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Full Length Research Paper

COMPARATIVE STUDY OF CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITIES OF THE LEAF ESSENTIAL OILS OF FIVE CITRUS SPECIES GROWN IN NIGERIA.

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Essential oils have broken ground in the field of natural product chemistry due to their great importance in pharmaceutics, beauty, food, liqueurs, flavours, and perfumery. In this study, essential oils were extracted from the leaves of five citrus species; Citrus hystrix, Citrus tangelo, Citrus reticulata, Citrus aurantifolia and Citrus limon using hydro-distillation method, the chemical composition of the oils was determined using GC-MS and the oils were tested against Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Candida albicans, and Aspergillus niger to determine their antimicrobial activities at different concentrations to determine their medicinal potentials. 57 compounds were identified in all the citrus species investigated, Citrus hystrix, Citrus tangelo, Citrus reticulata,Citrus aurantifolia, and Citrus limon were found to contain linalool, β-ocimine,γ-terpinine, phytol and 1-ethoxyhexane as the highest occurring components with 98.135%, 83.920%, 29.294%, 20.381%, and 15.180% respectively. All the oils showed strong antimicrobial potentials against all the microorganisms and this indicates that essential oils of the citrus species investigated are potential sources of drugs to cure diseases caused by test organisms.

Keywords: *Essential Oil, Citrus, GC-MS, Antimicrobial, Drugs*

INTRODUCTION

Citrus species belong to the family Rutaceae, is one of the most consumed fruits in the world [1, 2]. It contains 130 genera in the seven subfamilies with many important fruits [3]. These fruits contain vitamin C which is reported as an antiscorbutic and possess antioxidant properties [4, 5, 6].

Citrus plants are shrubs or tree usually spinous with alternate 1-foliolate leaves and coriaceous persistent. The trees are evergreen trees that produce fruits of different forms and sizes (from round to oblong) which are full of fragrance, flavor, and juice. The leaves are very green and the fruit has a rough, robust and bright colour from green to yellow skin or rind which contains

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essential oils which give the fruit and the leaf its characteristic citrus fragrance [2,7]. In the southwestern part of Nigeria, the dried rind of all the species of citrus is burnt in glowing charcoal to repel mosquitoes while leaves are used in combination with other medicinal herbs to make an anti-malaria concoction.

There are several reports on the essential oil composition of citrus peels from every part of the world, [8] reported on the essential oil composition of citrus from Tunisia, [9] reported on Turkish citrus peel oils. Many researchers had worked on the essential oil composition and the antimicrobial activities of several species of citrus in Nigeria.

[13] extracted fragrance from *Citrus recticulata* peels and found out that it contains 90.2% of limonene, [14] extracted the essential oil of citrus paradise peel and found out that D-Limonene was the component with the highest percentage (75.05) [15] also worked on the peels of *Citrus reticulata* and *Citrus paradisi*, from the reports of all these researchers, citrus peel majorly consist of D – limonene, β – myrcene, α – pinene, β – pinene, γ – terpinene, α – terpinolene, α – Caryophyllene, copaene, β – Phellandrene [8,9,10,11,12,13,14,15,16,17,128,19] secondary metabolites; flavonoids such as hesperidin, harirutin, naringin and eriocitrin and also polyphenols, such as caffeic acid, P – coomaric acid, ferulic acid and sinapinic acid [20, 21; 22, 23].

The antimicrobial effect of essential oils from the peels of the citrus genus has also been reported by many researchers and they found out that all the oils were active against gram-positive and gram-negative bacteria, fungi and it has the ability to kill the larvae of mosquitoes [14, 24, 25].

[26, 27] extracted the essential oil of the leaves of *Citrus sinesis*, *C. nobilis* loures. var deliciosa swingles; β-phellandrene was found to be the major constituent [22.85%]

The leaf essential oil was found to ameliorate the impaired renal and liver function and there had been claims by herbalist on the use of the leaves in managing several ailments [27]. The extract has been used in Nigeria to facilitate food digestion and it is also used as anti-bacteria, anti-fungal, anti-diabetic, hypotensive, antioxidant, anti-lavarcedal insect repellent, anti-hepatotoxic and anti-mutagenic agent [28,29,31,3,17]. However to the best of our knowledge, from the literatures available much work has been done on the analysis of the volatile oil constituents of the peels of many citrus species especially *C. sinesis* and *C. paradisi* but much work has not been done on the analysis and the antimicrobial activities of the leaf essential oil and also there is no much data on the comparative study of the chemical composition of the leaf essential oil in the genus.

