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Biological Properties And GC-MS Identification of Compounds of Ethanol Extracts and Volatile Oils From *Citrus sinensis*, *Citrus paradisi* and *Citrus reticulata*

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ABSTRACT

The numerous health advantages of citrus fruits are generally well-known. Therefore, this study investigated bioactive properties of ethanol extracts and volatile oils of three citrus fruits (orange, grape and tangerine) wastes. The antioxidant activities of the extracts and volatile oils were assessed using the 2, 2-diphenylpicrylhydrazyl (DPPH) radical scavenging test, while the antimicrobial qualities were determined using agar-well diffusion method. Gas chromatography-mass spectrometry (GC-MS) was used to identify the chemicals constituents. The sample peel extracts exhibited effective inhibition against certain bacterial isolates, according to the results of the antibacterial assay. The mycelial inhibitory activity of all the citrus fruit waste extracts against *Corynespora* sp. was significantly higher (60–84%) than that of any other fungal pathogens under investigation. All the extract exhibited significant ($p < 0.05$) increases in antioxidant activity in a concentration-dependent manner. Bioactive compounds with known antibacterial properties were identified in the extracts' GC-MS fingerprints. 1,2-benzenedicarboxylic acid and diisooctyl ester were prevalent in grapes, accounting for 22.9% in pomace, 21.64% in seed, 15.07 percent in peel, and 23.15% in volatile oil, respectively. However, in the tangerine samples, n-hexadecanoic acid was the most prevalent compound with peel (25.42%) and seed (23.31%) extracts, and (+)-spathulenol (22.30%) being the most dominant in the volatile oil. In contrast, n-hexadecanoic acid was most dominant in the orange samples with percent composition in pomace (20.94%), seed (21.00%), peel (21.07%), and volatile oil (19.71%). Thus, these findings revealed that the citrus fruits wastes could be alternative sources of bioactive ingredients with antimicrobial and antioxidant potentials.

***Keywords*:** Citrus Fruit Extracts, Bacterial and Fungal isolates, Antioxidant Activity, Boactive Compounds.

Introduction

Within the *Rutaceae* family, citrus trees are tiny, evergreen shrubs that thrive in tropical, subtropical, and temperate climates.[1,2] This comprises tangerines (Citrus reticulata), grape fruits (*Citrus paradisi*), citrus oranges (*Citrus sinensis*), limes (*Citrus aurantifolia*), lemons (*Citrus limon*), and several hybrids and varieties.[3-5] Due to their high production, affordability, nutritional content, flavor, sweetness, and other advantageous dietary characteristics, citrus fruits are among the most widely grown fruit crops worldwide. Secondary metabolites or bioactive compounds, such as phenols, flavonoids, terpenes, carotenes, coumarins, vitamins, and volatile oils, are found in citrus fruits and are well-known throughout the globe for their strong anti-oxidative and antimicrobial properties as well as their numerous protective health benefits.[3,5,6]

Many molecules with diverse structures, each defined by one or more phenol rings, have been identified to be members of the phenols and polyphenols group of chemicals.[6,7]

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In recent years, citrus fruit production has drawn more interest from across the world and has continued to rise. In the process of industrializing citrus juice processing, a significant quantity of citrus peel roughly half of the fruit mass is wasted, which presents major risks to human health and the environment.[8]However, researches suggested that it is a rich source of proteins, dietary fiber (such as cellulose, hemicelluloses, lignin, and pectin), limonoids, volatile oils, coumarins, terpenoids, and vitamins.[1,8] Antioxidants have been shown to reverse the harm caused by reactive oxygen species by converting free radicals into molecules that are either non-threatening or very slightly so through radical chain reactions on a regular basis.[9]Researchers are presently focusing a lot of effort on the discovery and development of new medications employing bioactive components derived from plants that have the potential to effectively treat complex illnesses. Due to their safety, non-toxicity, aromatherapeutic properties, and medical value, natural foods, herbs, and their derivatives have become more popular as a means of altering one's lifestyle and substituting pharmaceuticals.[1,8]

Citrus fruits, especially their peels, pomace, and seeds, have been shown in numerous studies to contain a variety of bioactive components that can be used as drugs or food supplements. These components, when combined with their antioxidant, antimicrobial, and other therapeutic properties, make citrus fruit extracts an ideal raw material for food packaging films.[2, 9,10] The principal flavonoids (flavanones and polymethoxylated flavones) present in citrus species, their by-products, and wastes include *eriocitrin*, *narirutin*, *naringin*, and *hesperidine*. These compounds protect against herpes infections and polioviruses, lower blood cholesterol, catalyze the breakdown of starches, function as antioxidants, facilitate the digestion of unsaturated fats, and restore liver lipid homeostasis. They also help prevent certain common and

prevalent illnesses, metabolic disorders, hypertension, obesity, and diabetes.[9,10]

Citrus fruit extracts therefore have high-value potentials and uses as natural food additives to raise the standard of food. Thus, this study evaluated the biological characteristics (such as antibacterial, antifungal and antioxidant properties) of ethanol extracts and volatile oils of sweet orange (*Citrus sinensis*), grapefruit (*Citrus paradisi*), and tangerine (*Citrus reticulata*).

