



Original Article

Characterisation of orange (*Citrus sinensis* L) mesocarp extract constituents as a potential storage agent for tomato fruits**^{1,3}Zubairu, A., ¹Zubair, A. B., ¹James, S., ²Egwim, E. C. and ^{*1}Ocheme, O. B.****¹Department of Food Science and Technology, Federal University of Technology, Minna****²Department of Biochemistry, Federal University of Technology, Minna****³Department Laboratory Science and Technology, Waziri Umaru Federal Polytechnic, Birnin Kebbi**

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ABSTRACT

Plant extracts have taken centre stage as alternatives to man-made preservatives in the food industry as they offer benefits such as safety, longer shelf life and consumer acceptance. This study characterised the constituents of ethanol and nhexane extracts of orange mesocarp. Orange mesocarp constituents were extracted using ethanol, n-hexane and an equal mixture of ethanol and n-hexane. Plant extracts have taken centre stage as alternatives to man-made preservatives in the food industry as they offer benefits such as safety, longer shelf life and consumer acceptance. The aim of this study was to identify the constituents of orange mesocarp extracts with a view to using the extracts for preservation of tomato fruit. The extracts' constituents were characterized using GC-MS analysis. Results obtained showed that mixture of ethanol/hexane recorded the highest extract yield while hexane had the lowest percentage yield (69% and 41% respectively). The ethanolic extract had the highest concentration of bioactive compounds while n-hexane extract had the lowest concentration. Bioactive compounds present in the extracts were flavonoids, alkaloids, glycosides, tannins and saponins. GC-MS analysis revealed that the ethanol, n-hexane and ethanol/n-hexane extracts contain 17, 15, and 20 bioactive compounds, respectively. Some of the compounds identified by GC-MS analysis include hexadecenoic acid, palmitic acid, erucic acid; stearic acid, octadecanoic acid and pentanoic acid. The constituents of the extracts indicate that *Citrus sinensis* mesocarp extract can be useful in fruit preservation.

Keywords: orange, mesocarp, extracts, constituents, characterized, bio-active, preservation

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INTRODUCTION

Tomato (*Solanum lycopersicum* L) is arguably the most consumed vegetable worldwide as it serves as an ingredient in

a wide range of raw, cooked and processed foods. Tomato plays significant roles in human nutrition due to their excellent antioxidant properties [1].

Regular consumption of tomatoes enhances immune system [2] and reduces the risk of chronic diseases such as cardiovascular diseases and cancer [3]; gastrointestinal disorders and diabetes mellitus4.

It is a good source of natural antioxidants such as carotenoids (β -carotenoids and lycopene), ascorbic acid, tocopherol and bioactive phenolic compounds [5]. These compounds possess many medicinal and pharmacological properties such as inhibition of reactive oxygen species (free radicals scavenging), modulation of enzymatic activities, cytokine expression and signal transduction pathways [6,7]. Unfortunately, tomatoes are highly perishable and substantial quantities are lost in the course of transportation and handling.

Tomato preservation is a crucial aspect of food processing, as tomatoes are highly perishable and susceptible to spoilage. Various methods have been employed to extend the shelf life of tomatoes, including canning, freezing, dehydration, pickling and high-pressure processing among others. Each preservation method has its advantages and limitations, and the choice of method depends on the desired outcome, resources, and intended use of the preserved tomatoes. The major limitation of some of these methods is the use of chemicals such as sodium metabisulphite and sodium benzoate. According to Shi et al. [8], there exists abundant evidence that some of these chemicals impacts health negatively in the form of obesity, cancer and heart issues. Consequently, there is a major shift towards natural plant extracts from synthetic chemicals due to their harmful effects on human health and the environment [9].

Plant extracts have gained a great deal of attention due to their various health

benefits and possible agricultural applications. According to Pinto *et al.* [10] plant extracts have taken centre stage as alternatives to man-made preservatives in the food industry as they offer benefits such as safety, longer shelf life and consumer acceptance. Mann *et al.* [11] reported that Aloe vera can be used for active packaging due to its antimicrobial and antioxidant properties and it has been utilized as an edible coating in functional food development. According to Ali *et al.* [12], application of aloe vera gel to Litchi chinensis not only reduced weight loss and browning index but also increased ascorbic acid and total phenolic contents. Same authors [12] also reported a reduction in moisture loss and a good glossy appearance when 20% aloe vera gel was applied to grapes. Shah and Hashmi [13] reported that aloe vera gel reduced weight loss, rate of respiration and ethylene production in mango. Aloe vera extracts did not only delay weight loss and enhanced firmness in banana, it also enhanced banana's phenolic content and antioxidant activity [14].

