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RESEARCH PAPER

ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF VOLATILE OILS FROM FOUR CITRUS SPECIES ON SELECTED ORAL PATHOGENS AND GC-MS IDENTIFICATION OF ASSOCIATED COMPOUNDS

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ABSTRACT

This study evaluated the in-vitro antioxidant and antimicrobial activities of peel volatile oils of Citrus aurantifolia, Citrus limon, Citrus paradisi and Citrus sinensis on clinical isolates of Methicillin-resistant Staphylococcus aureus, Methicillin-sensitive Staphylococcus aureus, Ciprofloxacin resistant Escherichia coli, Ciprofloxacin sensitive Escherichia coli and Candida albicans obtained from the oral cavity. Gas chromatography-Mass spectrometric (GC-MS) analysis of the oils was also carried out. The agar well diffusion method was used to determine the antimicrobial activity of the oils while the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method was used to evaluate the antioxidant activity. Total flavonoid and phenol contents of oils were equally determined. The composition of the oils was determined by GC-MS analysis. The volatile oil of C. aurantifolia exhibited the most activity against the test pathogens with a minimum inhibitory concentration (MIC) range of 2 – 4 mg/ml, while C. limon had the least antimicrobial activity as its MIC could not be determined against Methicillin-resistant Staphylococcus aureus, Ciprofloxacin resistant and sensitive Escherichia coli. C. limon and C. paradisi had MICs in the range of 4-16 mg/ml and 4-32 mg/ml respectively.C. sinensis peel oil had the highest DPPH radical scavenging activity of 47.86±2.22% and C. aurantifolia the least with a value of 25.27±1.23%. Methylisobutyl ketone was identified in C. paradisi, C. aurantifolia and C.sinensis. This study revealed that the peel volatile oils of the investigated citrus species possess antioxidant and antimicrobial activities against oral pathogens and have the potential to be used in the management of oral diseases caused by the selected pathogens.

Keywords: Oral pathogens, Antimicrobial, Antioxidant, Citrus peel, Volatile oils

INTRODUCTION

The mouth, also known as the buccal cavity, is the opening through which food is taken into the alimentary canal and vocal sounds are made. It also serves as an important means of psychosocial interaction (Aredt, *et al*., 2001; Le-Blanc, *et al*., 2013). The environment of the human mouth allows the growth of characteristic bacteria and other microorganisms that lead to the formation of dental plaque and periodontal diseases such as gingivitis, caries and eventual tooth decay and loss (Firas, *et al*., 2008: Avila, *et al*., 2009). Tooth decay and gum diseases are the most frequently reported chronic infective dental diseases, caused by microorganisms living in the oral cavity of public health importance (Nimako-Boateng *et al*., 2016). Antibiotics are prescribed to combat infective conditions of the mouth. Inappropriate antibiotic prescribing and use in oral diseases are a leading cause of antimicrobial resistance in pathogens such as *Staphylococcus aureus* and *Escherichia coli* that infect the oral cavity (Louise *et al.,* 2004). Antimicrobial resistance is a primary cause of treatment failure, increased burden of disease and expenditure on health (Micheal *et al*., 2014).

An increase in the production and level of reactive oxygen species in bacteria and fungi infections of the mouth have been reported (Ivanov *et al*., 2017); such that it has been suggested that interactions that reduce the ability of bacteria and other microorganisms to generate reactive species could be an effective therapeutic strategy in combating microbial infections (Novaes, *et al*., 2019).

Citrus volatile oils (VOs) are notable for their fragrance and have found application in aromatherapy and the cosmetic industry (Pallazollo *et al*, 2013; Ali *et al*., 2015). They have also been used as natural preservatives (Pandey *et al*., 2017) and flavouring agents (Mustafa, 2015). In the continued search for natural products with potential activity against oral pathogens, this study aimed at evaluating the antimicrobial activities of the VOs of *Citrus sinensis, Citrus aurantifolia, Citrus limon,* and *Citrus paradisi* against clinical isolates of Methicillin-sensitive and resistant *Staphylococcus aureus* (MSSA and MRSA), Ciprofloxacin-sensitive and resistant *Escherichia coli* (CSEC and CREC) and the fungi; *Candida albicans*. The *in-vitro* antioxidant activity and chemical compositions of the oils were also determined. This study seeks to answer the specific questions of; do volatile oils from the peel of the studied citrus fruits have antimicrobial activity against test organisms. Do the volatile oils under investigation have antioxidant properties? What could be the composition of the volatile oils?