Materials and Methods

Plant collection and preparation

Citrus fruits of the orange (*Citrus sinensis*), grape (*Citrus paradisi*), and tangerine *(Citrus reticulata*) species were purchased at a local market in Ado-Ekiti, located in Ekiti State, Nigeria (7° 37′ 15.9996″ N and 5° 13′ 17.0004″ E). The plant components were identified and authenticated at the Department of Plant Science and Biotechnology, Ekiti State University, Ado-Ekiti, Nigeria. The vouchers were deposited at the departmental herbarium with the following numbers: UHAE2023006 (*Citrus sinensis*), UHAE2023004 (*Citrus paradisi*), and UHAE2023005 (*Citrus reticulata*).

Preparation of peels, pomace, seeds and volatile oils

The peels, pomace, seeds, and volatile oils of each fruit were used. The fruit samples were cleaned many times with distilled water before being used to get rid of any dirt or debris. The pomace and seeds were extracted from the samples by chopping, peeling, and pressing out the juice. Following a period of 14 days of air drying, the corresponding fruit portions were ground up using a sterile electric blender (Marlex, Kil, Dabhel, Daman, India) and weighed (Mettler-Toledo, GmbH, model: ME104E, Switzerland). To produce the extracts, 200 g of the powdered peels, pomace, and seed were each steeped for 24 h in 400 mL of absolute ethanol.

To obtain the produced extracts, the solution was filtered using Whatman No. 1 filter paper and subsequently concentrated using a rotatory evaporator (BOSCH, model RE-52A, Changsha, Hunan, China). For the extraction of volatile oils,[11] the Soxhlet process and Association of Official Analytical Chemists (AOAC) method 920.85 were used. Diethyl ether was used to extract the oils from a 5 g sample of citrus fruit peels (with 80 mash) that was sealed in a thimble for 6 h.

Collection of microbes

All the bacterial and fungal pathogens were obtained from the Microbiology Laboratory in the Department of Biological Sciences, AfeBabalola University, Ado-Ekiti, Nigeria.

Antimicrobial assay

For the microbiological tests, six bacterial and five fungal pathogens were utilized. *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pnuemoniae*, *Bacillus subtilis*, and *Staphylococcus aureus* were the bacteria employed, and *Aspergillus niger*, *Alternaria* sp., *Corynespora* sp., *Fusarium* sp., and *Rhizopus* sp. were the fungi used. The bacterial and fungal isolates are common pathogens of humans and plants, respectively. Prior to use, the bacterial and fungal cultures were plated on nutrient agar and potato dextrose agar plates, respectively to ascertain their purity and viability. Pure colonies were then subcultured and stored as agar slants at 4 ± 2ºC till when needed.

Antibacterial assay of the extracts and volatile oils was carried out using the agar well diffusion method. To a 100 mL sterile nutrient agar in a 150 mL capacity conical flask, 0.5 mL of 18 h old broth cultures of a respective isolate was added, after cooling to a temperature of 45 ºC. The inoculated flasks were swirled gently to mix the organism with agar medium. Following mixing, 20 mL of the medium was dispensed into sterile Petri dishes and allowed to solidify at room temperature.

After solidifying, two holes were bored in each plate, after which 0.2 mL of a respective extract or volatile oil was added and allowed to diffuse. The plates were then incubated (Electro Thermal Incubator, model: MCL-25, China (Mainland)) at 37 ºC for 24 h and observed for zones of inhibition. Zones of inhibition were measured and recorded in milliliters. For all setups, a well in the bored agar that contained only dimethylsulfoxide (DMSO) was used as control. For all the extracts, concentration of 50 mg/mL was used, using DMSO was the diluent.[12]

For antifungal assay, mycelial discs of young actively growing respective fungal cultures were cut separately with a sterile cork borer and inoculated at the center of already prepared plates containing the different extracts and the control plates (without extracts) and incubated at 28 ± 2°C for 3 days. The mycelial growth diameter (cm) of each pathogen was measured and the percentage of growth inhibition was calculated using equation (1) as reported by earlier workers.[13]

$$\% \text{ Mycelial growth inhibition} = \frac{D_O - D_t}{D_O} \text{ x } 100 \quad (1)$$

Where D_O = Diameter of mycelial growth of fungal pathogen in the control plates; D_t = diameter of mycelial growth of fungal pathogen in the treatment plates.