According to Monika *et al.* [15], lemongrass oil extract possesses antioxidant, antimicrobial, anti-inflammatory and anti-cancer properties. The authors further reported that the extracts can serve as anti-fungal agent in foods and went ahead to strongly recommend its use in the food industry. Da Silva *et al.* [16] reported that lemongrass essential oil sustains the quality traits of fruits throughout postharvest handling and that spraying lemongrass essential oil on blackberries in combination with micro-fibrillated cellulose at different concentrations-maintained blackberry fruits' freshness and colour. The authors further reported that applications of lemongrass essential oil significantly preserved the physical

quality parameters of cucumber during storage.

Pirozzi *et al.* [17] reported that neem extract contains several bioactive compounds which are effective as insect repellents. The authors reported that application of neem extract to tomatoes as an edible coating caused an improvement in texture,

colour and flavour. A study done by Siddiqua *et al.* [18] revealed that application of 20-40% neem leaf extract enhanced physico-chemical parameters of banana. Mbili *et al.* [19] 1% carboxyl methyl cellulose and two percent moringa leaf extract on avocado and reported lower weight loss, lower respiration rate and lower ethylene production thereby extending the shelf life of avocado.

Oranges (*Citrus sinensis L*) is one of the most widely grown fruit crops in Nigeria. The fruit consists of the exocarp (the outermost layer), mesocarp (the middle layer) and endocarp (the innermost layer). The only edible part of the orange is the endocarp which is filled with juice while the exocarp and mesocarp are considered as waste. The exocarp is the outermost peel which is underlined by a mesocarp which is a sponge-like layer made up of various pore spaces and connected colorless cells, occurring close to the peel. Oranges are locally abundant in Nigeria, and its mesocarp constitutes a large portion of the waste biomass [20]. Oikeh *et al.* [21] reported that orange mesocarp is a rich source of bioactive compounds such as flavonoids phenolic acids, glycoside, saponins, organic acids, steroids, esters, alcohols and alkaloids. The use of orange mesocarp extract as a protective barrier and preservative for tomato fruit and paste respectively will help convert orange mesocarp from a waste material to a useful raw material

for tomato preservation thereby mitigating its negative effects on the environment. The extension of the keeping quality of tomato fruit and paste will help reduce losses of tomato fruit and paste during transportation and after processing/storage respectively. This will in turn reduce farmers' losses and improve the country's balance of trade and also strengthen the local currency through import reduction. Also, the use of orange mesocarp extract in preserving tomato paste will reduce the use of sodium benzoate thereby eliminating the risk of high sodium intake and its attendant health risks. The aim of this study is to identify the constituents of orange mesocarp extracts with a view to using the extracts for tomato preservation.

MATERIALS AND METHODS

Materials

Fresh and mature orange (*Citrus sinensis L*) fruits were purchased from *Kasuwan Gwari* in Minna, Nigeria. All the samples were packed in polyethylene bag and transported to the Food Processing Laboratory, Department of Food Science and Technology, Federal University of Technology Minna, Niger state, Nigeria for processing and analyses. All reagents used were of analytical grade and were purchased from a supplier in Minna, Nigeria

Extraction of orange mesocarp constituents using ethanol, n-hexane and equal mixture of ethanol and n-hexane

Ethanol, n-hexane, and equal mixture of ethanol and n-hexane were used to extract constituents of *Citrus sinensis* mesocarp [22]. The orange fruits were thoroughly washed with distilled water and then peeled. The mesocarp and the endocarp components of the fruits were

separated using sharp knife. The mesocarp were cut into small pieces (1-2 cm) and then dried in oven at 40°C for 5 hours. The dried fruits mesocarps were pulverized into fine powder using mortar and pestle. 50 g of the orange mesocarp flour was separately extracted with 250 mL of ethanol, n-hexane and mixture of ethanol and n-hexane solvent using Soxhlet apparatus for 5 hours. The extracts were filtered using Whatman filter paper No 1 and the filtrates were concentrated under reduced pressure at 40°C using rotary evaporator. The extracts obtained were stored in the refrigerator at 4°C for further analysis.

