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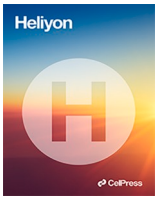

Research article

Characterization and antioxidant activity of peel extracts from three varieties of *citrus sinensis*

Ogo Ogo [a,b,1,*], Ngutor Hembafan [b,1], Raphael Amokaha [a], Oloche Jeremiah [c], Bawa Inalegwu [d]

[a] *Department of Biochemistry, Benue State University, Makurdi, Nigeria*
[b] *Centre for Food Technology and Research, Benue State University, Makurdi, Nigeria*
[c] *Department of Pharmacology & Therapeutics, Benue State University, Nigeria*
[d] *Department of Biochemistry, Federal University of Health Science, Otukpo, Nigeria*

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ABSTRACT

High volume of postharvest materials including peels from citrus fruits is periodically generated, which contributes to environmental pollution. Investigating the chemical composition cum antioxidant property of these 'wastes' would be instructive in achieving value addition in the food and pharmaceutical value chain. On this premise, this study carried out phytochemical screening and antioxidant activity of three (3) commonly cultivated citrus varieties namely *Citrus sinensis* 'valencia', *Citrus sinensis* 'washinton' and *Citrus sinensis* 'thompson navel'. The peels were extracted using ethanol and hexane in a Soxhlet extractor and thereafter subjected to phytochemical and Gas Chromatography/Mass Spectrometry (GC/MS) analyses, ferric ion reducing antioxidant power (FRAP), hydrogen peroxide scavenging and cupric ion reducing antioxidant capacity (CUPRAC) assays to evaluate their antioxidant potentials. Results show that *Citrus sinensis* peel extracts contain alkaloids, flavonoids, phenols, phytosterols, diterpenes, tannins and glycosides. GC/MS analysis identified about 48 compounds in each extract; with the predominant bioactive compounds being limonene (16.5%), ascorbic acid (17.7%), stearic acid (26.3%), linalool (4.7%), linoleic acid (16.18%), palmitic acid (15.23%), pentadecyclic acid (1.1%). Ethanol and hexane extracts of Valencia exhibited higher FRAP (9.09 ± 0.13) and CUPRAC (2.04 ± 0.06) values while the ethanol extract of Ibadan sweet demonstrated greater hydrogen peroxide scavenging activity (1.39 ± 0.00). Citrus peels are rich in bioactive compounds with excellent antioxidant activity and may serve as potential sources of natural antioxidants for food products or pharmaceutical formulations.

1. Introduction

Citrus belongs to the family Rutaceae with species such as oranges (*Citrus sinensis*), lemons (*Citrus limon*), grapefruits (*Citrus paradisi*), limes (*Citrus aurantifolia*) widely cultivated. Citrus fruits, especially oranges, are globally significant crops with annual production exceeding 150 million metric tons [1]. Nigeria is a major orange producer, generating 4.19 million metric tons in 2022 with

* Corresponding author. Department of Biochemistry, Benue State University Makurdi, Nigeria.
E-mail address: oogo@bsum.edu.ng (O. Ogo).
[1] Both authors have equal contribution to this work.

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the processed orange juice industry witnessing increasing growth to a projected annual growth rate of 19.11% between 2024 and 2028 [2]. However, large quantities of waste are produced during citrus processing, mainly in the form of peels and pomace [3]. As juice production scales up to meet demand, even more of this bio-waste will be generated. At present, this represents lost value and causes environmental pollution [4]. Citrus peel waste is rich in health-promoting phytochemicals like polyphenols, which have antioxidant and antimicrobial properties [5]. There exists an opportunity to extract and utilize these 'waste' as natural food preservatives [6], rather than discarding same. This will not only add value, but could also substitute synthetic additives with eco-friendly, circular alternatives aligned to achievement of sustainable development goals. This will benefit the citrus industry value chain from farmer to consumer and building on promising global precedents [7].

Fruit wastes have been reported to possess nutritional and functional properties [8], which when properly deployed in value addition could be a useful vehicle for combating malnutrition [9]. Additionally, phytochemicals such as polyphenols are reported to have preventive effect on cardiovascular diseases, cancer, aging, osteoporosis, diabetes mellitus and oxidative stress related neuro-degenerative diseases [10]. Furthermore, polyphenols exhibit food preservative properties [11] and are receiving attention for application in the food industry. This study investigated the phytochemical profile and preservative potential of peel extracts from major Nigerian sweet orange cultivars. The overarching aim was to provide data to support optimal valorization of citrus by-product waste into antioxidants for application as bio preservatives in the food, beverage, pharmaceutical and cosmetic industries. By ensuring full utilization of harvested fruit, it will create additional revenue streams while addressing the current issues of pollution and value loss posed by untreated citrus biomass residues. Consequently, this study was specifically aimed at investigating the phytochemicals present in the peel extracts of three popular varieties of *Citrus sinensis* and their potential as natural antioxidants with preservative properties.