Qualitative phytochemical and *in vitro* antioxidant assays

Phytochemical screening of the respective extracts was determined,[14] while antioxidant assay was carried out using the diphenyl-picrylhydrazyl (DPPH) free radical scavenging activity assay of the extracts.[15] A properly diluted portion (1 mL) was combined with an equal volume of a methanol-based 0.4 mM DPPH solution. The mixture was measured at 516 nm for absorbance after 30 min of dark incubation, by using quercetin as a standard. The DPPH free radical scavenging activity was reported as a percentage (%) inhibition using equation (2).

$$\% \text{ Inhibition} = \frac{\text{Absorbance of Control} - \text{Asorbance of sample}}{\text{Absorbance of control}} \text{X100} \quad (2)$$

Gas chromatography-mass spectrophotometric (GS-MS) analysis

Gas chromatography-mass spectroscopy (GC-MS) analyses of the respective samples and volatile oils were carried out using a Varian 3800/4000 gas chromatography mass spectrometer equipped with an agilent of a BP5 (30 m × 0.25 mm × 0.25 microns) capillary column. Organic compounds in the samples were identified in Wiley's NIST 08 Mass Spectral Library, the obtained comparison scores were higher than 95%. The fragmentation peaks of the compounds were evaluated and compounds were identified using the documented data background for the identification of the compounds that appeared in GC-MS chromatograms.[16]

Determination of IC_{50}

The concentration required to cause 50% inhibition (IC_{50}) was calculated using a linear regression curve generated from a plot of the percentage inhibition values versus different concentrations (mg/ml) of the extract used.[17]

Statistical analyses

Data were analysed in duplicate and results expressed as mean values ± standard deviation (SD) where appropriate. Differences and levels of significance were evaluated by one-way analysis of variance (ANOVA) followed by Duncan's multiple tests. The level of significance was considered at $p < 0.05$.

Results and Discussion

With the exception of tangerine peel extract, *Pseudomonas aeruginosa* demonstrated resistance to the corresponding extracts and volatile oil, as shown in Table 1. Most bacterial species were not inhibited in their proliferation by the corresponding volatile oils. Generally, highest zones of inhibition of 18 and 28 mm (peel and pomace extracts, respectively) and 23 mm (peel and pomace) against *Klebsiella pneumoniae* were recorded in presence of orange and grape extracts, respectively. Similarly, all the test bacterial pathogens were resistant to the tangerine seed extract (Table 1). These findings showed that the peel extracts of the three citrus fruits were highly effective against *E. coli*, *S. typhi*and *S. aureus* unlike their volatile oils. However, in the similar reports,[9,12,18]orange peel extract exhibited effect against *E. coli* and *S. aureus*.

Furthermore, extracts from citrus fruit waste demonstrated a greater proportion of fungal inhibitory action against *Corynespora* sp. in comparison to *Aspergillus niger*. Table 2 displays that every extract and volatile oil exhibited exceptional inhibition against mycelial growth of

the fungal pathogens investigated. For *Corynespora* sp., the highest percentage of inhibition was noted. Whatever the extracts and volatile oils, this observation held true. In the presence of orange and grape extracts as well as volatile oils, the lowest percentage of inhibition against *Aspergillus niger* was generally found. This discovery aligns with previous research demonstrating citrus fruit extracts' capacity to suppress several tested fungus strains.[6,19] Citrus extracts include a variety of bioactive chemicals with strong antifungal qualities, such as osthole, *α*-terpineol, and furfural, which have been linked in several studies to the antifungal activity of citrus fruits.[19,20]These active ingredients influence fungi through a variety of mechanisms. For example, n-hexadecanoic acid, methyl ester, 2-(1-ethoxyethoxy)-3-methyl-, and 5-Acetoxymethyl-2-furaldehyde affect several vital enzymes involved in metabolism, like aldehyde dehydrogenase, which damage mitochondrial membranes and causes an accumulation of reactive oxygen species that leads to microbial cell death.[12,19] The presence of various phytochemicals in grape and grape pomace were also investigated. According to the results (Table 3), presence of the screened phytochemicals were confirmed in the grape pomace. Similarly, the presence of carbohydrates was confirmed in all the extracts examined. However, terpenoids were present in all the extracts except excluding peel of grape. Similarly, tangerine peel indicated the presence of all the screened phytochemicals except saponins. In addition, tannins were observed to be absent in the orange peels and seeds and the grape and tangerine seed extracts. This is similar to past report.[9]Alkaloids are important group of nitrogenous compounds used in the treatment of different human and animal diseases. Flavonoids and polyphenols are beneficial human dietary components with anti-cancer, anti-inflammatory potential, as well as ability to prevent radiation-induced damage and cardiovascular diseases while tannins are high molecular weight polyphenols that exhibits antimicrobial properties by causing microbial protein indigestion.[2, 9,10]

