

aid of sterile forceps and transferred into a 5 mL sterile Sabouraud dextrose liquid medium containing 0.5% glycerol and incubated at 30 °C for 48 h. Minimum fungicidal concentrations were determined as the lowest concentration resulting in no growth of subculture.

### 2.6 Determination of Effects of varying pH and temperature on the Antifungal Activity of the oil extract

Ten (10.0) mL of the oil was dissolved in 10.0 mL of 10.00% v/v DMSO. This gives a concentration of 50.00% v/v and this was further diluted to obtain 6.25% v/v (1/16 dilution). The setup was maintained at different temperatures (25 °C, 37 °C, 45 °C, 60 °C, and 100 °C) in the water bath for three hours.

To determine the effect of pH, the oil (6.25% v/v) was dispensed in three sets of test tubes, and 1.0 N HCl was added drop-wise until the pH of the oil extract becomes 4.0 and 6.0 (pH was determined using the pH meter) or 1.0 N NaOH until the pH reaches 7.0 and 9.0 and the oil was then allowed to soak for 1.0 h. The antifungal activity of the test oil was determined using zone of inhibition methods and the results were documented after 48 h at 30 °C.

### 2.7. Statistical Analysis

All experiments were performed in triplicate and data were expressed as mean values  $\pm$  standard deviation (SD).

## 3. Results

### 3.1. Yield, phytochemical, and physicochemical Essential Oil

The obtained EO yield was 18.77% (v/w) and the physicochemical and organoleptic characteristics showed to be colorless, acidic, low density, and with a lemon smell. The phytochemical revealed the presence of steroids, triterpenes and glycosides only

### 3.2 Chemical Composition of the Essential oil

As shown in **Table 1 and 2**, the gas chromatography/mass spectrometry (GC/MS) analysis identified 13 (crude EO) and 14 (EO subjected to heat) components, respectively, which accounted for 100% of the total composition of the EOs. For both the EOs, the seven main detected components were D-Limonene (26.64-63.27%),  $\beta$ -linalool (2.82-13.42%),  $\alpha$ -terpineol (2.38-39.36%), cis -Carveol (1.36-4.42%), Carvone (3.62-3.80%), 1, 3-Cyclopentadiene, 1, 3-bis (1-methyl ethyl) (0.94 - 2.17%), Famesene (1.29-1.83%). D-Limonene was found to be predominant in crude EO from (63.27%), while  $\alpha$ -terpineol (39.36%), D-Limonene (26.64%), and  $\beta$ -linalool (13.42%) were the major component in EO subjected to boiling temperature.

### 3.3. Antidermatophytic Activity

The agar diffusion, MIC and MFC results (**Tables 3 and 4**) showed that all the EOs exhibited good antidermatophytic activity against both reference (*Trichophyton rubrum* ATCC 28188) and MDR dermatophyte strains. The MDR *Trichophyton tonsurans*, *Ctenomyces serratus*, and *Trichophyton simii* strains were more susceptible than the standard *Trichophyton rubrum* (ATCC 28188) strain. Oil volatiles has been demonstrated to inhibit the sporulation of fungi. It has been suggested that this inhibition of sporulation, as with cell wall damage, is also associated with alterations to the cell membrane or cell wall damage, leading to increased permeability and subsequent loss of cytoplasmic content (perhaps during synthesis). It has been proposed that this inhibition is due to components either damaging the cell wall or altering the membrane permeability of the microconidia, which results in loss of cytoplasm, which in turn would lead to cell death. Thus, the activity observed in MDR *Trichophyton tonsurans*, *Ctenomyces serratus*, and *Trichophyton simii*

strains which were more susceptible than the standard *Trichophyton rubrum* ATCC 28188 strain) could be attributed to the above-mentioned reasons stated.

When the EO was diluted at 1/4 and 1/8, the inhibition zones of the EO was 20-23 mm, while the positive control terbinafine (30 µg) was more effective, The MIC/MFC against all strains is at 3.125-12.5% v/v (1/32-1/8) dilution.