MATERIALS AND METHODS

Solvents and other chemicals

All solvents were obtained from Pharmatrend® Chemicals, Edo State Nigeria, while growth media (Nutrient agar, Sabouraud dextrose agar and Muller Hinton broth) were products of Sigma Aldrich, Germany.

Collection and identification of the plant samples

The citrus fruits were bought from New Benin Market, in Benin City, Edo State Nigeria. They were identified and authenticated by Dr Henry A. Akinnibosun, of the Department of Plant Biology and Biotechnology (PBB), University of Benin, Edo State, Nigeria. The following voucher numbers; *C. sinensis* (UBH_M 565), *C. aurantifolia* (UBH_M566), *C. limon* (UBH_M567) and *C. paradisi* (UBH_M569) were issued for the different fruits.

Preparation and extraction of the volatile oils

The fruits were washed under running water, dried with a napkin and peeled. The peels were chopped into smaller pieces, extracted by steam distillation using a Clevenger apparatus for 2 h. The oils obtained were individually dried over anhydrous sodium sulfate and stored in amber-coloured bottles at 4°C till ready for use. The percentage yield was calculated using the formula below;

$$\text{Percentage yield of oil} = \frac{\text{Volume of oil}}{weight\ of\ \boldsymbol{fruit}\ peel} \times \frac{100}{}$$

Preparation of microorganisms

Cultures of MSSA, MRSA, CSEC, CREC and *C. albicans* were prepared from isolates obtained from the Dental Clinic, University of Benin Teaching Hospital and held in the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Benin. Fresh cultures of representative organisms were prepared by sub-culturing bacteria into freshly prepared nutrient agar and fungi, into freshly prepared Sabouraud dextrose agar. Agar plates were incubated at 37ºC for 24 h and, 28ºC for 48 h for bacteria and fungi cultures respectively. Organisms were transferred into the freshly prepared nutrient broth and the turbidity of the freshly prepared pure isolates was adjusted to 0.5 McFarland turbidity standard and further diluted in 1:100 serial dilution with normal saline to give approximately 1×10^7 CFU/mL microbial suspension.

Antibacterial assay

The agar well diffusion method according to Murray, *et al*., (2009) was used to evaluate the antibacterial activity of the oils. Wells of 7 mm diameter were made into solidified Agar media that had been inoculated with the test organisms, with a flamed cork borer. Oils in a concentration of 5, 10, 20 and 40 mg/ml in 20 µl volume were delivered into the wells. Ciprofloxacin (10 µg/ml) and Itraconazole (10 µg/ml) served as the positive control for the antibacterial and antifungal testing, respectively. Oils were solubilized in 20% DMSO, which also served as the negative control. The plates were incubated for 24 h at 37ºC and 48 h at 28°C for bacteria and fungi, respectively. Zones of inhibition were measured in mm with the aid of a Vernier caliper and, tests were carried out in triplicates.

Determination of minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of oils was determined according to the Agar dilution method described by Andrews, (2001). Serial dilutions of test oils were prepared to obtain concentrations in the range of 2 – 64 mg/ml, which were added to molten nutrient agar for bacteria and Sabouraud dextrose agar for fungi. Overnight culture of microorganisms in Muller Hinton broth agar, adjusted to 0.5 McFarland to contain $1\text{x}10^7$ CFU/mL of microbial suspension was applied on the surface of agar plates and evenly distributed with a spreader. Plates were incubated at 37°C for 24 h and 28°C for 48 h for bacterial and fungal plates, respectively. The lowest concentration of oils at which there was no growth was taken as the minimum inhibitory concentration.

