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Antimicrobial activities and chemical composition of the essential oils from dry peels of *C. aurantium*

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Abstract
This study was aimed to look at the *in vitro* antimicrobial activities and chemical components of the volatile oil obtained from dry peels of *Citrus aurantium* (bitter orange). The essential oils of this plant were obtained by steam distillation technique and chemical compositions were analyzed by exploring chromatography – mass spectrometry (GC-MS) method. The susceptible effects of this oil were tested against *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Candida albican* (*C. albican*) by using agar disc diffusion and dilution broth techniques. The results obtained were able to reflect its antimicrobial activities against *P. aeruginosa* and *C. albican* with zone inhibition diameter of 13 mm and 22 mm when compared with standard antibiotic. In contrast, the oil exhibited wide range of antimicrobial activity against *P. aeruginosa* with minimum inhibitory concentration (MIC) and maximum bacteriocidal concentration (MBC) of 250 µg/mL when compare to *C. albican* with minimum inhibitory concentration of 500 µg/mL and maximum fungicidal concentration (MFC) greater than 500 µg/mL. The GC–MS analysis discovered the presence of D-Limonene with 38.13% that had highest share space follow by (-)-β-Fenchol with 6.83%. These might accounted for the antimicrobial properties of the essential oils. The encouraging results indicate the essential oils of *C. aurantium* may well be exploited as natural antibiotic for the management of many infectious disease elicited by these microorganisms.

Keywords: antimicrobial, activities, chemical, composition, essential

1. Introduction
Plant products like essential oils are important and they have been exploited for their aromatic, flavor, bactericidal, preservative, and healthful properties (Stohs *et al*., 2011). Bitter orange tree is an ever green of 2–2.5 m high, having white perfumed flowers and orange fruits, cultivated in tropical and semitropical zones. Immature peels are used to treat upset stomach and different enteric diseases (Kim *et al*., 2008) [8]. Their peels and flowers are frequently used in food as perfumes. They are also known to be rich in flavonoids, coumarins, triterpenes and vitamins (Haggag *et al*., 1999). Essential oils have been employed in controlling bacterial and fungal infections though not clinically regulated due to lack of awareness and data to support the reported therapeutic claims (Rehman *et al*., 2011) [10].

More than 90% of the citrus essential oil constitutes D-limonene which is an important terpene that has specific aromatic smell of citrus. Hosni *et al*. (2010) [7] did research on the functions of D-limonene and found that it:

1. Aids digestion and system detoxification
2. Eases constipation
3. Relieves water retention
4. Promotes circulation
5. Increases absorption of vitamin C
6. Supports immunity to fight colds and flu
7. Strengthens and rejuvenates skin

Choi *et al*. (2000) [4] also reported Limonene and β-myrcene as other major components of the peel oil while linalool, linalyl acetate and α-terpineol predominate in the leaf oil. The aim of this study is to examine the antimicrobial activities, chemical Composition of the essential oils from dry peels of *C. aurantium*.

2. Materials and Methods
2.1. Plant material and authentication
Fresh and fully matured fruit of the bitter orange was collected from *Oja Ipata* (Ipata Market) in Ilorin, Kwara State, Nigeria. The fruit was identified at the Herbarium Unit of the Department of Plant Biology, University of Ilorin, Kwara State, Nigeria with voucher number UILH/005/995. The peel of the plant was obtained, air dried at room temperature and pulverized into flour.

2.2 Distillation
The dry peel flour sample was subjected to steam distillation method with minimum water until the essential oil becomes non-existent in the condensate. The oil is then dried with anhydrous sodium sulphate and stored in an airtight container at 4°C.

2.3 GC-MS Analysis
The acquired essential oil was analyzed using GC-MS instrument with the standard protocol. The column conditions are as follows:

1. Rxi®-1 ms restech, 30 x 0.25mm ID, 0.25µm
2. 60°C column temperature
3. 250°C injection temperature
4. 300°C interface temperature

Helium was used as the carrier gas and dimethylpolysioxane was used as the stationary phase. 1mL of essential oil was diluted up to 2mL with dichloromethane for 50 minutes.

