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Gas Chromatography-Mass Spectrometry(Gc-Ms) Analysis Of Bioactive Components Present In Tangerine Citrus Peel In Nigeria

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Abstract
Tangerine (mandarin) is the second most important citrus genera, whose specific species is Citrus reticulate. Tangerine peels are usually treated as agroindustrial waste. However, they are rich in various nutraceutical components, including essential oils, flavonoids, pectin, and carotenes. The research work investigated the bioactive components present in an indigenous citrus peel, Tangerine (Citrus reticulata). The citrus fruits were purchased from fruits garden in Port-Harcourt metropolis, 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. This group of powerful instruments help characterize the various components. The gas chromatographic model: 7890A(GC) analysis was performed on an agilent technologies interfaced with mass selective detector model: 5975C(MSD). The results revealed 16 bioactive components in tangerine with n-Hexadecanoic acid having the highest component of 26.400% with a retention time of 18.529min. 9,12-Octadecadienoic acid, methyl esther, (E,E)- showed the lowest component of 0.574% and retention time of 19.947min. Results shows that tangerine (Citrus reticulata) 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.

Key words: *Citrus peels, bioactive, GC/MS,*

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I. 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 disaeases have been assessed (Proteggente *et al*., 2003; Gorinstein *et al*., 2004; Anagnostopoulou *et al*., 2006; Guimaraes *et al*., 2009) and it has been reported that citrus fruits and citrus fruits extracts and citrus flavonoids exihibits a wide range of promising biological properties due to their phenolic profile and antioxidant properties (Middleton and Kandaswami, 1994; Montanari *et al*., 1998; Samman *et al*., *1996*). 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 (USDA, 2010), and 34% of which was used for juice production, yielding about 44% peel by-product (Li *et al*., 2006). 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 dried, mixed with pulps and sold as cattle feed (Bucco *et al*., 1998). 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 was to investigate the bioactive wound healing properties of different extracts of citrus peel and their hematological effects.

II. Materiels And Methods

Preparation Ethanol Extract

Citrus fruits (orange, lime, lemon, tangerine, and grape) were procured from markets within Port Harcourt metropolis, Rivers State, Nigeria. The citrus fruits were 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 RadwagWagi electronic top loading balance (AS 220/C/2) to ensure uniformity in weight. The *citrus sinensis* peels were then pureed using MarlexElectroline (Excella) blender. Two hundred grams (200g) each 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°C, the weight of the concentrates were taken and the percentage yield calculated and kept at 4°C until usage.

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 ion source temperature at 250^{o}C. 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 60^{o}C held for 0.5 minute and ramped to 140^{o}C 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 8^{o}C /minutes. 1µ/l was auto injected.

III. Results

The results of the essential oils and bioactive components of the dichloromethane extract of tangerine citrus peel 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.

GC/MS and phytochemical analysis of dichloromethane extract of tangerine peel

Figure 1 shows the chromatogram of GC/MS of bioactive compounds of dichloromethane extract tangerine peel with highest peak observed at a retention time of 18.529min followed by retention time of 20.600min, 20.652 and 15.638min with the lowest peak observed at a retention time of 9.019min. Table 1 shows the presence of 16 active bioactive components in the dichloromethane extract of tangerine peel. n-Hexadecanoic acid was the highest in concentration (26.400% and retention time of 18.529min). This was followed by D-Limonene, 9,12,-Octadecadienoic acid(Z,Z)- and Oleic acid with concentrations of 23.049%, 18.849% and 7.272% with retention times of 4.202min, 20.600min and 20.652min respectively. 9,12-Octadecenoic acid, methyl esther(E,E)- concentration was the lowest value of 0.574% at a retention time of 19.947min.