In-vitro antioxidant assay

1,1-Diphenyl-2-picryl-hydrazyl (DPPH) antioxidant assay

The free radical scavenging effect of the citrus peel VOs on DPPH radical was estimated according to the method described by Jain *et al*., (2008). Briefly, 2 ml of appropriate concentrations of oils in 1 ml of methanol containing 0.5 mM of DPPH was vortexed thoroughly, left in the dark at ambient temperature for 30 min and the absorbance was measured at 517 nm. Ascorbic acid

served as the positive reference and all determinations were in triplicates.

The DPPH free radical scavenging ability was subsequently calculated for the negative reference (which contained all the reagents without the test sample) using the formula below:

$$\text{Per cent (\%) Inhibition of DPPH activity} = \frac{A0 - A1}{A0} \times \frac{100}{1}$$

Where A_O = Absorbance of DPPH radical + methanol (blank)

A_1 = Absorbance of DPPH radical + essential oils or standard

Determination of total flavonoid content

The total flavonoid content (TFC) of the VOs was determined according to the method previously described by Ebrahimzadeh, *et al.,* (2008). The oil solutions (0.5 ml) at a concentration of 1 mg/ml were mixed with 1.5 ml of methanol, 0.1 ml of aluminium chloride solution, 0.1 ml of 1M potassium acetate solution, and 2.8 ml of distilled water. The mixture was incubated at room temperature for 30 mins and the absorbance was measured at 415 nm. A standard curve with Quercetin in concentrations of 12.5, 25, 50, 75, 100 and 200 μg/ml was equally obtained. The result is expressed as milligram quercetin equivalent per gram of extract (mgQE/g extract). The assay was carried out in triplicate.

Determination of total phenol content

The total phenol content of different VOs under investigation was determined by the method described by Kim *et al.,* (2003). Appropriate concentrations of the oils were oxidized with 2.5 ml of 10% Folin-Ciocalteau's reagent (v/v) and neutralized with 2.0 ml of 7.5% sodium carbonate. The reaction mixture was incubated for 90 min at room temperature and the absorbance was measured at 750 nm. The total phenol content was subsequently calculated using gallic acid as standard. A standard curve was prepared with gallic acid in concentrations of 12.5, 25, 50, 75, 100, 150 µg/ml. The TPC is expressed as milligrams of Gallic acid equivalent (GAE) per gram of extract (mgGAE/g extract). The determinations were in triplicate.

Gas Chromatography-Mass Spectrometry (GC-MS) determination of the oils

The identification of individual components was based on their GC retention indices (RI) on polar columns and comparison of mass spectra by GC/MS to those described in the literature (Adams, 2007).

Gas chromatography mass spectroscopy analysis of the volatile oils was quantitatively determined using QP2010PLUS gas chromatograph (Shimadzu Japan) with specification listed as follows: injection temperature: 250.00°C, Column oven temperature: 60.0°C, Column flow: 1.03ml/min, Pressure: 59.7kPa, Linear velocity: 37.0 cm/sec, sampling time: 1.0 min, injection mode: split less, purge flow: 3.0ml/min, injection volume: 8.00 and total flow rate of 24.6ml/min. The mass peak was generated at a scan speed of 2500 and the mass to charge ratio of the peaks were obtained within the range of 50.00-700.00amu. Furthermore, the peak obtained were analyzed by direct comparison of the m/z and retention time to the compounds present on the National Institute of Standard and Technology (NIST) library (Kind *et al.*, 2018) Compounds showing the best percentage similarity index were tentatively denoted as the queried compound.

Statistical Analysis

Results of antimicrobial, antioxidant, total phenol and flavonoid content are expressed as mean ± standard deviation (SD). Data were analyzed with the one-way analysis of variance (ANOVA), followed by Turkey's test using the GraphPad Prism version 5.0 for Windows (Graph pad software Inc. San Diego, California USA). * $p < 0.05$ and ** $p < 0.001$ were considered statistically significant.

RESULTS

The yield of the oils is presented in Table 1. It shows that *C .sinensis* peel had the highest yield of oil, followed by the peel of *C. aurantifolia* while, *C. limon* gave the lowest yield.

Table 1: Percentage yield of volatile oils in tested citrus species

Citrus fruits	Percentage yield (%)
Citrus sinensis	0.92
Citrus limon	0.49
Citrus paradisi	0.52
Citrus aurantifolia	0.79

Antimicrobial activity of selected citrus volatile oils against test microorganisms

This study revealed the susceptibility of the test microorganisms to varying concentrations of the different citrus peel VOs as shown in Table 2.