2. Materials and methods

2.1. Sample collection, preparation and extraction

Three varieties of sweet orange (*Citrus sinensis*) namely: *Citrus sinensis* 'valencia', *Citrus sinensis* 'washinton' and *Citrus sinensis* 'thompson navel' were obtained from a commercial farm in Gboko Local government area of Benue State, Nigeria. The three orange samples were simply identified with their local names as 'Valencia', 'Washington Navel' and 'Ibadan Sweet' ('Thompson navel') respectively, and were chosen based on consumer preference revealed by the sellers.

The fruits were thoroughly washed under running water to remove any dirt after which they were peeled with a kitchen knife. The peels were reduced to smaller sizes and oven-dried at 40 °C for 72 h on aluminum foil paper. The dried peels were pulverized and 50 g of each orange peel powder was extracted with 250 ml of ethanol and then hexane using Soxhlet apparatus for 5 h. Thereafter, the extracts were filtered and the filtrates were evaporated under vacuum at 40 °C using a rotary evaporator. The resultant extracts were stored in the refrigerator at 4 °C for further analysis.

2.2. Determination of extract yield

To determine yield of extract, the weight of container and extract with container was taken separately and recorded using electronic weighing balance (ADAM). Thereafter, percentage yield of extract was obtained using the formular:

$$\%\ \text{yield of exract} = \frac{(W_2 - W_1)}{W_0}\ \text{x}100$$

where W_2 weight of the extract and container; W_1 the weight of container alone and W_0 is weight of the initial dried sample (extract).

2.3. Phytochemical screening

The extracts were analyzed using the methods described by Oluremi [12] for alkaloids, flavonoids, phenols, saponins, tannins, phytosterols, diterpenes and glycosides.

2.4. Gas chromatography/mass spectrometry analysis

The samples were analyzed using a GC/MS-QP2010 Plus (Shimadzu Japan) comprising of a Shimadzu QP-2010 GC with QP-2010 Mass Selective Detector (MSD), operated in the EI mode, electron energy of 70 eV, scan range of 40–300 amu, and Shimadzu GC/MS solution data system. The Gas chromatography column was Agilent HP-5 MS fused silica capillary with 5% phenyl-methylpolysiloxane stationary phase, with length of 30 m, internal diameter of 0.25 mm and film thickness of 0.25 μm. The carrier gas was helium 99.9% with flow rate of 1.61 ml/min. The program used for Gas chromatography oven temperature was 50–240 °C at a rate of 5 °C/min, then held at 240 °C for 1 min. The injection port temperature was 250 °C, interface temperature was 250 °C, the while ion source temperature was 200 °C. The sample filtered through 0.45 μm membrane filter and 1.0 μL subsequently injected into the GC using autosampler and the split mode set at a ratio of 20:1. Individual constituents separated in the GC columns were fed into the MS where they were identified by comparing their mass spectra with known compounds and NIST Mass Spectral Library. The percentage composition of each constituent is reported as raw percentage based on peak area of the total ion current as described previously [13].

2.5. Determination of antioxidant activity

2.5.1. Ferric ion reducing antioxidant power (FRAP) assay

The reducing power of the extracts was determined according to the method described by Bhatti [14] with slight modifications. A mixture of 0.5 ml extract and 2.5 ml 0.2 M phosphate buffer (pH 8.9) and 2.5 ml of 1% potassium ferricyanide [$K_3Fe(CN)_6$] was prepared and incubated at 50 °C for 20 min after which 2.5 ml of trichloroacetic acid (10%) was added and centrifuged at 3000 rpm for 10 min. Exactly 2.5 ml of the upper layer was mixed with 2.5 ml distilled water and 0.5 ml 0.1% $FeCl_3$, and absorbance measured at 700 nm using a PASCO PS-26000 spectrophotometer. L-Ascorbic acid (Vitamin C), $C_6H_8O_6$ = 176.13 Assay 99.0% min and Specific rotation +20.5 to + 21.5. procured from Nice chemicals PVT. LTD was used as a standard.

2.5.2. Hydrogen peroxide scavenging assay

This assay was carried out according to the method described by Keser [15]. Extract (0.5 mg/ml) in distilled water was added to 0.6 ml of hydrogen peroxide solution (40 mM, pH = 7.4) and absorbance of the mixture taken at 230 nm after 10 min of incubation against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of both peel extracts and standard compounds were calculated thus:

$$\text{Scavenged } H_2O_2 = \frac{(A_0 - A_1)}{A_0}$$

where Ao is the absorbance of the control and A_1 the absorbance of the sample.

2.5.3. Cupric ion reducing antioxidant capacity (CUPRAC) assay

Equal volumes (0.25 ml) of $CuCl_2$ (0.01 M), neocuproine ethanol solution (7.5×10^{-3} M) and CH_3COONH_4 (1 M) buffer solution were mixed in a test tube, and then 0.5 ml of the extracts were added. The final volume was made up to 2 ml with distilled water and vortex-mixed. The tubes were closed and left to stand at room temperature for 30 min and the absorbance thereafter measured at 450 nm against a blank reagent (water) [16]. The Cupric ion (Cu^{2+}) reducing power was calculated as:

$$\Delta A = A_{30}\text{-}A_0$$

where A_0 - absorbance of the test reagent, A_{30} - absorbance after 30 min of reaction.