Determination of extract yield

The yield of the extracts was calculated as follows [22]. Three empty containers were firstly weighed (w_0) using digital weighing balance. The ethanol, n-hexane, and ethanol/n-hexane extracts of *Citrus sinensis* mesocarp were separately transferred into the containers. The weight (w_2) of each container containing the extract was then measured and recorded. Finally, the weight of each extract (w_1) was then obtained by subtracting the weight of empty container from the weight of container with extract. The yield of each extract was obtained using the formula:

$$\% \text{ yield of extract} = \frac{w_2 - w_1}{w_0} \times 100\%$$

Where w_0 is the weight of empty container, w_1 is the weight of extract and w_2 is the weight of extract and container.

Qualitative phytochemicals screening

The extracts were tested for the presence alkaloids using Wagner's test method [23]; flavonoids, glycosides, tannins,

steroids and terpenoids presence were measured using procedures described by Ibrahim *et al.* [24]; saponins' presences were tested using the procedure described by Abubakar *et al.* [25]; and the method of Trease and Evans [26] was used to test for the presence of anthroquinones and phenols.

Quantitative phytochemicals analysis

Quantitative analyses were also done to determine the quantity of the phytochemicals present in each of the extracts. Alkaloids, flavonoids and glycosides were quantified using the procedure described by Ibrahim *et al.* [24]; the saponin contents were measured using the method outlined by El-Olemyl *et al.* [27]; tannins were quantified using the method of AOAC [28].

Determination of specific constituents of the phytochemicals

The constituents of the phytochemicals were determined using gas chromatography/mass spectroscopy (GC-MS). This was done using a GC-MS instrument (Shimadzu Company's model GC/MS-QP2010-Ultra, serial number 020525101565SA). The crude sample extract was mixed with 5 mL of 96% ethanol and ultrasonicated for 30 minutes at 55°C. The extract was filtered using Whatman filter paper No 1 and then injected into GC-MS operating in EI mode at 70 eV. A capillary column (Rtx-5 ms-30m $\times$ 0.25mm $\times$ 0.25 μ m). Helium was used as the carrier gas at a flow rate of 1.69 ml/min. The temperature initially set at 50 °C with a rate of 7 °C/min, and then 180 °C. The rate was changed to 10 °C/min, reaching a final temperature of 280°C. The injection port temperature was 300°C, the ion source temperature was 200 °C, and the interface temperature was 250°C. The sample was

analyzed in scan mode in the range of m/z 40–500 charges to ratio, with a total run time of 28 minutes. The compounds in the sample were identified by comparing their retention index and mass fragmentation patterns.

Data analysis

The experiments were conducted in triplicate and the results obtained were statistically analysed using One-Way Analysis of Variance (ANOVA). The

significant differences among the mean values were determined using Duncan multiple range test at 95 % confidence level.

RESULTS

Orange mesocarp extracts yield

The yields of the three extracts are shown in Table 1. The Ethanol/hexane extract was significantly higher ($p < 0.05$) with 68.8% yield than ethanol with 59.6% and n-hexane with 41.1%.

Table 1: Effect of solvent on the yield of *Citrus sinensis* mesocarp extract

Sample	Yield (%)
Ethanol extract	59.57 ^b ±0.12
Hexane extract	41.12 ^c ±0.07
Ethanol/Hexane extract	68.82 ^a ±0.10

Means with different superscript are significantly different ($p < 0.05$)

Qualitative phytochemical composition of *Citrus sinensis* mesocarp extracts

The phytochemicals present in *Citrus sinensis* mesocarp extracts are shown in Table 2. Alkaloids were detected in high concentration in the ethanol extract but were of moderate concentration in hexane and ethanol/hexane extracts. Flavonoids were detected in high

concentration in ethanol and ethanol/hexane extracts but low in hexane extract. Glycosides and steroids were found in moderate concentrations in all the extracts while tannins and phenols were moderate in ethanol and ethanol/hexane extracts but low in hexane extract. Saponins, anthraquinones and terpenoids were found in low concentrations in ethanol and ethanol/hexane extracts but absent in hexane extract.

Table 2: Phytochemical Composition of *Citrus sinensis* mesocarp extracts

Phytochemical	Ethanol	n-Hexane	Ethanol + n-hexane
Alkaloids	+++	++	++
Flavonoids	+++	+	+++
Glycosides	++	++	++
Tannins	++	+	++
Saponins	+	-	+
Steroids	++	++	++
Phenols	++	+	++
Anthraquinones	+	-	+
Terpenoids	+	-	+

Key: +++ = High concentration; ++ = Moderate concentration; + = Low concentration; - = Not detected.