The VO of *C. limon* peel was active against *C. albicans* at all concentrations with a zone of inhibition of 12.33±0.58 mm at 5 mg/ml. The oil showed no activity against CREC and CSEC at all concentrations but inhibited the growth of MSSA to varying degrees at a concentration range of 10 – 40 mg/ml. Equally, *C. aurantifolia* peel VO was active against all the test organisms at different concentrations. The observed pattern of activity was concentration-dependent, but not significant. The highest zone of growth inhibition of the oil at the least concentration (5 mg/ml) was recorded against MSSA with a zone of inhibition of 24.00±1.00 mm. This was followed by its activity against MRSA with a zone of inhibition of 23.67±2.89 mm at same concentration. The least activity was recorded against CREC. The VO of *C. paradisi* peel was active against MSSA at all concentrations with the 5 mg/ml concentration creating a zone of growth inhibition of 16.00±1.00 mm. The oil showed activity against MRSA at 30 and 40 mg/ml only. No activity was recorded against CREC at all the working concentrations, while activity against CSEC was recorded at 40 mg/ml only. Furthermore, activity against *C. albicans* was observed in the 10 – 40 mg/ml concentration range. The Peel VO of *C. sinensis* exhibited no activity against MRSA, CSEC and CREC at all test concentrations. The oil was active against MSSA at 20 and 40 mg/ml only. Activity against *C. albicans* at the different concentrations was observed, with a zone of inhibition of 13.00±1.00 mm at 5 mg/ml.

Table 2: Antimicrobial activity of *C. limon, C.aurantifolia, C. paradisi and C. sinensis essential* oil at different concentrations

Organisms	Zones of Inhibition (mean ± S.D mm)						
	Concentrations (mg/ml)				Ciprofloxacin	Itraconazole	DMSO
	5	10	20	40	10 µg	50 µg/ml	(20%)
C. limon							
MSSA	NA	12.00±1.00	14.00±1.00	15.67±2.08	32.00±0.00*	NA	NA
MRSA	NA	NA	13.67±0.58	15.00±1.00	29.00±0.50*	NA	NA
CREC	NA	NA	NA	NA	NA	NA	NA
CSEC	NA	NA	NA	NA	24.00±0.00	NA	NA
C. albicans	12.33±0.58	13.67±1.15	14.67±0.58	17.67±1.15	NA	26.00±0.50	NA
C. aurantifolia							
MSSA	24.00±1.00	29.00±1.00	30.00±1.00	33.00±1.73	32.00±0.00	NA	NA
MRSA	23.67±2.89	26.33±1.57	29.00±1.00	31.33±1.15	29.00±0.50	NA	NA
CREC	12.33±0.58	14.67±0.58	15.67±0.58	17.67±0.58	NA	NA	NA
CSEC	12.67±0.58	15.33±2.52	15.66±1.53	18.00±2.00	25.30±1.00	NA	NA
C. albicans	18.33±0.58	19.33±1.15	21.33±1.15	23.67±0.58*	NA	26.00±0.50	NA
C. paradisi							
MSSA	16.00±1.00	17.33±0.58	18.33±0.58	19.67±0.58	32.00±0.00*	NA	NA
MRSA	NA	NA	13.00±1.00	14.00±1.15	29.00±0.50*	NA	NA
CREC	NA	NA	NA	NA	NA	NA	NA
CSEC	NA	NA	NA	16.33±1.15	24.00±1.00	NA	NA
C. albicans	NA	13.00±1.00	15.00±1.00	17.00±1.00	NA	26.00±0.50	NA

Table 2 continued

C. sinensis							
MSSA	NA	NA	12.33±0.58	14.33±0.23	32.00±0.00*	NA	NA
MRSA	NA	NA	NA	NA	29.00±0.50	NA	NA
CREC	NA	NA	NA	NA	NA	NA	NA
CSEC	NA	NA	NA	NA	24.00±0.00	NA	NA
C. albicans	13.00±1.00	14.67±0.58	16.33±0.58	17.67±0.58	NA	26.00±0.50*	NA

KEY: NA = No Activity; MSSA = Methicillin-sensitive *Staphylococcus aureus'* MRSA = Methicillin-resistant *Staphylococcus aureus*, CREC; Ciprofloxacin-resistant *Escherichia coli*, CSEC: Ciprofloxacin-sensitive *Escherichia coli)*. *Statistically significant = $p < 0.05$.