2.6. Statistical analysis

Where applicable, measurements were carried out in replicates and results expressed as mean ± standard error of mean (SEM). Data were analyzed by One-way analysis of variance (ANOVA) using SPSS program (version 20.0 SPSS Inc., Chicago, IL, USA). Statistical difference between antioxidant activity of citrus varieties and assays was compared using the Duncan Multiple Range Test, where P value of ≤ 0.05 was considered to be statistically significant.

3. Results and discussion

3.1. Percentage yield of Citrus sinensis peel extracts

The results of percentage yield of peel extracts of *Citrus sinensis* 'valencia', *Citrus sinensis* 'washinton' and *Citrus sinensis* 'thompson navel' in ethanol and hexane are shown in Table 1. The yield of ethanol extracts was significantly higher ($p < 0.05$) ranging between 42. 14 and 54.96% than that of hexane, which ranged from 6.55 to 861%. Samples extracted with ethanol, *Citrus sinensis* 'washinton'

Table 1
Percentage yield of peel extracts of *Citrus sinensis* 'valencia', *Citrus sinensis* 'washinton' and *Citrus sinensis* 'thompson navel'in ethanol and hexane solvents.

Sample	Percentage yield per 150 g
EEV	42.14 ± 0.21^{ab}
EEW	54.96 ± 0.13^{a}
EEI	46.13 ± 0.16^{ab}
HEV	8.61 ± 0.19^{a}
HEW	6.55 ± 0.18^{ac}
HEI	8.08 ± 0.15^{ab}

KEY: EEV = Ethanolic extract of Valencia, EEW = Ethanolic extract of Washington Navel, EEI = Ethanolic extract of Ibadan Sweet, HEV= Hexane extract of Valencia, HEW=Hexane extract of Washington Navel, HEI= Hexane extract of Ibadan Sweet.

gave the highest yield while *Citrus sinensis* 'valencia' gave the least yield. On the other hand, *Citrus sinensis* 'valencia' gave the highest yield while *Citrus sinensis* 'washinton' had the least yield among samples extracted with hexane solvent. This result is akin to documented report of similar experiment by Mohamed and coworkers [17]. The observed result may not be unconnected with the fact that ethanol with higher polarity may have dissolved more plant constituents than hexane, indicating that ethanol is a better solvent for the extraction of *Citrus sinensis* than hexane.

3.2. Phytochemical constituents of Citrus sinensis peel extracts

The phytochemical analysis of the peel extracts indicated the presence of variety of bioactive compounds such as alkaloids, flavonoids, phenols, saponins, tannins, phytosterols, diterpenes and glycosides (Table 2). Compared to the hexane extracts, ethanolic extracts contained higher number of secondary metabolites with high degree of precipitation (+++) with diterpenes being detected only in the ethanolic extracts of the samples. The hexane extracts had low concentration of phenols and tannins. This may be attributed to the high polarity of ethanol which allows it to extract higher variety of plant constituents as alluded to by previous investigation [18].

Phytochemicals present in citrus peels are reported to have antioxidant, biological and therapeutic properties. Several studies have suggested that flavonoids exhibit biological activities, including antiallergenic, antiviral, anti-inflammatory, and vasodilating actions [19,20]. However, interests have shifted to the antioxidant activity of flavonoids, which is due to their ability to reduce free radical formation and to scavenge free radicals [21]. Phenols including polyphenols and phenolic acids are known for their high antioxidant activity [22,23]. Terpenes and terpenoids possess a wide range of biological activities including anticancer, antimicrobial, anti-inflammatory, antioxidant, and antiallergic. There are reports that they exhibit food preservative properties [24,25], which are considered for potential application in the food industry. Tannins form complexes with proline-rich proteins that inhibit cell protein synthesis. Previous research has reported that tannins have potential as natural food preservatives [26].

Naturally occurring antioxidants in fruits and vegetables such as citrus can scavenge free radicals, and thus have a protective effect against oxidation improving the shelf-life and nutritional value of food [27–29]. The reactions that protect the food are the similar to the ones that protect cells in biological organisms and have a specific aim to avoid oxidation [30]. From the phytochemical analysis, orange peel extracts can be considered as a source of natural antioxidants, which can be utilized in the food industry as bio-preservatives.

3.3. Gas chromatography/mass spectrometry detection of phytochemical compounds present in Citrus sinensis peel extracts

GC/MS analysis was carried out on the extracts to further determine the specific phytochemical constituents, their structures and percentage composition in each sample. About 50 bioactive compounds were identified in each citrus peel extract. The names, retention time (RT), formula, molecular weight (g/mol), similarity index and percentage area composition of some of the prominent compounds identified in the extracts are given in Tables 3–8 and their chromatograms are illustrated in Figs. 1–6 respectively.