Table 1: Antibacterial potential of the samples and volatile oil

	Zones of inhibition (mm)					
Extracts	***E. coli***	***P. aeruginosa***	***S. aureus***	***K. pneumoniae***	***B. subtilis***	***S. typhi***
			Orange			
Peel	14.0± 0.28	R	8.0±0.57	18.0±0.71	R	11.0±0.28
Pomace	11.0±0.57	R	6.0±0.28	28.0±0.71	R	R
Seed	R	R	8.0±1.13	13.0±0.99	15.0±0.71	R
Oil	R	4.0 ± 0.28	8.0±0.14	R	R	R
			Grape			
Peel	8.0±1.13	R	8.0±0.71	23.0 ± 1.13	18.0±0.99	10.0±0.71
Pomace	5.0 ± 0.14	R	R	23.0 ± 1.27	R	R
Seed	7.0 ± 0.99	R	R	R	R	R
Oil	R	R	5.0±0.28	R	R	R
			Tangerine			
Peel	13.0±1.27	11.0±1.13	8.0±0.10	R	15.0±0.71	13.0±0.28
Seed	R	R	R	R	R	R
Oil	R	7.0±0.14	8.0±1.27	3.0±0.28	4.0±0.57	6.0±0.00
DMSO	R	R	R	R	R	R

Values are presented as mean ± SD (n=3); Key: R: Resistant.

Table 2: Fungal inhibitory potential of the extracts and volatile oil

	% mycelial inhibition				
Extracts	***Aspergillus niger***	***Alternaria* sp.**	***Corynespora*n sp.**	***Fusarium* sp.**	***Rhizopus* sp.**
			Orange extracts		
Peel	27.0 ± 0.707	67.0 ± 1.414	76.0 ± 1.132	25.0 ± 0.990	25.0 ± 0.566
Pomace	33.0 ± 0.849	38.0 ± 1.414	76.0 ± 1.414	18.0 ± 1.414	35.0 ± 0.424
Seed	27.0 ± 0.283	49.0 ± 0.849	82.0 ± 1.132	33.0 ± 0.283	65.0 ± 1.414
Oil	22.0 ± 0.990	49.0 ± 1.414	76.0 ± 0.707	62.0 ± 1.132	65.0 ± 0.566
			Grape extracts		
Peel	31.0 ±1.414	60.0 ± 1.414	84.0 ± 1.414	42.0 ± 2.828	50.0 ± 2.828
Pomace	38.0 ± 0.424	40.0 ± 0.990	73.0 ± 0.849	33.0 ± 0.566	60.0 ± 0.707
Seed	49.0 ± 0.283	40.0 ± 0.849	60.0 ± 2.828	25.0 ± 1.414	35.0 ±0.424
Oil	29.0 ± 0.707	49.0 ± 1.414	76.0 ±1.132	62.0 ± 1.132	65.0 ± 0.424
Tangerine extracts					
Peel	71.0 ± 1.132	60.0 ± 0.849	73.0 ± 0.424	30.0 ± 0.566	50.0 ± 1.414
Seed	33.0 ± 2.828	67.0 ± 0.990	84.0 ± 1.132	22.0 ±0.849	60.0 ± 0.990
Oil	76.0 ± 0.424	38.0 ± 0.849	76.0 ± 0.990	88.0 ±1.414	65.0 ± 1.132
DMSO	R	R	R	R	R

Values are presented as mean ± SD (n=3).

According to Figure 1, the results indicated that all extracts exhibited significant ($p < 0.05$) inhibitory potentials against the DPPH free radical. Notably, grape peel (IC_{50} values = 0.74 ± 0.31 mg/ml) and tangerine peel (0.49 ± 0.31 mg/ml) extracts exhibited about 2-folds inhibitory activity compared to quercetin (reference control) in a concentration-dependent manner.