**Table 1. Kovats retention index, retention time, and percentage chemical composition of the compounds identified in the GC-MS analysis of the oil extracted from Nigerian *Citrus sinensis* (L.) Osbeck fruit peel (CSC)**

RT	M/Z	Constituents	RI	%c
7.024	68	D-Limonene	1018	63.27
7.837	71	1,6-Octadien-3-ol, 3,7-dimethyl- (beta.-Linalool)	1082	2.82
8.966	59	alpha.-Terpineol	1143	2.38
9.067	84	2,3,4,5-Tetrahydropyridazine	967	0.83
9.261	109	2-Cyclohexen-1-ol, 2-methyl-5-(1-methyleth	1206	4.42
9.398	84	Carveol	1206	1.36
9.478	82	Carvone	1190	3.62
10.281	93	1,3-Cyclopentadiene, 1,3-bis(1-methylethyl)-	1012	2.12
10.427	107	1,3-Cyclopentadiene, 1,3-bis(1-methylethyl)-	1012	2.17
10.519	71	1,2-Cyclohexanediol, 1-methyl-4-(1-methyleth	1346	8.91
10.550	93	alpha.-Farnesene	1458	1.29
10.484	93	(2S,4R)-p-Mentha-6,8-diene 2-hydroperoxide	1340	4.13
11.013	93	Cyclohexanemethanol, 4-methylene-	1090	2.26
11.558	121	(p-Hydroxyphenyl)glyoxal	1438	0.42

Key: RT: Retention time (minute); RI: Kovats Retention Indices, %c: Percentage composition of a compound  
CSC = *Citrus sinensis* crude oil.

**Table 2. Kovats retention index, retention time, and percentage chemical composition of the compounds identified in the GC-MS analysis of the oil extracted from Nigerian *Citrus sinensis* (L.) Osbeck fruit peel (CSB)**

RT	M/Z	Constituents	RI	%c
7.024	68	D-Limonene	1018	26.64
7.434	56	1-Heptene, 6-methyl-	742	1.16
7.839	78	1,6-Octadien-3-ol, 3,7-dimethyl- (beta.-Linalool)	1082	13.42
8.271	67	Cyclopentanecarboxylic acid, 2-methyl-3-vinyl	1688	1.64
8.967	59	alpha.-Terpineol	1143	39.36
9.262	109	cis-Carveol	1206	3.91
9.390	69	1,6-Heptadiene, 3-methyl-	733	1.09
9.479	82	(-)-Carvone	1190	3.80
9.883	56	Heptyl carbinol	1059	1.41
10.283	107	1,5,6,7-Tetrahydro-4-indolone	1320	1.09
10.429	107	1,3-Cyclopentadiene, 1,3-bis(1-methylethyl)-	1012	0.94
10.550	69	Trifluoroacetyl-lavandulol	1104	0.87
10.849	93	(2S,4R)-p-Mentha-[1(7),8]-diene 2-hydroperoxide	1335	2.83
11.015	93	1,3,6,10-Dodecatetraene, 3,7,11-trimethyl-,	1458	1.83

Key: RT: Retention time (minute); RI: Kovats Retention Indices, %c: Percentage composition of a compound  
 CSB = *Citrus sinensis* crude boiled oil at 100 °C

**Table 3. Antidermatophytic activity of the EO from Nigerian *Citrus sinensis* (L.) Osbeck expressed as mean diameter inhibition zones (mm)**

MDR strain	EO/ (1/16) dilutions			
	CSC	CSB	CSCPH	TBF (30µg/mL)
<i>T. tonsurans</i>	18.67 ± 0.33	22.67 ± 0.33	12.67 ± 0.33	30.67 ± 0.33
<i>T. simii</i>	20.67 ± 0.33	21.67 ± 0.33	16.67 ± 0.33	32.67 ± 0.33
<i>T. rubrum</i> (ATCC 28188)	14.00 ± 0.00	18.67 ± 0.33	10.67 ± 0.33	28.00 ± 0.00
<i>Ctenomyces serratus</i>	19.67 ± 0.33	21.67 ± 0.33	15.67 ± 0.33	30.67 ± 0.33