This study is aimed at the comparative study of the chemical composition and antimicrobial activities of the leaf essential oils of five citrus species (*C. hystrix, C. tangelo, C. reticulata, C. aurantifolia, and C. limon*) grown in Nigeria.

MATERIALS AND METHODS

Plant Collection and Identification

The leaves of *Citrus hrystrix, Citrus tangelo, Citrus reticulata, Citrus aurantifolia, and Citrus limon* were collected from different parts of Oyo town, southwest Nigeria in May 2017 and these were identified at Biology Department, Emmanuel Alayande College of Education, Oyo state.

Essential Oil Extraction

Fresh mature leaves of the five species of citrus (*C. hystrix, C. tangelo, C. tangerina, C. aurantifolia* and *C. limon*) were washed separately in clean water and were pulverized separately. 700g of each of the five species were subjected to hydrodistillation in a 5-liter round bottom flask fitted to a-all-glass Clevenger apparatus for 3 hours. According to British specification, the oils obtained were stored in airtight bottles at 4^0c until it was needed for analysis.

The analysis of the essential oil component of the five samples was carried out using GC – Ms Agilent 7890A.

Gas Chromatography/ Mass Spectrometry (GC/MS) Analysis

The essential oil was analyzed using GC-MS Agilent 7890A gas chromatograph coupled with MS Agilent technologies 5975 series MSD.

Antimicrobial Screening

Preparation of Graded Concentration of the Sample

50mg of essential oil was weighed and dissolved into 0.5 ml of methanol for proper dissolution from which 0.5 ml was taken into another 0.5 ml of the solvent; this was taken to the 6^{th} test tube which was the last test tube for the extract. The 7^{th} and 8^{th} were test tubes were negative and positive control (solvent and gentamicin for bacteria, tioconazole for fungi control of the experiment).

Antibacterial Screening

Pour Plate Method was used in the determination of the antimicrobial activities of *Staphylococcus aureus, Escherichia coli, Bacillus subtilis,* and *Pseudomonas aeruginosa.*

Anti-Fungi Screening

Surface Plate method was used to determine the anti-fungi activities of *Candida albicans* and *Aspergillus niger*.

RESULTS

All the oils obtained from the five samples were colourless and their percentage yield is shown in the table below.

Table 1: Percentage yield of all the five species of citrus leaves obtained hydro distillation method

S/N	Citrus Specie	Oil yield (W/W)
1.	*C. reticulata*	0.29%
2.	*C. Limon*	0.31%
3.	*C. hystrix*	0.18%
4.	*C. tangelo*	0.15%
5.	C. *aurantifolia*	0.14%

Table II: GC – MS Profile of the components of *C. tangerina, C. limon, C. hystrix, C. tangelo and C. aurantifolia* essential oil obtained by hydro distillation