2.4 Antimicrobial screening

20 mL of prepared molten *Mueller Hinton's* agar and potato dextrose agar were poured into petri dishes and allowed to set. About 0.5 mL of standardized inoculums was drop onto the solidified agar surface and rocked so as to spread over the surface. Cork borer of 6 mm diameter was used to bore holes into the agar and 10µL of prepared concentrations of extracts were poured into each hole and labeled. Plates were left to diffuse for 30 mins and incubated at 37ºC for 18 hrs for the bacteria and 28ºC for 72hrs for the fungi. The zones of inhibitions were measured in millimeter (mm) using a transparent ruler. This was repeated in triplicates for each organism (Bamidele *et al*., 2013) [2].

Results

Table 1: Susceptibility of *Pseudomonas aeruginosa* to standard antibiotics

Standard Antibiotics	Zone of Inhibition (mm) *Pseudomonas aeruginosa*
Ofloxacin	24.00±0.83 S
Ciprofloxacin	26.00±0.83 S
Gentamicin	26.00±0.83 S
Ceftriaxone	22.00±0.82 I
Ceftazidime	20.00±1.61 I
Ampicillin	0.00±0.00 R
Nitrofurantoin	16.00±1.41 I
Amoxycillin/Clavulanate	0.00±0.00 R

Data represent the mean± standard deviation of triplicate readings values, Key: S-Sensitive, I-Intermediate, R-Resistance

Table 2: Susceptibility of *Candida albican* to standard antibiotics

Standard Antibiotics	Zone of Inhibition (mm) *Candida albican*
Ofloxacin	20.00±1.41 S
Ciprofloxacin	24.00±1.61 S
Gentamicin	26.00±1.41 S
Ceftriaxone	28.00±1.41 S
Ceftazidime	18.00±0.82 I
Ampicillin	0.00±0.00 R
Nitrofurantoin	27.00±1.41 S
Amoxycillin/Clavulanate	20.00±1.41 R

Data represent the mean± standard deviation of triplicate readings values, Key: S-Sensitive, I-Intermediate, R-Resistance

Table 3: Antimicrobial Activities of Different Essential Oils (Stock Concentration)

Test Organisms	Zone of Inhibitions (mm)
	C. aurantium
C. albican	22±1.83
P. aeuruginosa	13±1.41

Data represent the mean± standard deviation of triplicate readings values

Table 4: Minimum inhibitory and bactericidal concentrations of essential oils against *Pseudomonas aeruginosa*

Essential oils	MIC (µg/ml)	MBC (µg/ml)
Citrus aurantium	250	250

Table 5: Minimum inhibitory and fungicidal concentrations of essential oils against *Candida albican*

Essential Oils	MIC (µl/ml)	MFC (µl/ml)
Citrus aurantium	500	>500

Table 6: Chemical composition of essential oil from dry peel of *Citrus aurantium*. The main compounds were D-limonene (38.13%), (-)-_β-fenchol (6.83%), dodecane (5.31%), 4-carvomenthenol (4.21%), -terpinene (3.62%), cis-4-thujanol (3.49%), linalool (2.94%), 6,7-dihydrogeraniol (2.15%), and undecane (2.13%)

Compound	Area (%)	Molecular Formula	Molecular Weight (g/mol)
α-Pinene	1.50	$C_{10}H_{16}$	136
Decane	0.65	$C_{10}H_{22}$	142
Myrcene	0.96	$C_{10}H_{16}$	136
β-Pinene	1.54	$C_{10}H_{16}$	136
D-Limonene	38.13	$C_{10}H_{16}$	136
p-Cymene	0.72	$C_{10}H_{14}$	134
Undecane	2.13	$C_{11}H_{24}$	156
ϒ-Terpinene	3.62	$C_{10}H_{16}$	136
4-Terpineol	0.95	$C_{10}H_{18}O$	154
1-Dodecanol	0.54	$C_{12}H_{26}O$	186
1-Eicosanol	1.69	$C_{20}H_{42}O$	298
Linalool	2.94	$C_{10}H_{18}O$	154
cis-4-Thujanol	3.49	$C_{10}H_{18}O$	154
Dodecane	5.31	$C_{12}H_{26}$	170
7-Methyl pentadecane	1.12	$C_{16}H_{34}$	226
4-Carvomenthenol	4.21	$C_{10}H_{18}O$	154
Capraldehyde	0.93	$C_{10}H_{20}O$	156
(-)-β-Fenchol	6.83	$C_{10}H_{18}O$	154
6,7-Dihydrogeraniol	2.15	$C_{10}H_{20}O$	156
β-Citrylideneethanol	0.45	$C_{12}H20O$	180
trans-Carveol	0.83	$C_{10}H_{16}O$	152
Z)-Citral	1.42	$C_{10}H_{16}O$	152