Mean inhibition zone (mm) ± S.D of three replicates, TBF =Terbinafine, CSC = *Citrus sinensis* EO (Untreated at pH 4.3), CSB = *Citrus sinensis* crude boiled oil, CSCPH = *Citrus sinensis* EO (treated at pH 6), DMSO = Negative control

**Table 4. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of *Citrus sinensis* EO**

MDR strain	MIC (MFC)		
	CSC	TBF ( $\mu\text{g/mL}$ )	DMSO (10% v/v)
<i>T. tonsurans</i>	12.5 (12.5)	> 8	-
<i>T. simii</i>	3.125 (6.25)	4(8)	-
<i>T. rubrum</i> (ATCC 20288)	6.25 (6.25)	0.5(1)	-
<i>Ctenomyces serratus</i>	6.25 (12.5)	4(8)	-

Mean inhibition zone (mm)  $\pm$  S.D of three replicates, CSC = *Citrus sinensis* EO (Untreated at pH 4.3), TBF = Terbinafine, DMSO = Negative control

### 3.4 Effect of varying pH and temperature

The effect of varying pH on the antifungal susceptibility testing of *Citrus senensis* EO against dermatophytes resistant strains showed that the untreated EO at pH of 4.3 (slightly acidic) had diameter zones of inhibition ranging from 11-20 mm. There was a remarkable decrease in diameter zones when the pH of the EO was increased to 6 and no activity was recorded in the neutral and basic medium of the oil (7 and 9). The effect of varying temperature showed that the untreated EO at 6.25% v/v (1/16) dilution showed diameter zones of inhibition ranging from 11-20 mm across all the strains. The effect of temperature is observed at 60 °C to 100 °C where the diameter zones of inhibition had increased to a range of 18-22 mm. This clearly shows that there is an increase in the diameter zones of inhibition as the temperature is increased from 100 °C. There was no remarkable antidermatophytic activity that was observed at 25 °C to 60 °C.

## 4. Discussion

The determination of biologically active compounds from plant material is dependent on the type of solvent and method used in their

extraction procedure [16]. According to Ferhat *et al.*, in a study [17], the percentage yield of the oil from *Citrus senensis* fruit peel using water as solvent was reported to be 0.42%. This is much lower than the percentage yield of 18.77% recorded in this study. In another study by Bustamante *et al.*, [18] recorded a yield of 2.3% which is also lower than the result obtained from this study. In a study by Ezejiofor *et al.*, [19] in Nigeria, 0.6% yield using the distillation method of extraction was reported which is also very lower than the percentage yield of 18.77%. Hence, this could be due to the difference in the source and processing of the fruit peel. Fresh peels generally contain much oil because of the level of moisture content. Geographical locations are also known to impact yield. The yield and biological activity of extracts have shown to be highly dependent on solvent polarity [20].

The phytochemical characteristics of the oils varied in the constituents; this might not be far-fetched from the solubility in the solvents used for extraction. These phytochemicals are secondary metabolites produced by plants to combat microorganisms in their habitat [21]. The EO contains steroids, terpenes, and glycosides which agree with a previous study of Ajayi-

Moses *et al*, [22]. Phytochemicals are secondary metabolites produced by plants that fight with microorganisms in their environment [23, 24], and terpenes among others are known to be produced by plants in response to microbial infection [25]. According to Irshad *et al*, [26], terpenes' antifungal activity may be due to their ability to disrupt the cell wall membrane via ergosterol biosynthesis. *Citrus senensis* fruit peels oil has a high quantity of terpenes which in turn disrupts ergosterol biosynthesis in dermatophytes cell wall membrane.