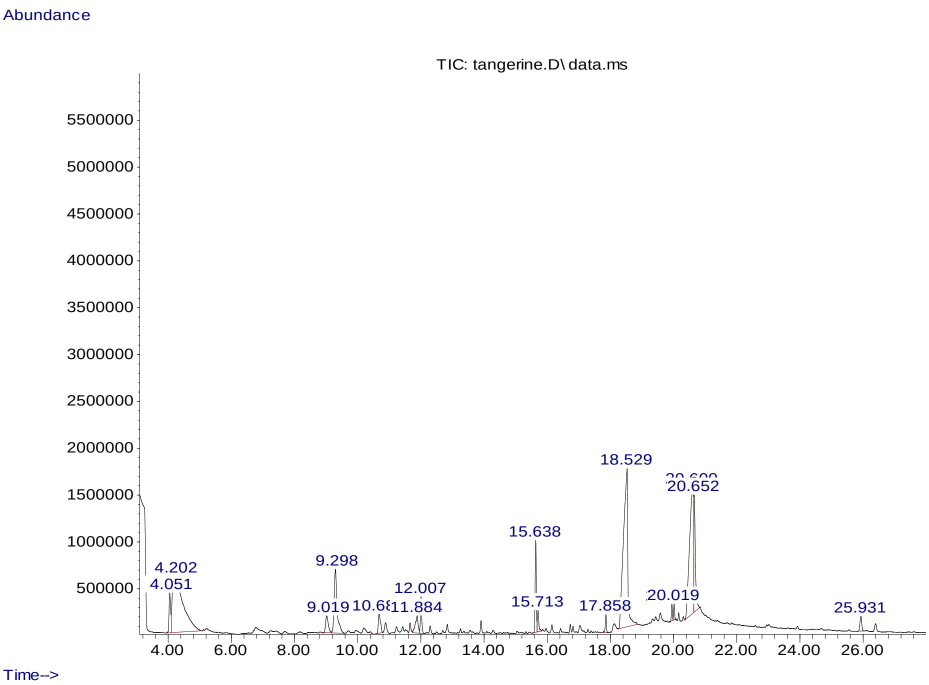
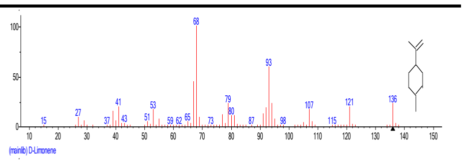
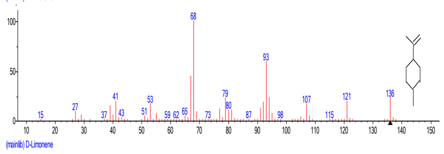
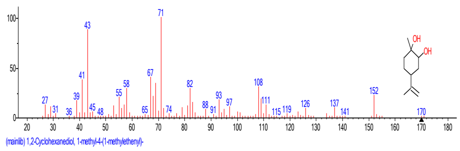
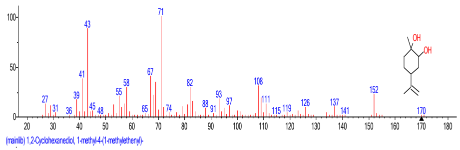
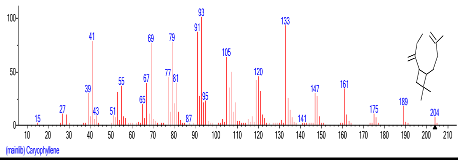


Fig. 1 GC-MS Chromatogram of Tangerine Peel

Table 1: Bioactive Components of Tangerine peel (*Citrus reticula*)

S/N	Compound	Retention Time (min)	Percentage of the total	Molecular formula	Molecular weight	Structure
1	D-Limonene	4.051	2.320	$C_{10}H_{16}$	136.24	
2	D-Limonene	4.202	23.049	$C_{10}H_{16}$	136.24	
3	1,2-Cyclo hexanediol, 1-methyl-4-(1-methylethenyl)	9.019	1.694	$C_{10}H_{18}O_2$	170.25	
4	1,2-Cyclo hexanediol, 1-methyl-4-(1-methylethenyl)-	9.298	7.151	$C_{10}H_{18}O_2$	170.25	
5	Caryophyllene	10.681	1.580	$C_{15}H_{24}$	204.3511	

