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Gas chromatography-Mass spectrometry (GC-MS) analysis of bioactive components present in grape citrus peel in Nigeria

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Abstract

Grapefruit (*Citrus paradisi*) is an important cultivar of the Citrus genus which contains a number of nutrients beneficial to human health. Grapefruit peels are usually thrown away in this part of Nigeria. The research work investigated the bioactive components present in an indigenous citrus peel, grape (*Citrus paradisi*). Grape fruits were purchased from the fruits garden market in D-Line, Port-Harcourt metropolis and washed with ionized water and allowed to shade dry. The peel of the fruits were separated and subjected to cold extraction using 95% ethanol. The extracts obtained were further extracted in dichloromethane and subjected to GC/MS analysis for characterization of various bioactive components. The gas chromatographic model: 789A (GC) analysis was performed on an agilent technologies interfaced with mass selective detector model: 5975(MSD). The results revealed 25 bioactive components in grape peel with n-Hexadecanoic acid showing the highest concentration of 20.36% and retention time of 18.522min. Nootkatone was the lowest component in the grape peel with concentration 0.74% and retention time of 16.459min. Results shows that grapefruit (*Citrus paradisi*) has considerable potential as a source of natural bioactive components with different retention times. These fruits residues which otherwise regarded as waste hold promising potentials for medicinal therapy and value added food supplements.

Keywords: Citrus peels; Bioactive; Gas chromatography; Mass spectrometry

1. Introduction

Citrus is a genus of flowering trees and shrubs in the rue family, Rutaceae which include fruits such as orange, lime, lemon, grapefruits, tangerine appear as a well-known promising source of multiple beneficial nutrients for human beings. Citrus fruits are one of the largest fruit crops in the world. These citrus fruits have a well-inscribed nutritional value, along with high levels of elemental bioactive compounds such as essential oils (EOs), flavonoids, limonoids and vitamins. Essential oils are a good source of several bioactive compounds, which possess antioxidative and antimicrobial properties. Essential oils are concentrated liquids of complex mixtures of volatile compounds and can be extracted from several plant organs. Citrus (Citrus L. from Rutaceae) is one of the most popular world fruit crops, contains active phytochemicals that can protect health. In addition to this, it provides an ample supply of vitamin C, folic acid, potassium and pectin. The contribution of citrus species in deterrence of life threatening diseases have been assessed [1] [2] [3] [4] and it has been reported that citrus fruits and citrus fruits extracts and citrus flavonoids exhibits a wide range of promising biological properties due to their phenolic profile and antioxidant properties [5] [6] [7] Global production of citrus fruits has significantly increased during the past few years and has reached 82 million tons in the years 2009-2010 of which oranges- commercially most important citrus fruits has accounts for about 50 million tons and 34% of which was used for juice production, yielding about 44% peel by-product [8] Therefore, a large amount of peels is produced every year. Citrus peel, the primary waste, is a good source of molasses, pectin and limonene and is usually

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dried, mixed with pulps and sold as cattle feed [9]. Uses of essential oils have received increased attention as natural additives for the shelf-life extension of food products, due to risk in using synthetic preservatives. The objective of this research is to investigate the bioactive components, their percentages, retention time, molecular weights and structures of the dichloromethane extract of grape citrus peel.

2. Material and methods

2.1. Preparation Ethanol Extract

A large quantity of grape fruits was procured from markets within Port Harcourt metropolis, Rivers State, Nigeria. The citrus fruit was washed with ionized water, dried up and peeled, after which the peels were blended using homemade grinder/blender into powder form. The peels were then weighed using a Radwag-Wagi electronic top loading balance (AS 220/C/2) to ensure uniformity in weight. The peels were then pureed using MarlexElectroline (Excella) blender. Two hundred grams (200g) of the milled sample was weighed and soaked in 100ml of 95% ethanol for 48 hours after which they were sieved using a muslin cloth and filtered with Whatman filter paper size 1. The filtrate was concentrated using rotary evaporator at 45℃ the weight of the concentrates were taken and the percentage yield calculated and kept at 4℃ until usage.