The GC/MS analysis of the samples revealed the presence of terpenes/terpenoids, esters, fatty acids, ketones, unsaturated polyhydroxy alcohols and oxygenated compounds nonvolatile compounds across the samples. These results are in consonance with the class of compounds recorded for GC/MS studies of orange peels in literature [31]. Some bioactive compounds of importance identified in the extracts include D-limonene, linalool acetate, linalool, 1-octanol, beta-cubebene, n-hexadecanoic acid, pentadecanoic acid, 9,12 octadecanoic acid, octadecanoic acid, 1-(+)-ascorbic acid 2,6-hexadecanoate, cubenol, menthol, terpineol, citronellol, copaene, muurolene, elemol, 3-hexen-2-one, oxazole etc. (Tables 3–8). The most abundant compound in the ethanolic peel extracts was D-limonene consistent with previous report [32]. The constituent has a peak area composition of 19.36% in EEV, 16.53% in EEW and 18.56% in EEI respectively. Meanwhile n-hexadecanoic acid was the most abundant compound in HEV comprising up to 15.23%; 9,12 octadecanoic acid (omega-6 fatty acid) had the highest peak area composition in HEW (26.36%) and HEI (16.77%). D-limonene was observed to be present in both ethanolic and hexane peel extracts but had lower composition in the hexane extracts, which may be due to use of different extraction solvent and the cultivar of sweet orange used to prepare the extracts.

Table 2
Qualitative phytochemical composition of *Citrus sinensis* peel extracts.

Constituent	Extract					
	EEV	EEW	EEI	HEV	HEW	HEI
Alkaloids	+++	+++	+++	+	+	+
Flavonoids	+++	+++	+++	+	+	+
Phenols	+++	+++	+++	+	+	+
Saponins	+++	+++	+	–	+	++
Tannins	+++	+++	+++	+	+	+
Phytosterols	+++	+++	+++	+++	+++	+++
Diterpenes	+++	+++	+++	–	–	–
Glycosides	+++	+++	+++	+	+	+

KEY: EEV = Ethanolic extract of 'valencia', EEW = Ethanolic extract of 'washington navel', EEI = Ethanolic extract of Ibadan Sweet, HEV= Hexane extract of valencia, HEW=Hexane extract of washington Navel, HEI= Hexane extract of Ibadan Sweet. +++ = High concentration, ++ = Moderate concentration, + = Low concentration, - = Not detected.

Table 3
Selected phytochemical components of ethanol extract of *Citrus sinensis* 'valencia'(EEV) identified by GC/MS analysis.

S/N	Name of compound	Molecular Formula	Molecular weight	Similarity Index	Retention Time	Peak Area %
1	Limonene	$C_{10}H_{16}$	136	93	7.482	19.36
2	1-Octanol	$C_8H_{18}O$	130	93	8.686	0.44
3	Linalool	$C_{10}H_{18}O$	154	91	9.295	1.30
4	Linalool acetate	$C_{12}H_{20}O_2$	154	95	9.538	4.70
5	Terpineol	$C_{10}H_{18}O$	154	95	12.098	1.00
6	Citronellol	$C_{10}H_{20}O$	156	89	13.120	0.35
7	n-Decanoic acid	$C_{10}H_{20}O_2$	172	90	17.092	0.41
8	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	80	28.762	0.43
9	Oleic Acid	$C_{18}H_{34}O_2$	282	79	30.453	0.66
10	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	652	87	31.039	9.13
11	Octadecanoic acid	$C_{18}H_{36}O_2$	652	85	32.817	1.21
12	Menthol	$C_{10}H_{20}O$	156	81	33.793	0.37
13	Linoleic acid	$C_{18}H_{32}O_2$	280	80	34.381	16.18
14	E,E,Z-1,3,12-Nonadecatriene-5,14-d	$C_{19}H_{34}O_2$	294	86	34.487	10.47
15	Octadecanoic acid	$C_{18}H_{36}O_2$	284	86	34.802	3.30

Table 4
Selected phytochemical components of ethanol extract of *Citrus sinensis* 'washinton'(EEW) identified by GC/MS analysis.

S/N	Name of compound	Molecular Formula	Molecular weight	Similarity Index	Retention Time	Peak Area %
1	Limonene	$C_{10}H_{16}$	136	93	7.469	16.53
2	1-Octanol	$C_8H_{18}O$	130	96	8.678	0.26
3	Linalool	$C_{10}H_{18}O$	154	95	9.510	4.71
4	2-Furancarboxaldehyde, 5-(hydroxyr	$C_6H_6O_3$	126	87	13.555	8.54
5	1-(+)-Abscorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652	77	26.627	0.85
6	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	79	28.750	0.30
7	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	652	86	30.462	0.69
8	-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652	88	30.938	9.34
9	Octadec-9-enoic acid	$C_{18}H_{34}O_2$	282	84	33.897	0.35
10	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	85	34.275	8.80
11	9-Octadecenoic acid, 1,2,3-propanetriyl	$C_{57}H_{104}O_6$	884	87	34.381	8.48
12	Octadecanoic acid	$C_{18}H_{36}O_2$	284	83	34.722	2.85
13	Ethyl Oleate	$C_{20}H_{38}O_2$	310	77	34.813	0.55
14	1,2-Benzenedicarboxylic acid, mono	$C_{13}H_{16}O_4$	278	93	35.210	7.36
15	Hexadecanoic acid, tetradecyl ester	$C_{30}H_{60}O_2$	452	79	36.352	2.55

Table 5
Selected phytochemical components of ethanol extract of *Citrus sinensis* 'thompson navel' (EEI) identified by GC/MS analysis.