Phytochemical components of *Citrus sinensis* mesocarp extracts detected by GC-MS analysis

Tables 3, 4 and 5 show the compounds identified in the extracts of *Citrus sinensis* mesocarp while their chromatograms are illustrated in figures 1, 2 and 3. The ethanol extract chromatogram (Figure 1) showed 17 peaks which indicate the presence of 17 different compounds. The results showed that hexadecenoic acid was the major compound identified with highest percentage up to 38.77 %. Other compounds are Pyrogallol, Fouramine Brown AP, Fourrine 85, Pyrogallol acid; palmitic acid, 1-pentadecanecarboxylic acid; 9-Tetradecenal; and 9-tetradecenal.

The major compounds identified in the n-hexane extract were erucic acid; stearic acid, hydrofol acid 150, hystrene T-70; octadecanoic acid, hystrene S-97; and palmitic acid, pentadecanecarboxylic acid among other compounds. A total of twenty peaks were observed in the GC-MS chromatogram (Figure 3) of the ethanol/n-hexane extract indicating the presence of twenty different compounds majorly among which are erucic acid, oxacyclotetradecan-2-one, 13-methyloxacyclotetradecan-2-one; stearic acid, hystrene S-97, hystrene T-70; and 2-octadecadecen-1-ol, 11-tridecen-1-ol, 2-tridecenal, pentanoic acid.

Table 3: Phytochemical Components of Ethanol Extract of *Citrus sinensis* Mesocarp

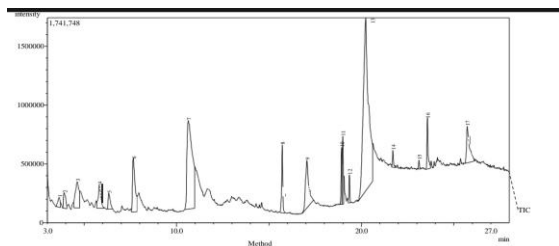
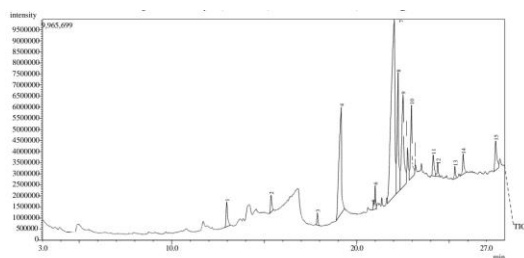
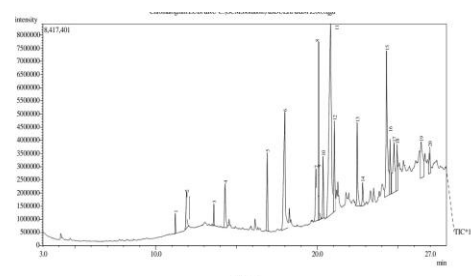
S/ N	Bioactive Compounds	Molecular Formula	Molecular Weight	Retention Time (min)	Peak Area (%)
1	Cyclopentanone, 2-Methylcyclopentanone	C ₆ H ₁₀ O	98	3.656	0.92
2	1-Nonanol, Octyl carbinol, Pelargonic alcohol	C ₉ H ₂₀ O	144	3.913	1.48
3	1,5-Cyclooctadien-4-one, 2,6-Cyclooctadien-1-one	C ₈ H ₁₀ O	86	4.630	4.03
4	1,3-Butadiene-1-carboxylic acid, 2,4-Pentadienoic acid.	C ₅ H ₆ O ₂	98	5.820	2.65
5	1,3-Dioxolane, 2,4,5-Trimethyl-1,3-dioxolane	C ₆ H ₁₂ O ₂	116	6.328	1.39
6	Borane, Triisopropylborane	C ₉ H ₂₁ B	140	7.656	6.06
7	Pyrogallol, Fouramine Brown AP, Fourrine 85, Pyrogallol acid	C ₆ H ₆ O ₃	126	10.640	20.54
8	Hexadecanoic acid, Metholene, Methyl hexadecanoate	C ₁₇ H ₃₄ O ₂	270	15.716	3.28
9	Palmitic acid, Pentadecanecarboxylic acid	C ₁₆ H ₃₂ O ₂	256	17.050	6.60
10	Linolelaidic acid, Methyl linolelaidate	C ₁₉ H ₃₄ O ₂	294	18.925	2.05
11	11-Octadecenoic acid, 11-octadecenoate	C ₁₉ H ₃₆ O ₂	296	18.987	2.83
12	Behenic acid, Methyl behenate.	C ₂₃ H ₄₆ O ₂	354	19.348	1.03
13	Hexadecenoic acid.	C ₁₆ H ₃₀ O ₂	254	20.231	38.77
14	1-Fluorononane,.	C ₉ H ₁₉ F	146	21.699	0.74
15	cis-Undec-4-enal.	C ₁₁ H ₂₀ O	168	23.115	0.39
16	10-Undecenal	C ₁₁ H ₂₀ O	168	23.572	2.62
17	9-Tetradecenal.	C ₁₄ H ₂₆ O	210	25.723	4.62