The quality of EO is expressed in terms of Physico-chemical properties via saponification value, iodine value, peroxide value, acid value, free fatty acid, specific gravity, and kinematic viscosity [27]. The saponification value of the EO is 137.445 mg of KOH/g of the oil, though it varies among EOs. This is less than the value of 180 mg of KOH/g of *Citrus senensis* oil reported by Anwar *et al*, [28]. Triglycerides with high values of saponification are considered to make better quality soaps than those with low saponification values [29]. This implies that EO from *Citrus senensis* fruit peel using water as solvent might not be suitable for soap making because of its low triglyceride level. Generally, saponification values change depending on the origin of the lipid and what the weather conditions were for that particular plant material, and the processing techniques used [30]. Iodine numbers are often used to determine the amount of unsaturation in fatty acids. The iodine value obtained in this study (5.076) indicates that the oil is relatively saturated and thus does not possess the property of absorbing oxygen on exposure to the atmosphere with time to undergo oxidative rancidity. The acid values found in *Citrus senensis* fruit peel EO are within acceptable values for most essential oils 6.171. The value is less than the value reported by Ezejiofor *et al*, [19] of 12.34. The high acid value of the extracted essential oil simply

indicates that the oil does not have good storage life. The peroxide value obtained in this study (0.56) is far below the maximum acceptable value of 10 mEq peroxide/kg and signifies that the oil will be more stable, and if stored properly, the shelf life will be extended. This value is very low compared with the peroxide value of the same oil 16 mEq O<sub>2</sub>/kg as reported by Fakayode and Abobi, [32]. These values are far more than the values obtained in this study and this might be due to geographical variation.

*Citrus senensis* has been of interest for the extraction of essential oil by many researchers; however, the fruit peels have been less studied. GC/MS analysis of *Citrus senensis* (L.) Osbeck carried by Cholke *et al*, [33] reported 15 compounds while Monsef-Esfahani *et al*, [34] reported the presence of 38 compounds. In the present research, the GC-MS chromatogram of *Citrus senensis* fruit peel oil displayed 15 peaks indicating the presence of fifteen (15) compounds which is in tandem with a work done by Cholke *et al*, [33]. GC-MS analysis discovered that the presence of 15 compounds were mainly D-Limonene, 1,6-Octadien-3-ol, 3,7-dimethyl-( $\beta$  Linalol),  $\alpha$ -Terpineol, 2,3,4,5-Tetrahydropyridazine, cis-Carveol, Carveol, Carvone, 1,3-Cyclopentadiene, 1,3-bis(1-methyl ethyl)-, 1,3-Cyclopentadiene, 1,3-bis(1-methyl ethyl)-, 1,2-Cyclohexanediol, 1-methyl-4-(1-methyl phenyl)-,  $\alpha$ -Farnesene, (2S,4R)-p-Mentha-6,8-diene 2-hydroperoxide, Cyclohexanemethanol, 4-methylene-, (p-Hydroxyphenyl)glyoxal. The GC-MS analyses discovered that *Citrus senensis* fruit peel oil is mainly composed of terpene hydrocarbons, oxygenated compounds, and non-volatile compounds [35]. The terpene fraction in the EO is 79.16% which agrees with Choke *et al*, [33] that terpene can constitute from 50 to more than 95% of the EO. The EO contains 63.26% D-limonene which is close to 65% as reported by Cholke *et al*, [33]. But Dugo *et al*, [36] reported



70% D-limonene which is close to the value obtained from this study, hence D-limonene might be responsible for the antidermatophytic activity of this EO. GC-MS chromatogram of *Citrus sinensis* fruit peel EO subjected to 100 °C boiling point temperature displayed also 15 peaks indicating the presence of fifteen (15) compounds, D-Limonene, 1-Heptene, 6-methyl-, 1,6-Octadien-3-ol, 3,7-dimethyl-(Beta.-Linalool), 4'-fluorophenyl ester, alpha.-Terpineol, cis-Carveol, 1,6-Heptadiene, 3-methyl- (-)-Carvone, 1-Octanol (Heptyl carbinol), 1,5,6,7-Tetrahydro-4-indolone, (2S,4R)-p-Mentha-[1(7),8]-diene 2-hydroperoxide, alpha.-Farnesene, 1,3-Cyclopentadiene, 1,3-bis(1-methyl ethyl)-, Trifluoroacetyl-lavandulol. The boiled EO contains 26.64% D-limonene, 13.42%  $\beta$ -linalool, and 39.36%  $\alpha$ -terpineol. The main constituent in this sample is  $\alpha$ -terpineol, not D-limonene. There was an increase in antidermatophytic activity when this oil was subjected to 100 °C boiling temperature thus  $\alpha$ -terpineol and linalool might be responsible for such increase in activity, this is in agreement with a study by Monsef-Esfahani *et al*, [34] who reported 20% composition of  $\alpha$ -terpineol in *Citrus sinensis* fruit and Guo - Xing Jing [37] reported that terpineol possesses antifungal activity.