2.2. Determination of Essential Oils Content

The milled sample was extracted in dichloromethane after soaking for 5 days. Ten grams of the sample was weighed into a well dried stopper bottle and 20mls of the organic solvent was added. The mixtures were vigorously agitated and were left to stand for 5 days. The crude extract was collected by fitting into a quartz beaker, the process were repeatedly carried out for two consecutive times. The combined aliquot collected was concentrated on a steam bath to about 5ml and purified by passing through a pasture pipette packed with silica gel and anhydrous sodium sulphate on a membrane and air dried to about 2ml for gas chromatographic analysis.

The extract of the sample was subjected to GC/MS analysis, this group of powerful instruments interfaced helped to characterize the various compositions. The gas chromatographic Model: 7890A (GC) analysis was performed on an Agilent Technologies interfaced with Mass Selective Detector model: 5975C (MSD). The electron ionization was at a 70v with an iron source temperature at250℃ highly pure helium gas (99.9% purity) was used as carrier gas, while HP-5 (30mm X 0.25mm X 0.320μm) was used as the stationary phase. The oven temperature was at at 60℃ held for 0.5 minute and ramped to at 140℃ at the rate of 4^{o}C/minutes holding for a minute, then ramped to 280 degrees while holding for 5 minutes at the rate of at 8℃ /minutes. 1μ/l was auto injected.

3. Results

The results of the essential oils and bioactive components of the dichloromethane extract of grape citrus peels analyzed are shown in Figure 1 and Table 1 respectively. The chromatogram shows the various peaks while the tables consist of the bioactive components, their retention time, percentages, molecular weights and structures.

3.1. GC/MS and phytochemical analysis of dichloromethane extract of grape peel

Figure 1 shows the chromatogram of GC/MS of bioactive compounds of dichloromethane extract grape peel with highest peak observed at a retention time of 18.522min followed by retention time of 20.630min, 21.755min and 27.606min with the lowest peak observed at a retention time of 4.220min. Table 1 shows the presence of 25 active bioactive components in the dichloromethane extract of grape peels. N-Hexadecanoic acid was the highest in concentration (20.360% and retention time of 18.522min). This was followed by 9,12,-Octadecadienoic acid(Z,Z)-(11.710% and retention time of 20.573min), Isoauraptene(9.28% and retention time of 21.758min) and 2H-1-Benzpyrane-2-one, 7-[(3,7,-dimethyl-2,6,-octadienyl)oxy]-,(E)- (7.990% and retention time of 27.606min) respectively. Nootkatoone concentration was the lowest value of 0.74% at a retention time of 16.459min.

