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Research Article

Chemical Composition and Anti-tubercular Activity of the Essential Oil of Orange (*Citrus sinensis* L.) Peel from North Central Nigeria

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ABSTRACT
The peels of *Citrus sinensis* obtained from a market in Abuja, North-central Nigeria, was hydro-distilled to obtain its essential oil. GC-MS analysis of the oil reveals a new chemo-type rich in α-terpineol (35.39%), D-limonene (17.74%), linalool (9.73%), Citronellol (4.88%), γ-Muurolene (4.44%) and Isopiperitenone (3.58%). The oil was screened against local strains of *Mycobacterium tuberculosis* and was found to be active at a strength of 25% (v/v) (or 0.25 ml/ml). This is the first time the essential oil of *C. sinensis* with lower limonene than α-terpineol content, and with anti-TB activity would be reported. The findings suggest that the oil could be used as source of industrial feeds for chemical synthesis, preservatives as well as for flavor and fragrance. This could also open up a new research direction in anti-TB drug development and use of essential oil in drug development and foods.

Keywords: *Citrus sinensis*, sweet orange, *Mycobacterium tuberculosis*, terpineol, limonene, essential oil

INTRODUCTION

Citrus sinensis (Orange) belonging to the Rutaceae family, which comprises of about 140 genera and 1,300 species. Like other *Citrus* spp., it grows in tropical, subtropical and mediterranean climate and region. The fruit, which is rich in vitamin C, is highly consumed worldwide in form of juice and edible vegetable [1]. The consumption of *Citrus* either locally as peeled orange or as processed and package juice usually create huge environmental waste inform of peels or extracted pulps. These wastes, especially the peels could be harnessed as a veritable source of industrial raw materials for the chemical, pharmaceutical and cosmetic industries instead of the not too productive use as animal feeds as currently being practiced in some part of the world [2,3,4].

For instance Citrus peel essential oils had been reported to be a rich sources of bioactive compounds such as mono- and sesqui-terpenes, coumarins, flavonoids, carotenes, etc. [5]. Citrus peels, especially *Citrus sinensis* remains the most abundant natural source of limonene with composition usually above 60% to over 96% of essential oil composition. Djenane had reported 77.37% limonene from Algeria orange peel oil [1,6,7]. Other usual components of essential oil of *Citrus sinensis* include linalool, linalyl acetate, caryophyllene, γ-muurolene, citronellol, carvone, terpineol, undecanal, etc. Citrus peel essential oils have also been scientifically reported to possess antioxidant, insect-repellant and antimicrobial activities [6,8,9,10]. Polymethoxyflavones, hydroxylated polymethoxyflavones and hydroxylated polymethoxychalcones from *C. sinensis* had been reported to exhibit anticancer and antioxidant activities [11]. The aim of this study was to establish the chemical profile of the essential oil of orange peel around Abuja, North central Nigeria and determine its anti-tubercular activity with a view for its use in the pharmaceutical drug development as active ingredient or flavouring excipient.

MATERIALS AND METHODS

Materials

All reagent used were of analytical grade and prepared according to manufacturer's specification. The GC-MS used was Shimadzu QP2010 SE (Japan). Isolate cultures of Mycobacterium tuberculosis from National Hospital Abuja, Nigeria were used as test organisms.

Collection of plant material and Hydro-distillation

Fresh orange peels were collected from market vendors at Karimo Market 22nd July, 2013. The collected peels were sorted to remove foreign matters and pulp and subjected to hydro-distillation using NIPRD multipurpose pilot extraction facility. The hydro-distilled oil was collected in a sterile amber bottle and kept in a cool dark place until required.