When an electron or a hydrogen atom is accepted from antioxidants, the comparatively stable nitrogen-containing free radical known as DPPH

free radical is reduced. The number of electrons received is then quantified by measuring the shift in light absorption at 516 nm.[9,15] All the extracts showed remarkable DPPH scavenging activities in concentration-dependent manner. According to our finding, grape and tangerine peel extracts revealed the highest DPPH scavenging activity in favorable comparison with a known standard, quercetin. The higher antioxidant activity of grape and tangerine peel extracts may be due to the available important phytochemicals like polyphenols, flavonoids, glycosides which are commonly known to be responsible for the antioxidant activity.[9,10,12]

Chromatograms of the extracts showed the presence of many chemicals with potential antibacterial properties. 1,2-Benzenedicarboxylic acid, n-hexadecanoic acid, di-isooctyl ester, and 9,12-octadecadienoic acid (Z, Z)-were the main chemicals found in the extracts and volatile oils (Table 4-6 and Fig. S1-S3). Diisooctyl ester and 1,2-benzenedicarboxylic acid were the most prevalent compounds found in the grape samples. Their percentage compositions were 22.95% in the pomace, 21.64% in the seed, peel extracts, and 23.15% in the volatile oil (Table 4 and Fig. S1). Whereas, the orange samples, n-hexadecanoic acid was observed to be the most dominant compound with percent composition of 20.94 % (pomace extract), 21.00 % (seed extract), 21.07 % (peel extract) and 19.71 % in the volatile oil (Table 5 and Fig. S2). In the case of the tangerine samples, n-hexadecanoic acid was detected to be the most dominant compound in the peel (25.42 %) and seed (23.31 %) extracts, while (+)-spathulenol (22.30 %) was observed to be most dominant in the volatile oil (Table 6 and Fig. S3). Several studies have reported chemical composition of volatile oil extracted from citrus peels to comprise monoterpenes, sesquiterpenes compounds and oxygenated derivatives such as alcohols, ketones, aldehydes and esters with major bioactive compounds like n-hexadecanoic acid, 1-octadecanol, furfural, 1,2-benzenedicarboxylic acid, 2-(1-ethoxyethoxy)-3-methyl-, 5-Acetoxymethyl-2-furaldehyde and others which are also present in the extracts and volatile oil used in this study.[2,21,22]This observation suggests that the potential of citrus fruit ethanol extracts and volatile oils for antibacterial, antioxidant and phytochemical (secondary metabolites) properties could be credited to the availability of all the aforementioned bioactive compounds detected by GC-MS technique. The established biological and pharmacological effects as well as the traditional use of certain citrus species in the treatment of various infectious illnesses and disorders may be supported by these findings.

Table 3: Qualitative phytochemical screening of the extracts

Extracts	Alkaloids	Saponins	Carbohydrates	Reducing sugars	Flavonoids	Terpenoids	Phenols	Tannins
			Orange					
Peel	+	-	+	-	+	+	-	-
Pomace	-	+	+	-	+	+	+	+
Seed	-	+	+	+	-	+	-	-
			Grape					
Peel	+	+	+	+	+	-	+	+
Pomace	+	+	+	+	+	+	+	+
Seed	+	+	+	-	-	+	-	-
			Tangerine					
Peel	+	-	+	+	+	+	+	+
Seed	+	+	+	+	-	+	-	-

Keys: '+' indicates present and '- '; absent.

Table 4: GC-MS identified compounds in the grape samples

S/N	Compound Detected	Peak area	Comp	Compound detected	Peak area	Comp
	Pomace			**Seed**		
1	Methyl dichlorosilane	2.07	0.41	Methyl dichlorosilane	1.20	0.44
2	2-Hydroxy-5-methylbenzaldehyde	3.46	1.28	2-Hydroxy-5-methylbenzaldehyde	3.16	3.39
3	1,4-Butanediol,2-(1-ethoxyethoxy)-3-methyl-	6.45	5.43	1,4-Butanediol, 2-(1-ethoxyethoxy)-3-methyl-	4.85	4.63
4	5-Acetoxymethyl-2-furaldehyde	1.38	2.17	5-Acetoxymethyl-2-furaldehyde	0.96	1.17
5	Tetradecanoic acid	4.61	5.05	Tetradecanoic acid	3.89	4.75
6	Norethandrolon	4.15	4.63	Norethandrolone	3.40	3.82
7	Benzoic acid, 4-hydroxy-3,5-dimethoxy-	5.53	7.95	Benzoic acid, 4-hydroxy-3,5-dimethoxy-	7.81	9.06
8	9,12-Octadecadienoic acid (Z, Z)-	10.14	11.02	9,12-Octadecadienoic acid (Z, Z)-	9.76	11.24
9	Octadecane	4.17	4.25	Octadecane	3.16	5.08
10	Diethyl Phthalate	4.82	5.21	Diethyl Phthalate	6.09	3.17