Minimum Inhibitory Concentration

The tested VOs showed varying degree of minimum inhibitory concentrations (MIC) against the test organisms, with *C. aurantifolia* recording an MIC of 5 mg/kg against *C. albicans,* MSSA and MRSA. *Citrus limon* VO had MICs of 16, 8 and 5 mg/ml against MRSA, MSSA and *C. albicans* respectively. All results are shown in Table 3**.**

Table 3: Minimum Inhibitory Concentration (MIC) (mg/ml) of tested citrus oils

Essential Oils	MRSA	MSSA	CREC	CSEC	*Candida albicans*
C. limon	16*	8	-	32*	4
C. aurantifolia	4	2	4	4	2
C. paradisi	16*	4	-	32*	8
C. sinensis	-	-	-	16*	8

KEY: MSSA= Methicillin-sensitive *Staphylococcus aureus* MRSA= Methicillin-resistant *Staphylococcus aureus*; CREC = Ciprofloxacin-resistant *Escherichia coli*; CSEC= Ciprofloxacin-sensitive *Escherichia coli*; - (could not be determined)

In-Vitro Antioxidant Assay

DPPH Scavenging activity

The ability of the VOs to scavenge the DPPH free radical was compared with the standard ascorbic acid. At 200 µg/mL, ascorbic acid had the highest scavenging capacity with a value of 97.77±4.42%. Among the tested VOs, the VO of *C. sinensis* had the highest scavenging activity of 48.47±3.23% at 200 µg/mL followed by the VO of *C. paradisi* (37.60±2.01%). Other results are shown in figure 1.

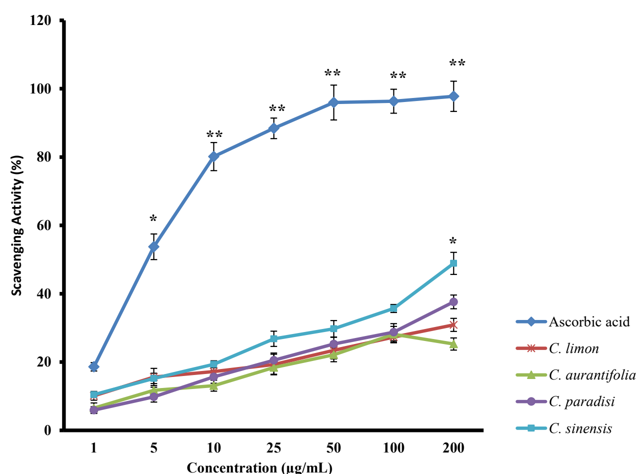

Fig. 1: Free Radical Scavenging activity of the different volatile oils and Ascorbic acid

Total Flavonoid content

The total flavonoid contents in the VOs of the different citrus peels were examined in this experiment. The VO of *C. limon* peel had flavonoid content of 89.35±3.52 mgQE/g extract, followed by the VO of *C. sinensis* (76.02±0.18 mgQE/g extract). The VO of *C. paradisi* peel had the lowest flavonoid content at 72.87±1.16 mgQE/g extract. These are shown fully in Figure 2.