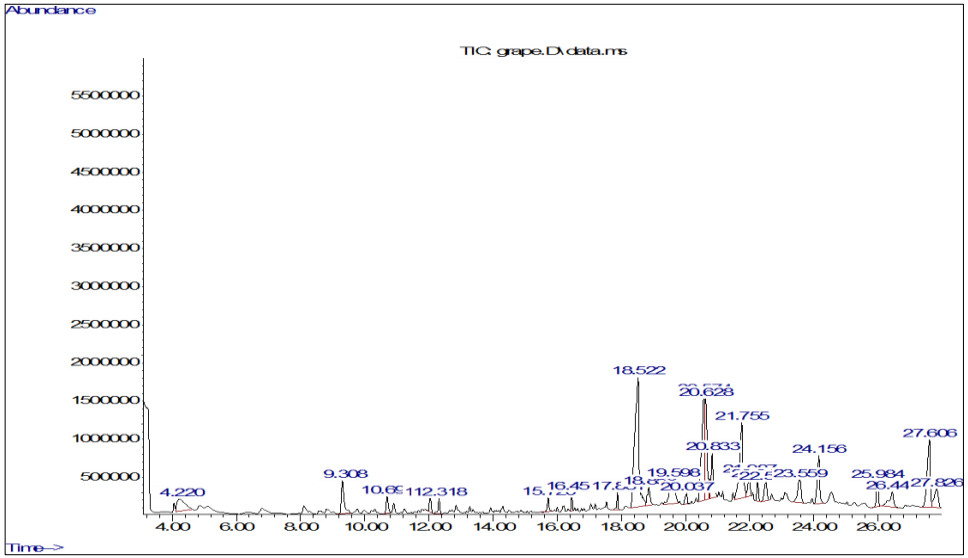
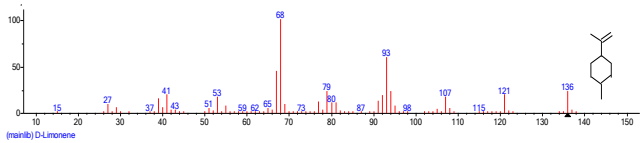
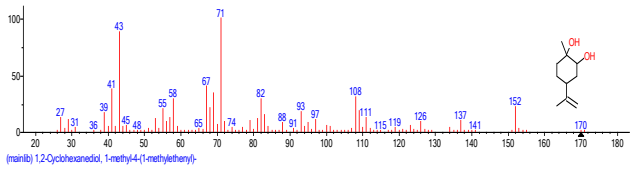
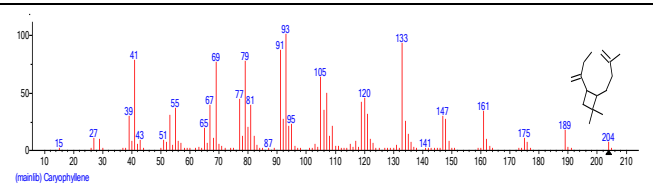
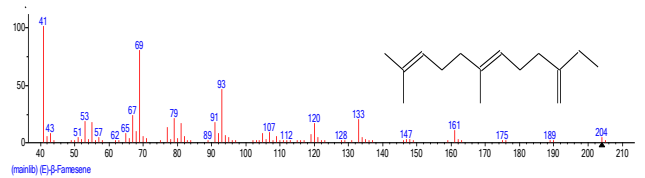
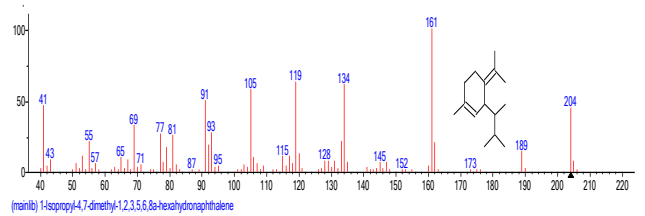
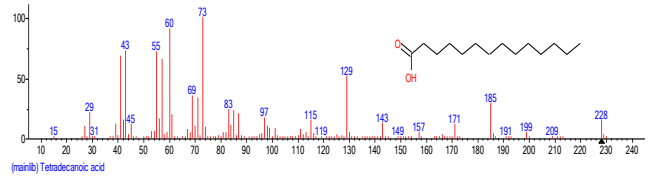
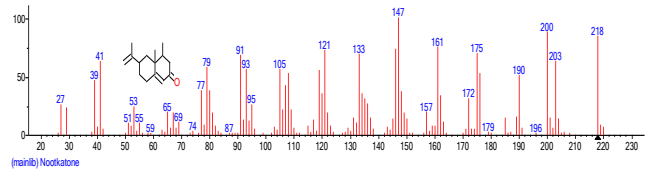
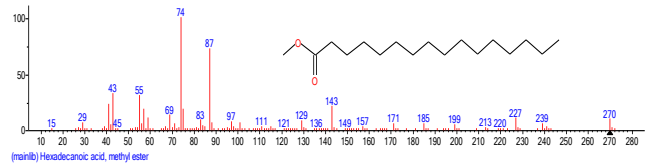
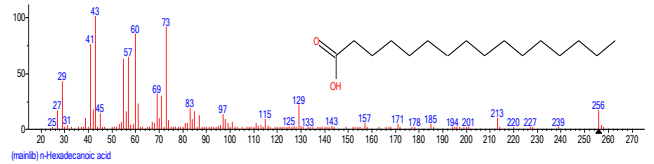
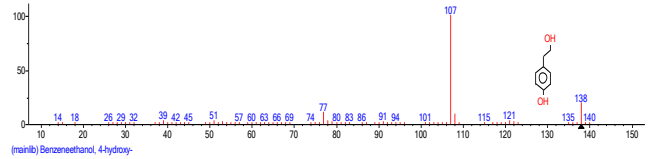
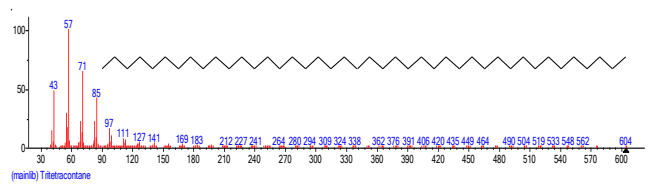
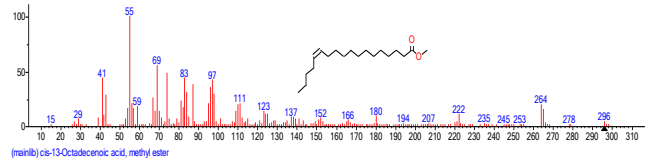
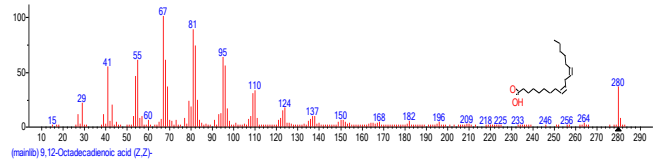