11	Mannitol, 1,3,4-tri-O-methyl-, triacetate, D-	6.68	4.64	Mannitol, 1,3,4-tri-O-methyl-, triacetate, D-	5.60	3.92
12	n-Hexadecanoic acid	12.59	16.38	n-Hexadecanoic acid	16.12	18.00
13	1,2-Benzenedicarboxylic acid, diisooctyl ester.	18.82	22.95	1,2-Benzenedicarboxylic acid, diisooctyl ester.	19.79	21.64
14	Stigmasterol	5.30	3.20	Stigmasterol	5.97	3.18
15	Lignoceric acid, TMS derivative	6.37	4.11	Lignoceric acid, TMS derivative	6.29	2.95
16	Octadecanoic acid, octadecyl ester	2.76	1.18	Octadecanoic acid, octadecyl ester	1.94	2.88
	Peel			**Oil**		
1	Methyl dichlorosilane	2.10	0.51	Methyl lactate	2.22	0.48
2	2-Hydroxy-5-methylbenzaldehyde	3.48	1.30	Cyclohexanol, 5-methyl-2-(1-methylethyl)-	3.88	1.41
3	1,4-Butanediol, 2-(1-ethoxyethoxy)-3-methyl-	6.47	5.42	(+)-spathulenol	6.65	4.91
4	5-Acetoxymethyl-2-furaldehyde	1.40	2.25	Tetradecanoic acid	1.94	2.83
5	Tetradecanoic acid	4.63	5.12	Dibutyl phthalate	4.43	5.96
6	Norethandrolon	4.17	4.58	9,12-Octadecadienoic acid (Z, Z)-	4.16	3.71
7	Benzoic acid, 4-hydroxy-3,5-dimethoxy-	5.55	7.88	(Z, E)-β-Farnesene	8.86	10.14
8	9,12-Octadecadienoic acid (Z, Z)-	10.24	11.13	3,7,11,15-tetramethyl-2-hexadecen-1-ol	4.02	7.21
9	Octadecane	4.18	4.27	Octadecane	3.05	3.74
10	Diethyl Phthalate	4.85	5.20	9-Octadecenoic acid (Z)-, methyl ester	7.20	5.63
11	Mannitol, 1,3,4-tri-O-methyl-, triacetate, D-	6.70	4.61	Phytol	5.70	4.83
12	n-Hexadecanoic acid	12.64	16.48	n-Hexadecanoic acid	15.51	18.25
13	1,2-Benzenedicarboxylic acid, diisooctyl ester.	18.86	23.04	1,2-Benzenedicarboxylic acid, diisooctyl ester.	22.26	23.15
14	Stigmasterol	5.54	3.24	Stigmasterol	5.79	3.26
15	Lignoceric acid, TMS derivative	5.40	3.81	Lignoceric acid, TMS derivative	2.77	3.06
16	Octadecanoic acid, octadecyl ester	2.66	1.21	Octadecanoic acid, octadecyl ester	1.33	1.28

Keys: PA, peak area; comp, % composition.

Table 5: Compounds detected in the orange samples

S/N	Compound Detected	PA	Comp	Compound detected	PA	Comp
	Pomace			**Seed**		
1	Methyl dichlorosilane	4.50	3.25	Methyl dichlorosilane	5.01	4.04
2	2-Hydroxy-5-methylbenzaldehyde	3.50	2.42	2-Hydroxy-5-methylbenzaldehyde	3.71	4.47
3	1,4-Butanediol, 2-(1-ethoxyethoxy)-3-methyl-	8.31	9.36	1,4-Butanediol, 2-(1-ethoxyethoxy)-3-methyl-	8.16	7.35
4	5-Acetoxymethyl-2-furaldehyde	8.50	9.35	5-Acetoxymethyl-2-furaldehyde	8.15	7.25
5	Tetradecanoic acid	8.49	6.32	Tetradecanoic acid	8.14	7.07
6	Norethandrolone	3.00	3.32	Norethandrolone	2.97	3.14
7	Benzoic acid, 4-hydroxy-3,5-dimethoxy-	6.01	7.40	Benzoic acid, 4-hydroxy-3,5-dimethoxy-	5.97	5.31
8	9,12-Octadecadienoic acid (Z, Z)-	19.02	20.91	9,12-Octadecadienoic acid (Z, Z)-	19.31	20.92
9	Octadecane	6.41	5.48	Octadecane	5.93	6.33