Analysis of the hydro-distilled essential oil

The analysis of the essential oil to determine its constituent compounds was carried out on a Shimadzu GC-MS model QP2010 SE (Japan) at the Shimadzu Training Center (STC) for Analytical Instruments, Lagos, Nigeria. The GC-MS was equipped with Optima 5MS capillary column of length 30 m, internal diameter of 0.25 mm and a film

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Table 1: Result of GCMS analysis of *C. sinensis* peel

S/N	RT (min)	% Composition (Area)	MW (Da)	Names Of Identified Compounds
1	5.264	17.74	136	D-Limonene
2	6.306	9.73	154	Linalool
3	6.429	0.18	152	*trans-p*-Mentha-2,8-dienol
4	6.534	1.71	152	Limonene 1,2-epoxide
5	6.628	0.65	154	(R)-(+)-Citronellal
6	7.574	35.39	154	α-Terpineol
7	7.746	4.88	156	β-Citronellol
8	7.954	1.21	150	D-Carvone
9	7.957	2.25	154	Geraniol
10	8.131	3.53	150	Isopiperitenone
11	8.482	2.18	170	Undecanal
12	8.662	0.77	268	Methyl 11-cyclopentylundecanoate
13	9.628	4.44	204	γ-Muurolene
14	10.027	2.18	204	Caryophyllene
15	10.331	1.33	204	β-Sesquiphellandrene
16	10.726	1.22	204	2-methylene-4,8,8-trimethyl-4-vinyl-bicyclo[5.2.0]nonane
17	11.178	2.18	204	Cadina-1(10),4-diene
18	11.462	0.64	222	Elemol
19	11.931	1.68	220	Spathulenol
20	12.885	0.36	222	Eudesm-7(11)-en-4-ol
21	13.400	2.27	218	Sinensal
22	13.615	0.40	264	Linalyl anthranilate
23	14.435	0.19	220	Z-α-trans-Bergamotol
24	16.310	0.44	270	Methyl palmitate
25	16.660	0.41	218	3,7,11-Trimethyl-dodeca-2,4,6,10-tetraenal
26	17.060	0.27	222	D-nerolidol
27	17.300	0.06	272	Biformene
28	17.735	0.13	270	Abieta-8(14),9(11),12-triene
29	17.912	0.17	294	Methyl linoleate
30	17.977	0.16	320	8,11,14-Eicosatrienoic acid, methyl ester
31	18.085	0.19	296	Phytol
32	19.654	0.19	386	(3β,5α)-Cholest-14-en-3-ol
33	19.910	0.15	286	Dehydro-4-Epiabietol
34	20.834	0.12	390	Bis(2-ethylhexyl)phthalate

thickness of 0.25µm. The carrier gas was Helium with a flow rate of 6.2 mL/min. The injector mode was split (1.0). The injector temperature was 250 °C and the detector (ion source) temperature was 200 °C. The conditions for analysis were set as follows; column oven temperature was programmed from 60-280°C (temperature at 60°C, raised to 180°C at 10°C/min and held for 2 min, and then finally to 280°C at 15°C/min and held for 4 min). The M/Z was set at 40-600. The constituents of the essential oil were identified by matching their mass spectra with NIST 11 mass spectral library collection.

Anti-TB screening - Tetrazolium microplate assay (TEMA)

The stock was filter-sterilized with 0.45 µm membrane filter and 50 µl were dispensed into well 1 in triplicate. Dilution of stock (1:2) was made in well 2 through to well 9 with double strength Middlebrook 7H9 broth, also in triplicate. This was followed by addition of 50 µl prepared test organisms using suspensions of *M. tuberculosis* prepared by emulsifying growth from slants (7H11) with 100 µL of Tween 80 into 0.2% bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.). The turbidity was adjusted to McFarland standard no. 1 (approximately 3×10^7 CFU/mL) by adding Tween 80 and bovine serum albumin. The medium sterility, stock and organism viability controls were included. After 5 days incubation at 37°C, 50 µl of the Tetrazolium-Tween 80 solution was dispensed each into few wells for colour change to indicate growth and colourless if there is no growth of the *Mycobacterium* strains. Rifampicin was used as the standard antibiotic.

RESULTS AND DISCUSSION

The essential oil extraction yield was 0.78% (v/w) and the result of GC-MS analysis is as depicted in Table 1. The GC-MS analysis revealed a total of 34 chemical components with α-terpineol as the major component with a percentage composition of 35.39%, followed by D-limonene (17.74%), linalool (9.73%), Citronellol (4.88%), γ-Muurolene (4.44%) and Isopiperitenone (3.58%) (Figure 1). Other components were between 0.06% (Biformene) and 2.27% (Sinensal). The profile of constituents of the orange peels oil suggests that it is a different chemo-type

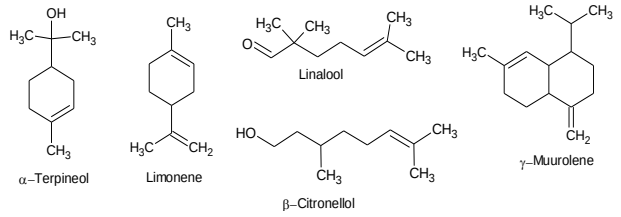

Figure 1: Structures of major compounds found in *C. sinensis* oil of North Central Nigeria

with terpineol as the major constituent. Thus this can be branded terpineol chemo-type.