Table 4: Phytochemical Components of n-Hexane Extract of *Citrus sinensis* Mesocarp

S/N	Bioactive Compounds	Molecular Formula	Molecular weight	Retention Time (min)	Peak Area (%)
1	n-Undecoic acid, Undecylic acid, Hendecanoic acid	C ₁₁ H ₂₂ O ₂	186	15.370	1.42
2	Palmitic acid Pentadecanecarboxylic acid.	C ₁₆ H ₃₂ O ₂	256	12.969	2.28
3	Capric acid, Metholene 2095, Methyl caprate.	C ₁₁ H ₂₂ O ₂	186	17.874	0.73
4	Stearic acid, Hydrofol Acid 150, Hystrene T-70	C ₁₈ H ₃₆ O ₂	284	19.156	16.05
5	9,12-Octadecadienoic acid, Methyl linolelaidate.	C ₁₉ H ₃₄ O ₂	294	20.905	0.47
6	9-Octadecenoic acid, Methyl trans-9-octadecenoate	C ₁₉ H ₃₆ O ₂	296	20.992	1.23
7	Erucic acid, Prifrac 2990	C ₂₂ H ₄₂ O ₂	338	22.025	38.57
8	Palmitic acid, Pentadecanecarboxylic acid.	C ₁₆ H ₃₂ O ₂	256	22.235	11.26
9	Octadecanoic acid, Hystrene S-97,	C ₁₉ H ₃₄ O ₂	294	22.498	12.47
10	9,12-Octadecadienoic acid.	C ₁₉ H ₃₄ O ₂	294	22.959	6.66
11	10-Undecenoyl chloride	C ₁₁ H ₁₉ ClO	202	24.136	2.00
12	n-Hexadecanoic acid, 1-Pentadecanecarboxylic acid	C ₁₆ H ₃₂ O ₂	256	24.371	1.00
13	10-Undecenal, n-Undecylenic aldehyde.	C ₁₁ H ₂₀ O	168	25.304	0.80
14	Hexadecanoic acid. Palmitin.	C ₁₉ H ₃₈ O ₄	330	25.749	1.81
15	9-Octadecenal	C ₁₈ H ₃₄ O	266	27.507	3.25

Table 5: Phytochemical Components of Ethanol/n-Hexane Extract of *Citrus sinensis* Mesocarp