10	Diethyl Phthalate	6.48	5.51	Diethyl Phthalate	5.91	6.30
11	Mannitol, 1,3,4-tri-O-methyl-, triacetate, D-	6.40	5.47	Mannitol, 1,3,4-tri-O-methyl-, triacetate, D-	5.94	6.56
12	n-Hexadecanoic acid	19.27	20.94	n-Hexadecanoic acid	20.79	21.00
	Peel			**Oil**		
1	Methyl dichlorosilane	5.02	4.42	Methyl lactate	5.81	4.82
2	2-Hydroxy-5-methylbenzaldehyde	3.51	4.42	Cyclohexanol, 5-methyl-2-(1-methylethyl)-	4.62	4.69
3	1,4-Butanediol, 2-(1-ethoxyethoxy)-3-methyl-	8.02	8.34	(+)-spathulenol	8.10	8.95
4	5-Acetoxymethyl-2-furaldehyde	8.01	8.00	Tetradecanoic acid	8.07	6.90
5	Tetradecanoic acid	8.00	6.00	9,12-Octadecadienoic acid (Z, Z)-	8.05	10.88
6	Norethandrolone	3.01	3.40	Dibutyl phthalate	3.89	3.00
7	Benzoic acid, 4-hydroxy-3,5-dimethoxy-	6.02	6.24	(Z, E)-β-Farnesene	5.74	4.99
8	9,12-Octadecadienoic acid (Z, Z)-	19.07	20.86	3,7,11,15-tetramethyl-2-hexadecen-1-ol	18.68	19.69
9	Octadecane	6.42	6.60	Octadecane	6.37	5.43
10	Diethyl Phthalate	6.40	5.09	9-Octadecenoic acid (Z)-, methyl ester	5.86	5.32
11	Mannitol, 1,3,4-tri-O-methyl-, triacetate, D-	6.41	5.54	Phytol	5.64	5.56
12	n-Hexadecanoic acid	20.07	21.01	n-Hexadecanoic acid	18.97	19.71

Keys: PA, peak area; comp, % composition.