S/N	Retetion Time(min	Compound	*Citrus reticulata*	*Citrus limon*	*Citrus hystrix*	*Citrus tangelo*	*Citrus aurantifolia*
1	5.038	α-pinene	0.022				
2	5.639	4-methylene-1-(1-methylethyl)-hexane		*1.426*			*10.096*
3		β-myrcene	0.590				
4	6.600	D-limonene		*16.294*			
5	6.772	1-methyl-3-(1-methylethyl)-benzene	4.571				
6	6.949	1,3,3-trimethyl tricyclo[2.2.1.0(2,6) heptane			*0.971*	*10.033*	
7	7.075	γ-terpinene		*1.063*			
8	7.493	γ-terpene	29.294	*0.741*			
9	7.802	β-ocimene			*0.174*	*83.920*	
10	7.842	3-carene		*5.360*		*4.908*	
11	8.059	(+)-4-carene	2.150	*3.547*	*0.529*		
12	8.380	Linalool	24.401		*98.135*		
13	8.442	3-ethyl-2,4-pentadione					*5.707*
14	8.649	Citronellal		*4.637*			
15	8.826	9-Oxabicyclo[6.1.0]non-6-en-2-one		*0.766*			
16	9.106	Isoneral		*1.296*			
17	9.535	3,5-dimethyl-2-Cyclohexen-1-one					*8.700*
18	9.545	2,6-dimethyl-3,7-octadiene-2,6-diol					*5.811*
19	9.558	4-methyl-1-(1-m ethylethyl)-, (R)- 3-Cyclohexen-1-ol	2.020				
20	9.570	γ-terpineol			*0.191*	*1.138*	
21	9.805	α-terpineol	3.189				
22	10.062	Citronellol	0.087				
23	10.148	3,7-dimethyl-(Z)- 2,6-Octadienal		*13.424*			
24	10.262	Citral	0.596	*12.491*			
25	10.417	Geraniol	0.208				
26	10.760	1-ethoxylhexane					*15.180*
27	10.863	4,5-dimethyl-3-heptanol					*8.913*
28	11.103	1-ethoxyl-2,4-hexadiene					*5.505*
29	11.223	Isoprene					*7.413*
30	11.487	Elemene	0.845				
31	11.807	4-methylene-5-hexenal					*4.388*
32	12.139	1,3-pentadiene					*6.245*
33	12.705	2-methylfuran					*3.962*
34	12.812	17α-hydroxyl-pregen-one		*1.939*			

35	12.912	Caryophyllene	9.079				
36	13.186	Humulene	1.335				
37	13.724	Azulene	2.436				
38	13.758	1,1,4,7-tetramethyldecahydro-1H-cyclopropa[e]azulene-4,7-diol	1.822				
39	13.884	α-farnescene	11.189				
40	14.033	7-hydroxy-3,7-dimethyloctanal					*9.232*
41	14.142	Naphthalene	0.784				
42	14.370	Succic acid					*4.465*
43	14.519	4-ethenyl-α,α-4-trimethyl-3-(1-methylethenyl)-[1R-(1α,3α,4β)]-cyclohexanemethanol	0.293				
44	14.605	Nerolidol	0.672				
45	14.948	2,6-dimethyl-5-heptenol					*0.672*
46	14.977	6-ethyl-3-octanol					*0.420*
47	15.103	Longifolene	0.623				
48	15.578	Cis-linaloloxide					*1.117*
49	15.698	Furanoid					*1.233*
50	15.835	2-furanmethanol					*0.941*
51	15.847	(-)-spathulenol	1.873				
52	16.030	Spirojatamol	0.662				
53	16.442	1-(cyclohexylmethyl)- 2-methyl-, trans- cyclohexane	0.293				
54	17.209	Phananthrene		*14.635*			
55	22.896	Phytol	0.340	*20.381*			
56	29.070	Heptacosane	0.626				

Table III: Anti-microbial Activities of the Leaf Essential Oil of *C. tangerina, C. limon, C. hystrix*, *C. tangelo,* and *C. aurantifolia*

Plant Sample	Conc.	*S. aureus*	*E. coli*	*B. subtlis*	*P. aeruginosa*	*C. albicans*	*A. niger*
Citrus tangerine	1	28	24	22	24	20	18
	2	24	20	18	20	18	16
	3	20	18	16	18	16	14
	4	16	14	12	14	12	12
	5	12	10	10	12	10	10
Citrus limon	1	26	28	26	26	20	18
	2	24	24	22	24	18	16
	3	18	20	18	20	16	14
	4	14	16	14	16	14	12
	5	12	12	10	12	12	10
Citrus hystrix	1	18	28	26	26	20	18
	2	16	24	22	24	18	16
	3	14	20	18	20	16	14
	4	10	16	14	16	14	12
	5	-	12	10	12	12	10
Citrus tangelo	1	24	22	20	22	18	16
	2	20	16	18	18	14	14
	3	18	14	14	14	12	10
	4	14	10	10	10	10	-
	5	10	-	-	-	-	
Citrus aurantifolia	1	20	18	20	18	16	14
	2	18	16	18	16	14	12
	3	14	14	14	14	12	10
	4	12	12	12	12	10	-
	5	10	10	10	-	-	-
	-ve	-	-	-	-	-	-
	+ve	38	36	38	38	28	28