S/ N	Bioactive Compounds	Molecular Formula	Molecular weight	Retention Time (min)	Peak Area (%)
1	Metholene 2296, Methyl dodecylate.	C ₁₃ H ₂₆ O ₂	214	11.210	0.56
2	Dodecylic acid, Lauric acid, Laurostearic acid.	C ₁₂ H ₂₄ O ₂	200	11.894	2.26
3	Methyl tetradecanoate, Tetradecanoic acid, Methyl myristate.	C ₁₅ H ₃₀ O ₂	242	13.594	0.63
4	Palmitic acid, 1-Pentadecanecarboxylic acid.	C ₁₆ H ₃₂ O ₂	256	14.291	2.76
5	Tridecanoic acid, Methyl tridecanoate, Methyl ester of tridecanoic acid.	C ₁₄ H ₂₈ O ₂	228	16.901	3.76
6	Stearic acid, Hystrene S-97, Hystrene T-70.	C ₁₈ H ₃₆ O ₂	284	17.987	11.25
7	7,10-Hexadecadienoic acid, Methyl-7,10-hexadecadienoate.	C ₁₇ H ₃₀ O ₂	266	19.913	2.53
8	5-Octadecenoic acid, Methyl-5-octadecenoate.	C ₁₉ H ₃₆ O ₂	296	20.017	7.71
9	Linolelaidic acid, Methyl linolelaidate.	C ₁₉ H ₃₄ O ₂	294	20.095	2.26
10	Hexadecanoic acid, Methyl 15-methylhexadecanoate.	C ₁₈ H ₃₆ O ₂	284	20.360	2.29
11	Erucic acid, Oxacyclotetradecan-2-one, 13-Methyloxacyclotetradecan-2-one.	C ₂₂ H ₄₂ O ₂	338	20.820	24.69
12	n-Pentadecanoic acid, n-Pentadecylic acid	C ₁₅ H ₃₀ O ₂	242	21.057	4.73
13	1-Octanol, 2-Butyl-1-octanol.	C ₁₂ H ₂₆ O	186	22.473	3.80
14	Triacantanoic acid, Methyl melissicate, Methyl triacantanoate.	C ₃₁ H ₆₂ O ₂	466	22.800	1.14
15	2-Octadecadecen-1-ol, 11-Tridecen-1-ol, 2-Tridecenal, Pentanoic acid.	C ₁₈ H ₃₆ O	268	24.296	11.08
16	3-Hexadecyloxycarbonyl-5-(2-hydroxyethyl)-4-methylimidazolium ion	C ₂₄ H ₄₅ N ₂ O ₃	409	24.509	3.02
17	Heneicosanoic acid, Methyl henelcosanoate, Methyl heneicosanoate.	C ₂₂ H ₄₄ O ₂	340	24.748	5.79
18	2-Benzenedicarboxylic acid, Diisooctyl phthalate, Hexaplas M/O.	C ₂₄ H ₃₈ O ₄	390	24.943	3.41
19	Octadecane, Octadecyl vinyl ether.	C ₂₀ H ₄₀ O	296	26.428	4.67
20	4-Nitrobenzoic acid, Cyclobutyl 4-nitrobenzoate.	C ₁₁ H ₁₁ NO ₄	221	26.957	1.67

**Figure 1: GC-MS Chromatogram of Ethanol Extract of *Citrus sinensis* Mesocarp****Figure 2: GC-MS Chromatogram of n-Hexane Extract of *Citrus sinensis* Mesocarp****Figure 3: GC-MS Chromatogram of Ethanol/n-Hexane Extract of *Citrus sinensis* Mesocarp**

DISCUSSION

Orange mesocarp extracts yield

The significantly higher yield observed with the ethanol/hexane extracts may be due to synergistic effect of the two solvents which enhanced their extractive power compared with the individual solvents. Also, the higher yield observed with the ethanol extract in comparison with hexane may be due to ethanol having a higher polarity than hexane and this outcome is in agreement with the finding of Ogo *et al.* [22] who also reported higher yield of

ethanol extract of orange peel than the hexane extract.

Composition of *Citrus sinensis* mesocarp extracts

The higher concentrations observed in the ethanol and ethanol/hexane extract may be due to ethanol's high polarity and the synergistic effect of combining ethanol and hexane.

Phytochemical components of *Citrus sinensis* mesocarp extracts

These results show that orange mesocarp contains fewer compounds compared to orange peel, *Xylopi aethiopica*, *Zingiber officinale* and *Curcuma longa*. Ogo *et al.* [22] reported up to 50 different compounds in different orange peels while Okhale *et al.* [29] reported the presence of forty, twenty and thirty eight compounds in the essential oil of *X. aethiopica*, *Z. officinale* and *C. longa* respectively. The compounds identified in the three extracts can be used as emollients, preservative agents, antioxidants, fragrances, flavouring agents, emulsifiers, clouding and coating agents [30]. The presence of these compounds in the extracts indicates that extracts of orange mesocarp can be put to useful applications in the food, pharmaceutical and cosmetic industries.

CONCLUSION

The study used ethanol, n-hexane and an equal mixture of ethanol and hexane to extract the constituents of *Citrus sinensis* mesocarp which were then characterized using GC-MS analysis. The major bioactive compounds found in the extracts were, alkaloids, flavonoids, glycosides, tannins, steroids and phenols. Seventeen, fifteen and twenty compounds were identified in the ethanol, n-hexane and ethanol/n-hexane extracts respectively. Some of the

compounds identified by GC-MS analysis include hexadecenoic acid, palmitic acid, erucic acid; stearic acid, octadecanoic acid and pentanoic acid. The constituents of the extracts indicate that *Citrus sinensis* mesocarp extract can be useful in the food industry.

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