Discussion

The antimicrobial activity of bitter orange peel may be due to the presence of D-limonene and (-)-β-Fenchol in the essential oil. It was reported from the present study that essential oil from the peel of *Citrus aurantium* possess antibacterial activity against *P. aureginosa* and antifungal activity against *C. albican* with inhibition zone diameter 13±1.41 mm and 22±1.83 mm. This result was in agreement with Ehigbai *et al*., (2015) reported that the bitter orange has a higher antifungal activity compared with citrus juice concentrates from grape but lesser when compared with lemon juice base on their respective zones of inhibition values. The antifungal activities of citrus juice concentrates revealed that *C. albican* was most susceptible to the juice concentrates with observed zones of inhibition ranging from 8 mm for grape juice to 24 mm for lemon juice.

Further results obtained in Table 5 showed that the minimum inhibitory concentration (MIC) value for the *C. albican* was lower than their maximum bactericidal concentration (MFC) values. Thus, suggesting that the concentrates were bacteriostatic at lower concentrations but bactericidal at higher concentrations. The *Citrus aurantium* essential oil was more effective in inhibiting the growth of *P. aeruginosa* compared to *C. albican* in Table 4. The present study when compared with Ehigbai *et al*. (2015) showed that tangerine and lemon juice concentrates significantly inhibited the growth of *P. aeruginosa* at MIC 12.5µg/mL and 25µg/mL which was significantly effective. The essential oil from *C. aurantium* may therefore hold promise in the management of *P. aeruginosa* infections.

The inhibitory and fungicidal concentrations of the *Citrus aurantium* peel oil concentrates against *Candida albican* were found to have a higher MBC than the MIC. It was less susceptible when compared to tangerine, grape, and lemon juices (Ehigbai *et al*. 2015). The results of this study showed that bitter orange was effective as an antibacterial against *P. aeruginosa* compared to an antifungal agent. This is evidenced by the larger zones of inhibition and the same MIC and MBC values obtained for concentrate.

The GC-MS analysis of the essential oil showed that the major components are (-)-β-Fenchol, Dodecane and D-limonene compounds. The present study was in agreement with the chemical components of *Citrus medica* essential oil with limonene and citral as the major constituents.

Karoui and Marzouk, (2013) [6] investigated the aroma compounds in peel and juice of bitter orange by GC-MS. Twenty-seven components were identified, amounting to 99.48% of the total oil. Limonene was determined to be the major volatile compounds of fruit peel (90.25%) and fruit juice (91.61%) which significantly differ in terms of percentage (%) when compared with the present study. Tounsi *et al*. (2011) [9] reported that most studies have emphasized the amount of limonene is high in citrus essential oils. Bourgou *et al*. (2012) [11] reported the D-limonene has been the predominant component of grape and other citrus peel oils. The major component of the oil was D-limonene and probably elicits the antibacterial and antifungal property of the oils. D-limonene has been reported to inhibit *E. coli*, *S. Aureus*, *Salmonella* sp and *Pseudomonas* sp. It has also been shown to inhibit some yeast species; *Sachharomyces elipsoideus*, *Wilia hansenula* and *Oidium lactis* (Unal *et al*., 2012) [13].

Conclusion

This study has shown that the oil extract of lime peels from Federal Republic of Nigeria contains some helpful potential antibiotic principles that are repressing to clinical isolates. Thus, it should be thought of as a natural supply of antimicrobials for therapeutic functions.

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