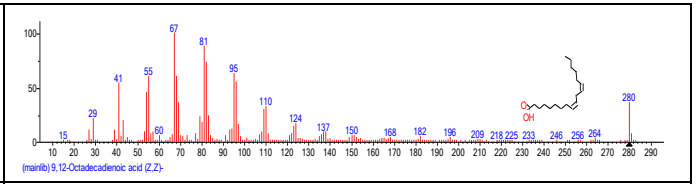
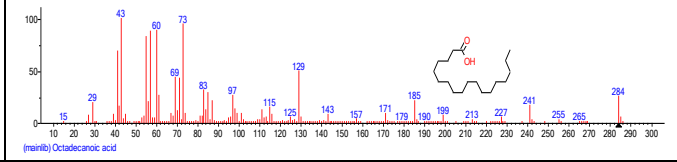
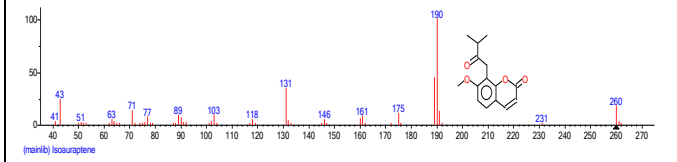
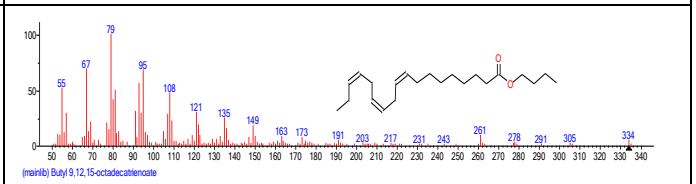
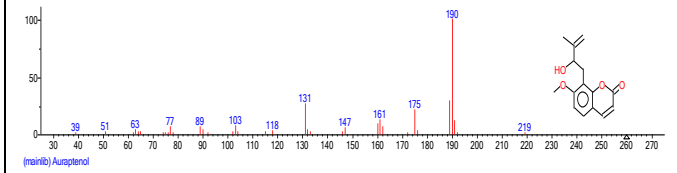
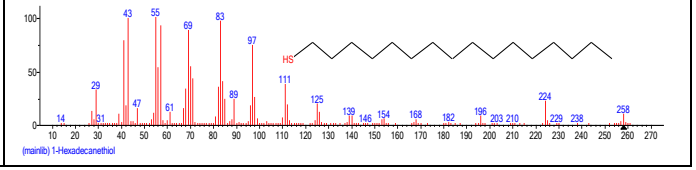
Figure 1 GC/MS Chromatogram of Grape Peel