Note: 1=100%, 2=50%, 3=25%, 4=12.5%, 5=6.25%, -ve = DMSO, +ve = Gentamicin ;10µg/ml (Bacteria) and Tioconazole;70% (Fungi).

DISCUSSION

The major components of the leaf essential oil of *C. reticulata, C. limon, C. hystrix, C. tangelo,* and *C. aurantifolia* are linalool 93.14%, β – ocimene 83. 92%, citral 12.491% γ – terpene 29.29%, D – limonene 16 .29% 1-ethoxythexane 15.18%, Phananthrene 14.635%, and phytol 20.381%, the minor components are α – pinene 0.021%, β-myrcene 0.59% geraniol 0.21%, 4-ethenyl-α,α-4-trimethyl-3-(1-methylethenyl)-[1R-(1α,3α,4β)]-cyclohexanemethanol 0.29%, 6-ethyl -3-3octanol 0.42%, heptacosane 0.6% and 1-(cyclohexylmethyl) – 2 – methyl – trans-cyclohexane 0.29%.

The percentage yield of the oils from the five species under investigation were higher than some previous reports [12, 27, 34] and this may be due to environmental factors, vegetative age of the leaves, origin, and species of the leaves and method of extraction employed [8,13,35,36,37,38]. Linonalool, a component that dominated the oil of the Nigerian *Citrus hystrix* was not found at all in the new Caledonian sample and α-pinene, a major constituent in the new Caledonian *C. hystrix* was not found at all in the Nigerian sample under investigation [34]. Also, limonene and Geranial which dominated the

Malaysian *C. aurantifolia* was not found at all in the Nigerian sample under investigation [12].

In a research conducted in two different parts of Lagos State, Nigeria by [38] on the comparative analysis between the leaf essential oil of *C. aurantifolia* swingle obtained from two different parts of Lagos State, Nigeria, there was a great variability in the essential oil component of the two samples and this result is at a great variance. [38] found out that limonene 33.7%, Geranial 21.5%, Nerol 6.1% and β-pinene 5.5% were a major component of the leaf essential oil of *C. aurantifolia* in a place in Lagos while limonene 43.1% and β-pinene 14.2% was the major constituent in the other part. These two results obtained were at variance with the result obtained for *C. aurantifolia* in the present study in which 1-etyhoxyl-hexane 15.18%, 4 – methylene -1-1 (1methylethyl) – hexane *10.096*% and 7-hydroxyl – 3,7- dimethyl octanal 9.232%were the major constituents and these may be due to the to the factors mentioned above.

All the five leaves essential oil inhibited all the test bacteria and fungi strains at all concentration. Expect *C. tangelo* and *C. aurantifolia* at 6.25% concentration on, *Pseudomonas aeruginosa*, *C. albicans* and *Aspergillus niger*, moreover *C. hystrix* at 6.25% was not active against *S. aureus* and this is due to the variation in the major and minor components of the leaf essential oil. [39] reported that *C. limon* leaf essential oil was active against gram-positive bacteria *S.aureus* and *B. subtilis* and this corroborate the present study but further reported that the oil was not active against gram-negative bacteria *E. Coli*, but in this present study, *C. limon* leaf essential oil was active against E. coli at all concentrations and this is due to high concentration of D- limonene 29.13% [40], also [34] reported that *C. hystrix oil* was inactive against bacteria strains but *C. hytrix* leaf essential oil in the present study is active against strains of gram-positive and gram-negative bacteria due to the high percentage of linalool. The antimicrobial activity of the leaf essential oils of these citrus species may be contributed by many bioactive components, each of which may affect different groups of micro-organisms, hence the broad-spectrum inhibition of the oil

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