The anti-TB screening gave a minimum inhibitory concentration (MIC) of 25% (0.25 ml/ml) for the essential oil while the MIC of the control drug rifampicin was 0.09 µg/ml. This means that the orange peel oil was active against *Mycobacterium tuberculosis* at a dilution of up to 400%. The findings in this study are similar to that of Esquivel-Ferriño *et al*., in composition and activity [11]. Esquivel-Ferriño *et al*. reported an anti-tubercular hexane extract of *C. sinensis* rich in D-limonine (24.35%), 1-methyl-4-(methylethenyl)-1,2-cyclohexanediol (9.80%), 2-methyl-5-(methylethenyl)-2-cyclohexen-1-one (8.71%), *trans*-L-carveol (7.10%), dodecanal (4.61) and *cis*-p-menth-2,8-dienol (4.15%) [11]. The study also demonstrated that D-limonene was inactive against *M. tuberculosis* whiles it derivative, limonene epoxide, caryophyllene oxide, palmitic acid and decanal was active at 50 µg/ml, 100 µg/ml, 50 µg/ml and 25 µg/ml, respectively. The activity of limonene oxide was attributed to the presence of oxygenated group. The Esquivel-Ferriño and her coworkers concluded that palmitic acid, decanal, caryophyllene oxide, and *cis*-limonene oxide contained in the hexane extract of *C. sinensis* peel contributed to the anti-TB activity of the extract [11]. The essential oil in this study contained limonene epoxide, methyl palmitate and other oxygenated compounds. The synergistic action of these compounds may be responsible for the anti-tubercular action.

Citrus limonum essential oil rich in limonene (51.39%), β-pinene (17.04%) and γ-terpinene (13.46%) had been reported to exhibit strong activity against *S. aureus* at a concentration of 0.25 µl/ml (0.03%) [6]. The same study also revealed a weaker activity for *C. sinensis* with a higher limonene content of over 77%. In fact bergamot oil (*Citrus aurantium*) with less than 3% limonene was more active [6]. Thus the antibacterial activities of *Citrus* species seemed to decrease with higher limonene content. However, most of the components such as linalool, caryphyllene, etc., had been shown to exhibit reasonable selective antibacterial activity individually. Linalool had been reported to be active against *Campylobacter jejuni, Esherichia coli* O157H:7, *Listeria monocytogenes, Acinetobacter spp.; Enterobacteriaceae, Morexella spp.* and *Vibrionaceae,* etc.[6]. Cineol, α-pinene, limonene, and α-thujone had been reported to be among the most active antimicrobial compounds found in essential oils. These compounds which are basically terpenoids are hydrophorbic and lipophilic, making them able to disrupt bacterial membranes. Hence their mode of action is more likely to be disruption of membrane integrity leading to leakage of cellular content [12,13]. The fact that most of these compounds are analogues of same family, their activity is believed to be synergistic rather than independent.

CONCLUSION

The essential oil of *C. sinensis* from North-central Nigeria was found to be a chemo-type rich in α-terpineol (35.39%), D-limonene (17.74%), linalool (9.73%), Citronellol (4.88%), γ-Muurolene (4.44%) and Isopiperitenone (3.58%). The oil was found to exhibit antibacterial activity against isolated local strains of *Mycobacterium tuberculosis* at strength of 25%. This is the first time the essential oil of *C. sinensis* with lower limonene than α-terpineol content, and with anti-TB activity is reported. The oil could serve as source of industrial feeds for chemical synthesis, preservatives as well as for flavor and fragrance [4]. The finding could help focus new research direction in anti-TB research and use of essential oil in drug development and foods.

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