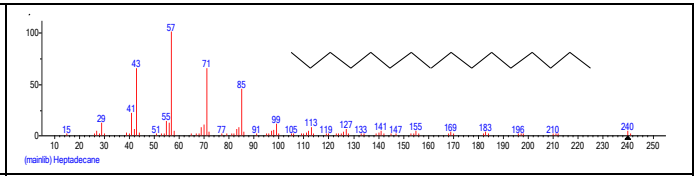
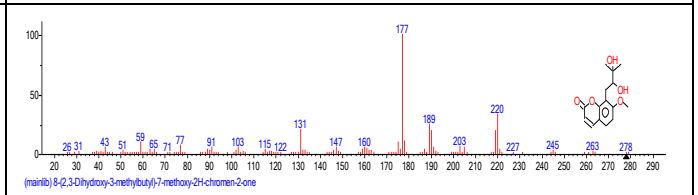
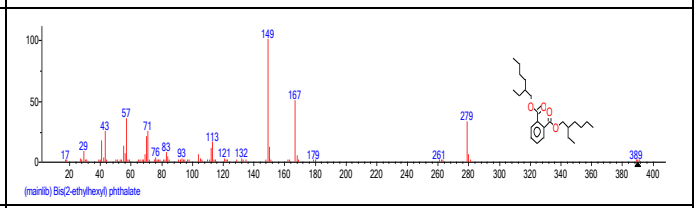
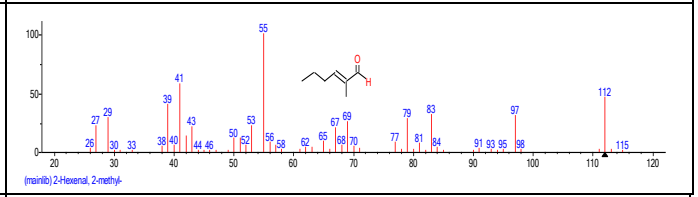
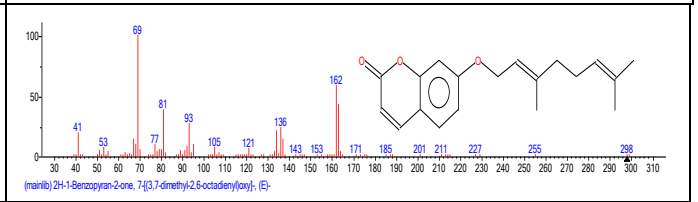
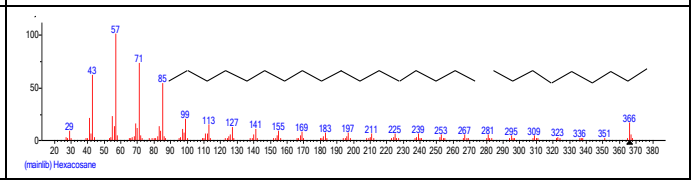
Table 1 Bioactive Components of Grape Peel (*Citrus paradisi*)

S/N	Compound	Retention Time (min)	Percentage of the total	Molecular formula	Molecular weight	Structure
1	D-Limonene	4.220	3.64	$C_{10}H_{16}$	136.24	
2	1,2-Cyclohexanediol, 1-methyl-4-(methylethenyl)-	9.307	3.40	$C_{10}H_{18}O_2$	170.2487	

3	Caryophyllene	10.697	1.28	$C_{15}H_{24}$	204.36	
4	(E)-.beta.-Famesene	12.047	1.04	$C_{15}H_{24}$	204.357	
5	1-Isopropyl-4,7-dimethyl-1,2,3,5,6 ,8a-hexahydronaphthalene	12.316	0.94	$C_{15}H_{24}$	204.351	
6	Tetradecanoic acid	15.721	0.82	$C_{14}H_{28}O_2$	228.3709	
7.	Nootkatone	16.459	0.74	$C_{15}H_{22}O$	218.34	

8	Hexadecanoic acid, methyl ester	17.878	0.81	$C_{17}H_{34}O_2$	270.4507	
9	n-Hexadecanoic acid	18.522	20.36	$C_{16}H_{32}O_2$	256.4241	
10.	Benzeneethanol, 4-hydroxy-	18.857	1.82	$C_8H_{10}O_2$	138.1638	
11.	Tritetracontane	19.600	4.53	$C_{43}H_{88}$	605.177	
12.	cis-13-Octadecenoic acid, methyl ester	20.035	0.81	$C_{19}H_{36}O_2$	296.4879	
13.	9,12-Octadecadienoic acid (Z,Z)-	20.573	11.71	$C_{18}H_{32}O_2$	280.4455	