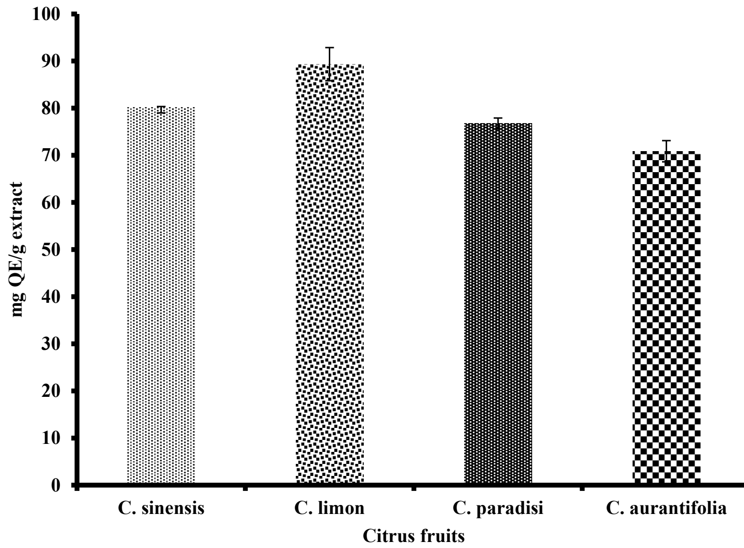

Fig. 2: Total flavonoid content of the different volatile oils

Total phenol content

The VO of *C. sinensis* peel had the highest total phenol content (TPC) of 19.18±2.15 mg GAE/g extract while the TPC of *C. paradisi* VO was lowest at 4.90±1.64 mg GAE/g extract. All results are shown in Figure 3.

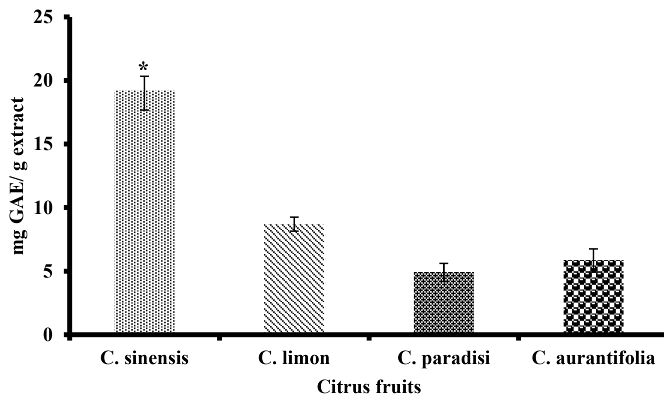

Fig. 3: Total phenol content of the different volatile oils

Results of the Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the different citrus volatile oils

The result of the GC-MS analysis of the oils is as presented in Table 5. Fifteen constituents were identified in *C. aurantifolia* while, four were identified in *C. sinensis*. Methyl isobutylketone was identified in *C. aurantifolia*, *C. paradisis* and *C. sinsnsis*. Limonene was present in *C. aurantifolia* and *C. paradisis* while, 1-octadycyne was identified in *C. limon* and *C. sinensis*. Other results are as presented in Table 4.

Table 4: Constituents of Citrus Peel volatile oils as determined by GC-MS

Percentage	Content in	the oils (%)		
Components	***C. limon***	***C. aurantifolia***	***C. paradisi***	***C. sinensis***
α-Phellandrene	nd	1.05	nd	nd
1,3,6-Octatriene	nd	0.88	nd	nd
1,3,7-Octatriene	nd	nd	nd	14.91
β-Pinene	nd	12.37	nd	nd
Limonene	nd	14.40	19.49	nd
2,6-Octadienal	nd	4.77	nd	nd
α-Bergamotene	nd	2.27	nd	nd
Bicyclo[2,2,2]octane-1-ol	nd	nd	6.35	nd
3-Cyclohexen-1-ol	nd	6.72	nd	nd
p-menth-1-en-8-ol	nd	31.72	nd	nd
Z,E-2-Methyl-3,13- octadecadien-1-ol	nd	nd	8.14	nd
Bicyclo[3.1.1]hept-3-en-2-ol	nd	3.39	nd	nd
1,6-Nonadien-3-ol	nd	4.56	nd	nd
Trans-2-Undecen-1-ol	4.01	nd	nd	nd
2-Methyl-ZZ-13-octadecadienol	33.33	nd	nd	nd
Bicyclo[2.2.1]heptan-2-ol	1.50	nd	nd	nd
Santolina triene	nd	3.69	nd	nd
1-Octadecyne	nd	9.18	nd	62.67
Cyclohexene	nd	nd	1.54	nd
2,2-Dimethyl-1-oxa-spiro[2.4] heptane	nd	nd	1.65	nd