S/No.	Name of compound	Molecular Formula	Molecular weight	Similarity Index	Retention Time	Peak Area %
1	Limonene	$C_{10}H_{16}$	136	93	7.579	18.56
2	1,6-Octadien-3-ol, 3,7-dimethyl	$C_{10}H_{18}O$	154	95	9.539	4.19
3	Pentadecanoic acid	$C_{15}H_{30}O_2$	652	71	28.796	0.40
4	9-Hexadecenoic acid, 9-octadecenyl	$C_{34}H_{64}O_2$	504	74	30.475	0.47
5	1,2-Benzenedicarboxylic acid, butyl	$C_{20}H_{30}O_4$	304	88	30.926	3.72
6	n-Hexadecenoic acid	$C_{16}H_{32}O_2$	652	88	31.055	5.99
7	1-(+)-Abscorbic acid, 2,6-dihexadecano	$C_{38}H_{68}O_8$	652	87	32.852	1.15
8	Hexadecanoic acid, 2-hydroxy-1	$C_{19}H_{38}O_4$	330	72	33.322	1.15
9	Phytol	$C_{20}H_{40}O$	296	93	33.819	0.56
10	9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	280	81	34.382	7.77
11	Octadecanoic acid	$C_{18}H_{36}O_2$	284	86	34.819	1.92
12	Hexadecanoic acid, tetradecyl ester	$C_{30}H_{60}O_2$	452	80	36.429	2.68
13	Butyl 9,12-octadecadienoate	$C_{22}H_{38}O_2$	336	83	36.925	8.40
14	9-Octadecanoic acid (Z)-, hexadecyl	$C_{34}H_{66}O_2$	506	87	37.159	4.04
15	Butyl 9,12,15-octadecatrienoate	$C_{22}H_{38}O_2$	334	80	37.348	3.43

The phytochemical D-limonene was detected in all the extracts. D-limonene has been reported to possess anti-inflammatory, antioxidant [33], antinociceptive, anticancer, antidiabetic, anti-hyperalgesic, antiviral, gastroprotective and antifungal properties [34,35]. Some studies have proposed the use of limonene in active food packaging [36] and as a food preservative [37]. N-Hexadecenoic acid, Pentadecanoic acid, 9,12 Octadecanoic acid and Octadecanoic acid with common names Palmitic acid, Pentadecyclic acid, Omega-6 fatty acid/Linolenic acid and Stearic acid respectively were present in all the citrus peels extracts. These are fatty acids reported to have diverse biological activities including antioxidant, hypocholesterolemic, nematicide, pesticide [38], anti-inflammatory, antibacterial and antifungal properties [39,40]. Other important phytochemicals such as 1-(+)-Ascorbic acid 2,

Table 6
Selected phytochemical components of hexane extract of *Citrus sinensis* 'valencia'(HEV) identified by GC/MS analysis.

S/N	Name of compound	Molecular Formula	Molecular weight	Similarity Index	Retention Time	Peak Area %
1	3 -Hexen-2-one	$C_6H_{10}O$	98	92	6.047	4.80
2	Limonene	$C_{10}H_{16}$	136	93	7.521	5.94
3	1-Octanol	$C_8H_{18}O$	130	97	8.640	0.31
4	.alpha.-Muurolene	$C_{15}H_{24}$	204	86	20.469	0.56
5	Isoledene	$C_{15}H_{24}$	204	93	21.054	1.31
6	Elemol	$C_{15}H_{26}O$	222	89	21.715	0.43
7	Dodecanoic acid	$C_{12}H_{24}O_2$	200	95	22.198	1.96
8	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	92	26.715	0.87
9	Pentadecanoic acid	$C_{15}H_{30}O_2$	652	81	28.788	0.83
10	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	652	88	31.246	15.23
11	Heptadecanoic acid	$C_{17}H_{34}O_2$	270	87	32.905	1.09
12	9,12-Octadecadienoic acid (Z,Z)-, methy	$C_{19}H_{34}O_2$	294	86	33.469	0.60
13	Phytol	$C_{20}H_{40}O$	296	83	33.820	0.39
14	Oxazole, 5-phenyl-	C_9H_7NO	145	89	34.567	9.62
15	Octadecanoic acid	$C_{18}H_{36}O_2$	284	84	35.009	3.47

Table 7
Selected phytochemical components of hexane extract of *Citrus sinensis* 'washinton'(HEW) identified by GC/MS analysis.