14.	9,12-Octadecadienoic acid (Z,Z)-	20.630	7.98	$C_{18}H_{32}O_2$	280.4455	
15.	Octadecanoic acid	20.831	3.59	$C_{18}H_{36}O_2$	284.4772	
16.	Isoauraptene	21.758	9.28	$C_{19}H_{22}O_3$	298.38	
17.	Butyl 9,12,15-octadecatrienoate	21.987	1.81	$C_{22}H_{38}O_2$	334.5433	
18.	Auraptenol	22.250	1.20	$C_{19}H_{22}O_3$	298.38	
19.	1-Hexadecanethiol	22.507	1.95	$C_{16}H_{34}S$	258.506	

20.	Heptadecane	23.560	2.99	$C_{17}H_{36}$	240.4677	
21.	8-(2,3-Dihydroxy-3-methylbutyl)-7-methoxy-2H-chromen-2-one	24.155	4.29	$C_{15}H_{20}O_6$	296.319	
22.	Bis(2-ethylhexyl) phthalate	25.986	1.74	$C_{24}H_{38}O_4$	390.56	
23.	2-Hexenal, 2-methyl-	26.444	2.49	$C_7H_{12}O$	112.170	
24.	2H-1-Benzopyran-2-one, 7-[(3,7-dimethyl-2,6-octadienyl)oxy]-, (E)-	27.606	7.99	$C_{19}H_{22}O_3$	298.376	
25.	Hexacosane	27.829	2.79	$C_{26}H_{54}$	366.718	

4. Discussion

Every part of citrus family, starting from the leaves, the root, the back of the tree, the fruits juice etc which the peels are not exclusive has their functions. The fruits peel; grape (*Citrus paradise*), which was investigated for its essential oils. Grape peels revealed a number of 25 bioactive compounds with n-Hexadecanoic acid having the highest concentration of 20.36% and a retention time of 18.522min. The results agrees with at [10] which states that citrus peels contain significant amounts of biologically active polyphenols, specifically phenolic acids and flavonoids, which have exhibited important antioxidants, anti-inflammatory, anti-proliferative, anti-allergic, anti-viral, anti-carcinogenic, neuroprotective and antimicrobial properties. The presence of D-Limonene in grape peel agrees with [11] which state that Limonene is the most abundant compound of monoterpene hydrocabons for all of the examined juices, and [12] which states that experimental studies have demonstrated its analgesic, antibacterial, antiviral, hepatoprotective, anti-atherogenic, radical scavenging activity, uricosuric, hypothensive agent, carminative and antilarvicidal. N-Hexadecanoic acid with the highest percentage in grape has its therapeutic function such as: inhibitory activity and anti-inflammatory agent [13]. N-hexadecanoic acid shows inhibitory activity against mycobacterium tuberculosis [14]. . 9,12, -Octadecadienoic acid(Z,Z)- acid which is also one of the bioactive component in grape peel has its own benefits: 9,12,-Octadecadienoic acid(Z,Z)-, acid has antiarthritic and anti-inflammatory property [15]. [16]. stated that 9,12,-Octadecadienoic acid(Z,Z)- has functions such as Hypocholesterolemic, Anticoronary, Nematicide, Hepatoprotective, Hypocholesterolemic etc. These bioactive components and others have a lot of medicinal and synthetic values.

5. Conclusion

Recent research concerning essential oils of citrus peels has added to our knowledge. Due to the low cost and easy availability of fruit residues which are commonly discarded as waste in our immediate environments should be regarded as potential medicinal and synthetic resources, capable of offering significant low-cost, medicinal and dietary supplements. In addition, an established use of bioactive components from grape citrus peel would also help alleviate environmental pollution problems caused due to poor disposal of such residues. This will go a long way to enhance the health stability and reduce environmental pollution.

Compliance with ethical standards

We confirm that we complied to all ethical standard that concerns with publishing an article.

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Disclosure of conflict of interest

The authors declared that there is no conflict of interest regarding the publication of this paper.

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