6-Octen-2-one	4.08	nd	nd	nd
n-Hexadecanoic acid	nd	nd	7.24	nd
9,12-Octadecadienoic acid	nd	nd	44.74	nd
Oleic acid	56.38	nd	nd	nd
Phthalic acid	nd	1.49	nd	nd
Methyl Isobutyl Ketone	nd	1.44	2.97	15.78
3,3-Dimethyl-1-(2-carboxyphenyl)triazene	nd	nd	3.43	5.63
2-Propen-1-amine	nd	nd	3.45	nd
TOTAL	99.30	99.43	99.00	98.99

nd= not detected

DISCUSSION

Steam distillation was applied in this study to extract the peel VOs of the studied citrus species. The fruit peels of *Citrus sinensis* had the highest VO yield (0.92%v/v), while *Citrus paradisi* had the least yield. Several factors are known to influence the yield of essential oil in citrus fruits. These include vegetative age, origin, specie of the fruit used, climatic conditions as well as soil quality (Yanqun Li *et al*., 2020; Uysal *et al.,* 2011; Bourgou *et al.,* 2012; Dorby *et al.,* 2008; Vekiara *et al.,* 2002). In our present study, any of these factors may have influenced the yield of the essential oils individually or in combination with other factors.

Antimicrobial activity

The agar well diffusion method is used qualitatively and quantitatively to evaluate the ability of antimicrobial agents to inhibit the growth of susceptible microorganisms (Bonev *et al.,* 2008). The formation of a clear diameter zone of inhibition on the surface of the seeded agar plate is indicative of the susceptibility of the organism/s to the agents. A direct correlation between the diameter of the zone of inhibition and antimicrobial activity has been reported (Balouiri *et al*., 2015).

Varied level of antimicrobial activity by the studied VOs against the test organisms, evidenced by the presence of growth inhibitory zones on seeded agar plate was recorded. Activities were observed to be concentration-dependent and VO specific. The VO of *C. aurantifolia* had the most activity with zones of inhibition observed at the lowest studied concentration against all the test organisms. This is in contrast to the findings with the VO of *C. sinensis* which at the highest test concentration did not produce zones of inhibition in three of the test organisms, showing it to have the least activity. The antimicrobial activity of *C. paradisi* and *C. limon* closely followed that of *C. aurantifolia*. Findings from the susceptibility testing were corroborated by the results of the MIC studies which showed that the VO of *C. aurantifolia* with the least MICs values was the most potent, compared to the other VOs. An inverse relationship between MIC value and antimicrobial potency has been cited (Das *et al*., 2010; Murray, 2015).

This study revealed the Gram-positive bacteria; MSSA and MRSA to be more susceptible to the test VOs compared to the Gram-negative organisms; CSEC and CREC. Gram-positive bacteria are inherently more susceptible to the action of antimicrobial agents

compared to Gram-negative bacteria (Lambert *et al*., 2001; Delaquis *et al.,* 2002). The presence of an outer membrane surrounding the peptidoglycan layer of Gram-negative bacteria restricts the diffusion of hydrophobic compounds through its lipopolysaccharide covering (Gutierrez *et al.,* 2009; Filomena *et al.,* 2013). However, it has been suggested that the simple relationship involving cell structure and microbial sensitivity to volatile oils is not well established. Possible antagonistic or synergistic effects among the various active constituents of the oils could play a role in observed activity (Vardar-Unlu *et al.,* 2003).

Bacteria resistance to antimicrobial agents develops via mechanisms identified to include; limitation of antimicrobial uptake, modification of target, inactivation of antimicrobial and active efflux of agents (Reygaert, 2018). The activity of the VOs of *C. aurantifolia*, *C. limon* and *C. paradisi* against the resistant organisms used in this study could be by interactions that interfered with one or more of these mechanisms to render them susceptible.