S/N	Name of compound	Molecular Formula	Molecular weight	Similarity Index	Retention Time	Peak Area %
1	3-Hexen-2-one	$C_6H_{10}O$	98	91	5.996	1.36
2	D-Limonene	$C_{10}H_{16}$	136	94	7.505	4.95
3	Copaene	$C_{15}H_{24}$	204	94	17.204	0.28
4	Isoledene	$C_{15}H_{24}$	204	93	21.055	1.04
5	Elemol	$C_{15}H_{26}O$	222	89	21.722	0.54
6	Dodecanoic acid	$C_{12}H_{24}O_2$	200	95	22.144	0.85
7	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	91	26.697	0.76
8	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	85	28.796	0.71
9	l-(+)-Ascorbic acid 2,6-dihexadecanoa	$C_{38}H_{68}O_8$	652	88	31.238	17.77
10	Heptadecanoic acid	$C_{17}H_{34}O_2$	270	88	32.907	1.35
11	Phytol	$C_{20}H_{40}O$	296	80	33.826	0.34
12	9,12-Octadecadienoic acid (Z,Z)	$C_{18}H_{32}O_2$	280	76	34.667	26.36
13	E,E,Z-1,3,12-Nonadecatriene-5,14-di	$C_{19}H_{34}O_2$	294	86	34.761	9.87
14	Octadecanoic acid	$C_{18}H_{36}O_2$	284	85	35.007	4.20
15	Hexadecanoic acid, hexadecyl ester	$C_{32}H_{64}O_2$	480	78	36.445	4.06

Table 8
Selected phytochemical components of hexane extract of *Citrus sinensis* 'thompson navel' (HEI) identified by GC/MS analysis.

S/N	Name of compound	Molecular Formula	Molecular weight	Similarity Index	Retention Time	Peak Area %
1	3-Hexen-2-one	$C_6H_{10}O$	98	92	5.974	2.63
2	Limonene	$C_{10}H_{16}$	136	93	7.476	2.04
3	n-Decanoic acid	$C_{10}H_{20}O_2$	172	93	17.015	0.59
4	Isoledene	$C_{15}H_{24}$	204	91	21.032	0.48
5	Dodecanoic acid	$C_{12}H_{24}O_2$	200	96	22.181	4.29
6	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	92	26.722	3.00
7	Pentadecanoic acid	$C_{15}H_{30}O_2$	652	79	28.752	1.10
8	18-Nonadecenoic acid	$C_{19}H_{36}O_2$	296	84	30.425	0.83
9	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	652	88	31.089	14.11
10	Heptadecanoic acid	$C_{17}H_{34}O_2$	652	88	32.833	2.40
11	9,12-Octadecadienoic acid (Z,Z)-, me	$C_{18}H_{32}O_2$	294	87	33.444	0.38
12	Phytol	$C_{20}H_{40}O$	296	93	33.793	0.91
13	9,12-Octadecadienoic acid (Z,Z)	$C_{18}H_{32}O_2$	280	79	34.421	16.77
14	6-Octadecenoic acid	$C_{18}H_{34}O_2$	282	88	34.529	11.48
15	Octadecanoic acid	$C_{18}H_{36}O_2$	284	86	34.840	4.09

6-hexadecanoate, linalool and phytol were also detected in the samples.

Considering the rich bioactivity profile of the compounds identified in the extracts by GC/MS analysis, especially their antioxidant, anti-inflammatory and antimicrobial attributes; it can be inferred that the compounds can work synergistically to bring about a preservative effect in fresh food and food products. The possibility of utilizing orange peel extracts independently or as a component of a food preservative may also enhance the functional properties, flavour and nutritional profile of food. This study also suggests the use of orange peels as a source of phytochemicals for pharmaceutical and cosmetic industries.

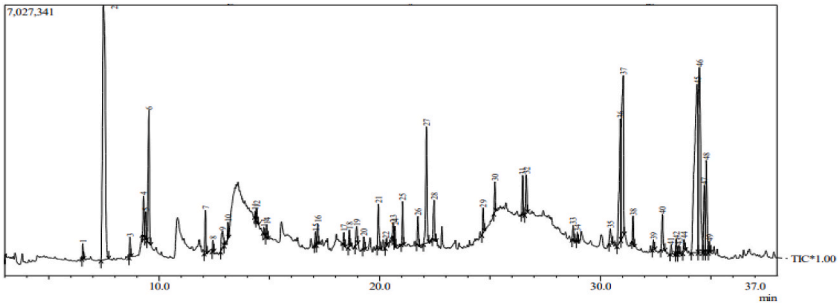

Fig. 1. Chromatogram of ethanol extract of *Citrus sinensis* 'valencia'(EEV).

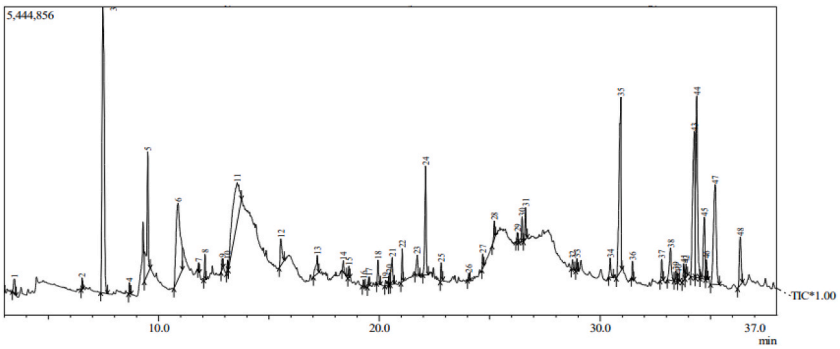

Fig. 2. Chromatogram of ethanol extract of *Citrus sinensis* 'washinton' (EEW).