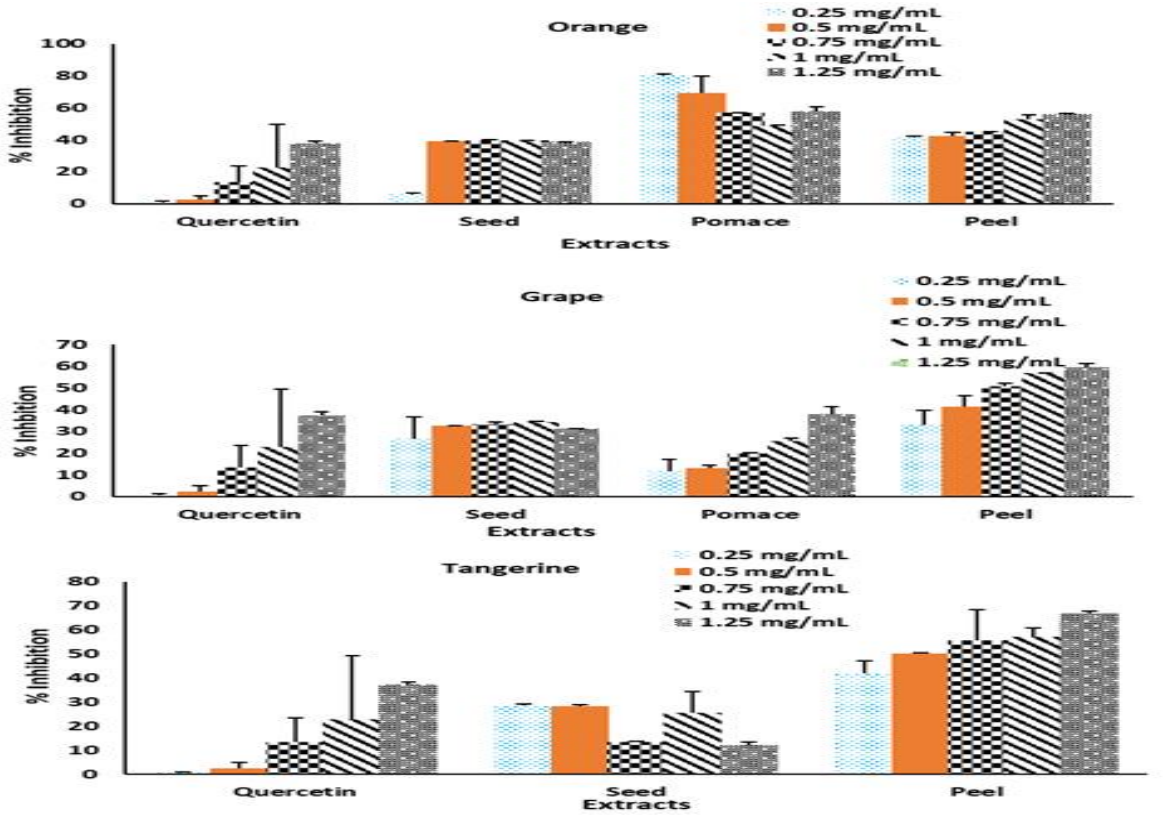

Figure 1: DPPH scavenging activity of the extracts at different concentrations

Table 6: Compounds detected in the tangerine samples

S/N	Compound Detected	PA	Comp	Compound detected	PA	Comp

	Peel			**Seed**		
1	Methyl dichlorosilane	7.37	4.91	Methyl dichlorosilane	7.43	4.88
2	2-Hydroxy-5-methylbenzaldehyde	3.90	3.42	2-Hydroxy-5-methylbenzaldehyde	3.91	4.41
3	n-Hexadecanoic acid	26.45	25.42	n-Hexadecanoic acid	26.21	23.31
4	5-Acetoxymethyl-2-furaldehyde	3.04	3.90	5-Acetoxymethyl-2-furaldehyde	3.52	4.85
5	Tetradecanoic acid	2.17	2.40	Tetradecanoic acid	2.35	3.40
6	Norethandrolone	9.11	9.43	Norethandrolone	9.39	6.50
7	Benzoic acid, 4-hydroxy-3,5-dimethoxy-	0.87	1.41	Benzoic acid, 4-hydroxy-3,5-dimethoxy-	0.78	1.41
8	9,12-Octadecadienoic acid (Z, Z)-	17.35	18.04	9,12-Octadecadienoic acid (Z, Z)-	17.21	18.11
9	Octadecane	3.47	4.78	Octadecane	2.74	3.17
10	Diethyl Phthalate	5.20	4.53	Diethyl Phthalate	5.09	4.57
11	Mannitol, 1,3,4-tri-O-methyl-, triacetate, D-	0.43	1.11	Mannitol, 1,3,4-tri-O-methyl-, triacetate, D-	0.39	1.15
12	1,4-Butanediol, 2-(1-ethoxyethoxy)-3-methyl-	0.78	1.41	1,4-Butanediol, 2-(1-ethoxyethoxy)-3-methyl-	0.71	1.44
13	1,2-Benzenedicarboxylic acid, diisooctyl ester.	1.73	2.20	1,2-Benzenedicarboxylic acid, diisooctyl ester.	1.17	2.24
14	9,12,15-Octadecatrienoic acid, methyl ester	9.10	10.05	9,12,15-Octadecatrienoic acid, methyl ester	9.78	9.10
15	1,2-Benzenedicarboxylic acid, diisooctyl ester	8.67	5.07	1,2-Benzenedicarboxylic acid, diisooctyl ester	9.00	10.08
16	Eicosanoic acid	0.35	1.11	Eicosanoic acid	0.31	1.12
	Oil					
1	Methyl lactate	5.16	2.86			
2	Cyclohexanol, 5-methyl-2-(1-methylethyl)-	5.63	3.40			
3	(+)-spathulenol	25.94	22.30			
4	Tetradecanoic acid	3.24	4.82			
5	Dibutyl phthalate	2.16	3.43			
6	9,12-Octadecadienoic acid (Z, Z)-	9.36	10.01			
7	(Z, E)-β-Farnesene	0.72	1.38			
8	3,7,11,15-tetramethyl-2-hexadecen-1-ol	17.29	18.04			
9	Octadecane	3.25	5.15			
10	9-Octadecenoic acid (Z)-, methyl ester	5.76	6.22			
11	Phytol	0.36	1.11			
12	n-Hexadecanoic acid	0.65	1.54			
13	1,2-Benzenedicarboxylic acid, diisooctyl ester.	1.44	2.23			
14	9,12,15-Octadecatrienoic acid, methyl ester	9.73	10.22			
15	1,2-Benzenedicarboxylic acid, diisooctyl ester	8.65	6.06			
16	Eicosanoic acid	0.29	1.10			

Keys: PA, peak area; comp, % composition.

Conclusion

This study confirmed the antimicrobial, antioxidant, and phytochemical (secondary metabolites) properties of ethanol extracts and volatile oils of orange (*Citrus sinensis*), grape fruit (*Citrus paradisi*) and tangerine (*Citrus reticulate*) for their inhibitory abilities against microbes and free radicals that have been implicated in causing various disease in humans and animals. Therefore, this report could invariably suggest the potentiality of the extracts and volatile oil for possible potential treatment against bacterial and fungal pathogens as well as management of free radical induced human diseases.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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