Findings from this study revealed that *C. albicans* displayed a varied level of susceptibility to the different VOs used in this experiment, with the highest susceptibility shown against the VO of *C. aurantifolia*. This corroborates the work of Aibinu *et al., (*2007), who reported the susceptibility of *C. albicans* to the VO and extract of *C. aurantifolia.*

Antioxidant activity

The DPPH free radical scavenging antioxidant test is an accepted and widely used method for testing the antioxidant activity of plant extract. It is based on the discolouration of the violet/purple DPPH solution to the yellow coloured reduced product. None of the test samples had a scavenging activity comparable to the standard ascorbic acid. Findings from this study revealed that the VO of *C. sinensis* had the highest antioxidant activity. It has been reported that the antioxidant activity of citrus fruit peel VOs is highly influenced by the method of extraction, composition and antioxidant testing method (Ahmed *et al*., 2019). Lu *et al*., (2019) reported that the cold-pressed peel VO of *C. limon* has a higher scavenging activity in the DPPH test compared to one obtained via steam distillation. The reverse was reported for the peel VO oil of *C. sinensis* in the same report.

This study failed to demonstrate a direct correlation between antioxidant activity, TPC and/or TFC. This is in agreement with similar reported findings by Anagnostopoulou *et al*., (2006) and Ghasemi *et al*., (2009) who studied the relationship between antioxidant activity, TPC and TFC in *C. sinensis*, *C. aurantifolia* and *C. limon* peel VOs. The antioxidant activity of flavonoids is a function of their structure, and –OH substitution pattern. Although we did not attempt to identify the different flavonoids in the studied VO samples, possibly those represented in the oils are not those with four, five or six -OH groups in the ring structure or those with -OH in position 3- or 4-; as these are known to favour proton donation and hence, antioxidant activity (Pannalla *et al.,* 2001).

Gas chromatography-mass spectrometry analysis

Gas chromatography-mass spectrometry (GC-MS) was employed to identify the components of the VOs. Constituents identified consisted majorly of monoterpenes, alcohols and hydrocarbons. Other minor components identified include; ketones, fatty acids and esters. Limonene, a monoterpene is a major component of the peel VO of several citrus species including; *C. sinenesis, C. aurantifolia*, *C. paradisi*, *C. reticulate*, *C. bergamia* and *C. limon* (Erasto and Viljoen, 2008). In the present study, it was identified in the VOs of *C. aurantifolia* and C. *paradisi* in relatively small amounts; 14.40 and 19.46% respectively. Methyl isobutyl ketone, identified

in the VOs of *C. aurantifolia*, *C. paradisi* and *C. senensis* in this study was not detected in the VO of *C. limon.* However, another report identified it in *C. limon* (Ajayi-Moses *et al*., 2019). Major constituents identified in the different VOs samples used in the present study include; oleic acid, a major component of *C. limon*, constituting up to 56.38% of identified components. P-menth-1en-8-ol; hydrocarbon alcohol was the major constituent in *C. aurantifolia* while 9,12-Octadecadienoic acid and 1-Octadecyne were identified as major constituents in the VOs of *C. paradisi* and *C. sinensis* respectively. Quantity and type of constituents present in citrus peel VOs are greatly influenced by cultivar, time of collection, method of extraction, stage of maturity of the plant at collection and peculiarities of analysis (Gonzalez-Mas, *et al*., 2019). Any of these factors individually or collaboratively may have played a role in the type and quantities of constituents identified in the tested oils. Some of the identified compounds, particularly the monoterpenes and sesquiterpene hydrocarbons and their oxygenated derivatives including limonene have been shown to contribute in whole or in part to the antimicrobial and antioxidant activities of VOs (Deba *et al.,* 2015). Hence observed antimicrobial and antioxidant activities of the different VOs can be attributed to their constituents working additively or synergistically.

CONCLUSION

This study has shown that the VOs of *C. sinensis, C. aurantifolia, C. limon and C. paradisi* fruit peel possess a varying degree of antimicrobial activities against studied oral pathogens. The oils also possess significant antioxidant potentials which however did not correlate with their total phenolic or flavonoid content. The VO of *C. aurantifolia* had the highest antimicrobial activity while *C. sinensis* had the highest antioxidant potential. Several constituents were identified in the sampled VOs, of which Methyl isobutyl ketone was identified in three of the oils. These oils are suggested for further study as potential sources of antimicrobial agent/s for the maintenance of good oral health and management of chronic diseases due to oxidative stress and oral pathogens.

Conflict of interests: The authors declare nil conflict of interests.

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