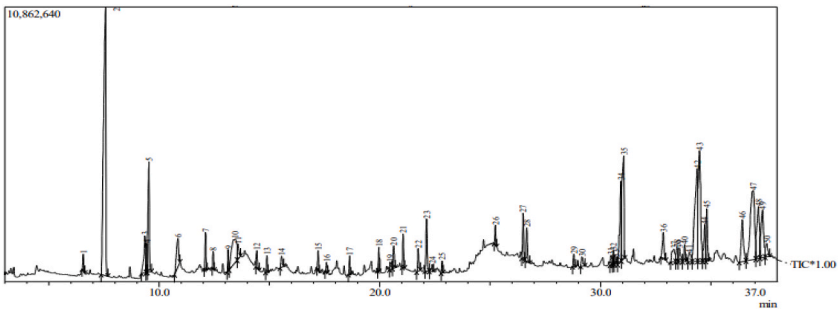

Fig. 3. Chromatogram of ethanol extract of *Citrus sinensis* 'thompson navel' (EEI).

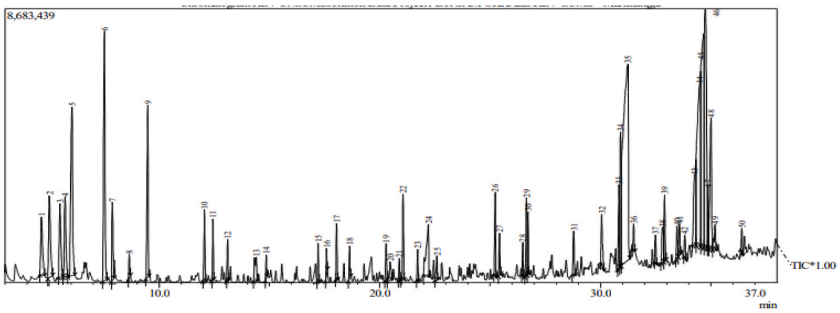

Fig. 4. Chromatogram of hexane extract of *Citrus sinensis* 'valencia'(HEV).

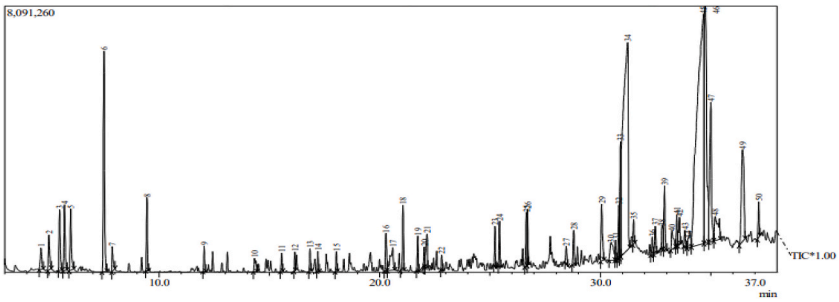

Fig. 5. Chromatogram of hexane extract of *Citrus sinensis* 'washinton' (HEW).

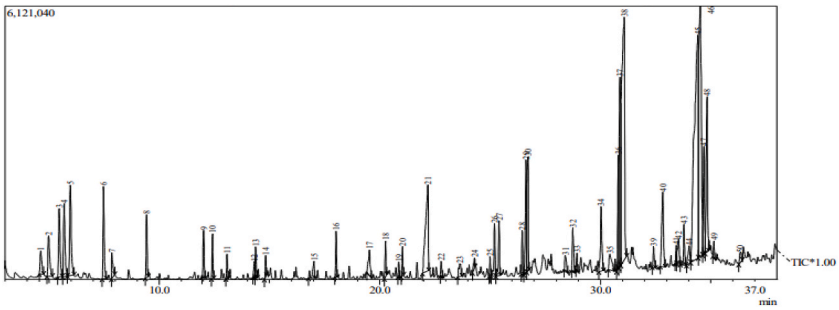

Fig. 6. Chromatogram of hexane extract of *Citrus sinensis* 'thompson navel' (HEI).

3.4. Antioxidant activity of Citrus sinensis peel extracts

The FRAP method is based on the reduction of Fe^{3+} to Fe^{2+} forming an intense blue coloured ferrous complex under acidic conditions. The antioxidant potential of a compound/sample can reliably be determined by measuring its reducing power; a higher reducing power indicates a better ability to donate the electron. Thus, free radicals accept the donated electron and form stable substances, which results in the termination of radical chain reactions [41].

The extracts in the present study demonstrated good antioxidant activity with the highest FRAP value of 9.09 mg/100g recorded in EEV and the least value of 1.45 mg/100g obtained in HEI (Table 9). It was also observed that the ethanolic extracts had slightly higher antioxidant activity than the hexane extracts. This may be due to the variation in the level of extraction of antioxidant compounds by the solvents. This result is comparable with literature [42] which reported that extracts from orange peels have good antioxidant activity. Other similar results were reported when reducing power of orange peels were assessed [43].