The EO of *Citrus sinensis* fruit peel was effective in inhibiting the growth of the clinical and standard dermatophytes. A lot of work has been done on the medicinal use of various parts of *Citrus sinensis*. Some parts of *Citrus sinensis* have been reported to possess antifungal activity [38]. Diánez *et al*, [39] reported antifungal activity of *Citrus sinensis* against eight pathogenic fungi. Jain and Sharma, [40] also reported EO from *Citrus sinensis* to be effective against certain human fungi, including *Trichophyton*, *Microsporum*, and *Candida*. The finding agrees with the result from this present

study as extracted EO from the fruit peel of *Citrus sinensis* possesses the ability to inhibit some human pathogenic fungi growth. The lipophilic nature of EOs allow them to pass through the cell wall and cytoplasmic membrane damage while disrupting various layers of polysaccharide, fatty acids, and phospholipids eventually making them permeable [41-43]. Antidermatophytic activity of the oil is increased by subjecting the EO to boiling than the untreated oil against all the dermatophytes as the diameter zones of inhibition were greatly increased when the temperature was raised from 45 °C to 100 °C. Rath and Alena, [44] reported an increase in the activity of EO with an increase in temperature. The increase in activity might be attributed to an increase in the concentration of  $\alpha$ -terpineol and  $\beta$ -Linalool in the boiled EO as this is in tandem with a work done by Kong *et al*, [45] who reported  $\alpha$ -terpineol as a potential antifungal compound [46] reported linalool as a potential and major compound of citrus having antifungal activity. This can be deduced that  $\alpha$ -terpineol and  $\beta$ -linalool have better antidermatophytic activity compared to D-Limonene and the presence of these compounds makes EO bioactive in the vapor phase along with the liquid phase thus making them potential natural antimicrobial agents

The oil of *Citrus sinensis* proved to have a higher effect against dermatophytes in acidic pH as compared to basic pH. As the pH increases from acidic towards basic, the activity decreases as seen with pH 4.5 to 6.0. No activity was seen at pH 7.0 and 9.0. This might be due to the hydrophobic components present in essential oil which could change the permeability of the fungi cell membrane for cations such as  $H^+$ , which change the flow of protons, modifying cellular pH and affecting the chemical composition of the cells and their activity [47, 48].

## Conclusion

In summary, D-limonene,  $\alpha$ -terpineol,  $\beta$ -linalool, Carveol, Carvone, and  $\alpha$ -Farnesene were the main components found in the EO, with a predomination of  $\alpha$ -terpineol,  $\beta$ -linalool, Carveol, Carvone 1-Octanol (Heptyl carbinols) in EO when subjected to heat. All EOs (*Citrus sinensis* EO Untreated at pH 4.3, *Citrus sinensis* crude boiled oil, and *Citrus sinensis* EO treated at pH 6) were active against all *dermatophytes* MDR strains at acidic pH of 4.3. The EO when subjected to heat (100 °C) contained the highest amount of  $\alpha$ -terpineol (39.36%), and showed higher antidermatophytic activity (diameters from 18.67±0.33 to 22.67±0.33 mm at dilution of 1/16). Thus, it is possible to formulate an herbal ointment or cream for its redeployment in the potential use as a natural antidermatophytic agent in treating or preventing dermatophytic infections (e.g. tinea capitis infections) with MDR strains.

## Declarations

### Ethics approval and consent to participate

Not applicable

### Consent to publish

Not applicable

### Availability of data and materials

All data generated or analyzed during this study are included in this published article in the main manuscript.

### Competing interests

No competing interests were declared by the authors.

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