Hydrogen peroxide is a highly reactive oxygen species that can destroy a wide range of biological substrates, including carbohydrates, DNA, proteins, or polyunsaturated fatty acids. Preventing such hazardous interactions is critical for human health as well as the shelf life of foods, cosmetics, and medications [44]. As shown in Table 9, citrus peels extract scavenged hydrogen peroxide. The ethanolic peels extracts had higher H_2O_2 scavenging activities of 1.23, 0.86 and 1.39 mg/100g, respectively while the hexane extracts had 0.94, 0.81 and 0.76 mg/100g and was significantly lower ($p < 0.05$). The findings revealed that all of the samples possess H_2O_2 scavenging activity, which might be attributed to the antioxidant nature of the samples, and the presence of phenolic groups that could donate electrons to hydrogen peroxide, thereby neutralizing it to water. This result is consistent with other research works which reported positive hydrogen peroxide scavenging ability of a plant extract using the same method [45–47].

The Cupric Reducing Antioxidant Capacity (CUPRAC) assay is based on the reduction of Cu (II) to Cu (I) by antioxidants present in a

Table 9
Antioxidant activity of *Citrus sinensis* peel extracts (mg/100g).

Sample	FRAP	H_2O_2	CUPRAC
EEV	9.09 ± 0.13^{de}	1.23 ± 0.08^{cd}	0.90 ± 0.02^{b}
EEW	7.93 ± 0.81^{c}	0.86 ± 0.06^{ab}	0.83 ± 0.02^{b}
EEI	7.98 ± 0.55^{d}	1.39 ± 0.00^{de}	0.43 ± 0.1^{a}
HEV	8.22 ± 0.23^{d}	0.94 ± 0.01^{c}	0.94 ± 0.02^{b}
HEW	7.06 ± 0.01^{b}	0.81 ± 0.00^{a}	1.15 ± 0.01^{c}
HEI	1.45 ± 0.04^{a}	0.76 ± 0.02^{a}	2.04 ± 0.06^{d}

*Values within the same column with the same superscript are not significantly different at $p < 0.05$. EEV = ethanol extract of Valencia peels; EEW = ethanol extract of Washington Navel peels; EEI = ethanol extract of Ibadan sweet peels; HEV = hexane extract of Valencia peels; HEW = hexane extract of Washington Navel peels; HEI = hexane extract of Ibadan sweet peels.

plant extract using copper (II) neocuproine reagent as the chromogenic oxidant. A significant variation of CUPRAC antioxidant capacity was observed in the peel extracts analyzed, as shown in Table 9. Citrus peels extracts gave CUPRAC values ranging between 0.43 and 2.04 mg/100g, representing variation of approximately 4-fold. In this assay, the hexane extracts had higher CUPRAC values compared to the ethanolic extracts. It is worth mentioning that the CUPRAC assay is useful for determining antioxidant capacity in a wide variety of polyphenols, including phenolic acids, flavonoids, carotenoids, anthocyanins, as well as for thiols (glutathione), synthetic antioxidants, vitamins C and E [48].

4. Conclusion

This study analyzed phytochemical profiles using gas chromatography/mass spectrometry analysis and determined antioxidant capacities of ethanol and hexane peel extracts from three common varieties of *Citrus sinensis*: 'Valencia', 'Washington', and 'Thompson Navel'. The phytochemicals identified include flavonoids, alkaloids, terpenoids, phenolic acids, tanins, saponins, phytosterols, diterpenes and glycosides with higher levels typically found in the ethanol extracts. Gas chromatography mass spectrometry validation confirms the presence of these bioactive phytochemicals in orange peels, while the antioxidant assay provides preliminary evidence that orange peels may have beneficial antioxidant properties. The presence of bioactive compounds such as D-limonene, ascorbic acid and several fatty acids including n-hexadecenoic acid, pentadecanoic acid, and 9,12 octadecanoic acid infers that these underutilized and otherwise wasted peels have great prospects in food and drug value addition. 'Valencia' exhibited the highest antioxidant capacity as well as uniquely high phenolic and ascorbic acid contents, which may confer its superior activity in pharmaceutical and food industries as bio-preservatives. While these findings provide useful foundational insights, examination of only three geographically limited Citrus sinensis varieties restricts generalized inferences across broader citrus diversity. Further isolation, purification, and testing of the specific phytochemical constituents would be required to fully characterize the functional bioactive properties cum underlying biochemical mechanisms of action of citrus sinensis peels. That notwithstanding, the results suggest that orange fruit peels contain compounds that may be further explored for their antioxidant, pharmaceutical, nutraceutical or other industrial applications.

CRediT authorship contribution statement

Ogo Ogo: Writing – review & editing, Validation, Supervision, Project administration, Methodology, Conceptualization. **Ngutor Hembafan:** Writing – original draft, Investigation, Funding acquisition, Data curation, Conceptualization. **Raphael Amokaha:** Writing – review & editing, Supervision. **Oloche Jeremiah:** Writing – review & editing. **Bawa Inalegwu:** Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Ngutor Hembafan reports financial support was provided by UNESCO International Center for Biotechnology, Nsukka, Nigeria. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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