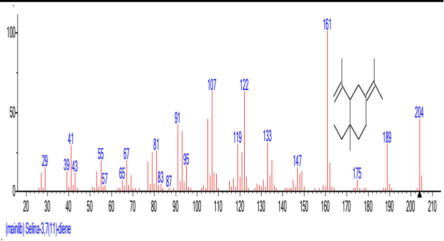
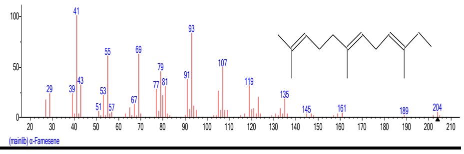
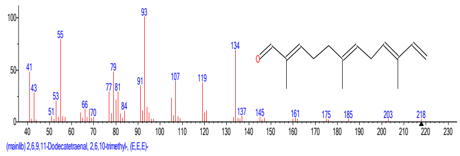
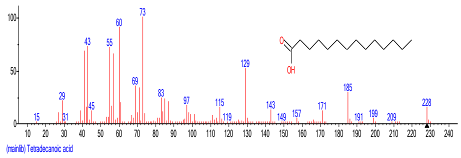
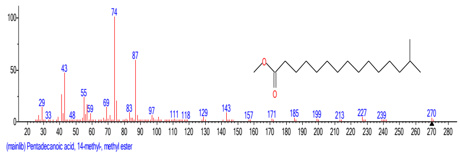
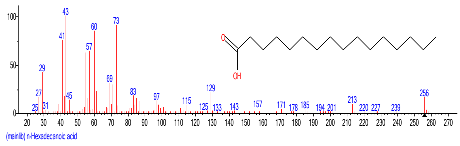
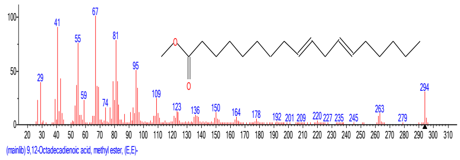
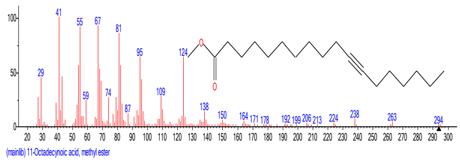
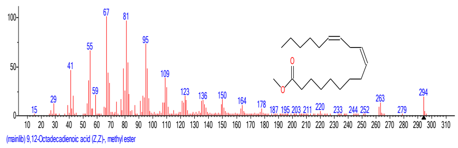
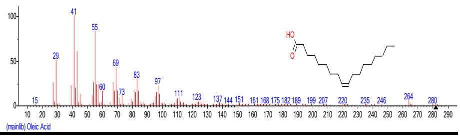
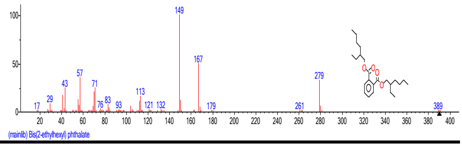
6	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]	11.884	1.834	$C_{15}H_{24}$	204.3511	
7	alpha.-Farnesene	12.007	1.802	$C_{15}H_{24}$	204.357	

Table 1: Bioactive Components of Tangerine peel (*Citrus reticula*)

S/N	Compound	Retention Time (min)	Percentage of the total	Molecular formula	Molecular weight	Structure
8	2,6,9,11-Dodeca tetraenal, 2,6,10-trimethyl-, (E,E,E)-	15.638	3.953	$C_{15}H_{22}O$	218.3346	
9	Tetradecanoic acid	15.713	1.147	$C_{14}H_{28}O_2$	228.3709	
10	Pentadecanoic acid, 14-methyl-, methyl ester	17.858	0.783	$C_{17}H_{34}O_2$	270.4507	
11	n-Hexadecanoic acid	18.529	26.400	$C_{16}H_{32}O_2$	256.4241	
12	9,12-Octadeca dienoic acid, methyl ester, (E,E)-	19.947	0.574	$C_{19}H_{34}O_2$	294.4721	
13	11-Octadecenoic acid, methyl ester	20.019	0.600	$C_{19}H_{36}O_2$	296.4879	
14	9,12-Octadeca dienoic acid(Z,Z)-	20.600	18.849	$C_{18}H_{32}O_2$	280.4455	
15	Oleic Acid	20.652	7.272	$C_{18}H_{34}O_2$	282.47	

16	Bis(2-ethylhexyl) phthalate	25.931	0.992	$C_{24}H_{38}O_4$	390.56	

IV. 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 function. The fruits peel; tangerine (*Citrus reticulata*) which were investigated for their essential oils. No previous work have been reported. The dichloromethane extract of tangerine peel revealed 16 bioactive compounds with N-Hexadecanoic acid having the highest percentage of 26.400 and a retention time of 18.529min, while 9,12,-Octadecadienoic acid, methyl esther (E,E)-had the lowest percentage of 0.574 and a retention time of 19.947min. This results agrees with Oboh and Ademosun (2012) 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 the tangerine peel agrees with Saidani and Brahim (2003) which states that Limonene is the most abundant compound of monoterpene hydrocabons for all of the examined juices. N-hexadecanoic acid shows inhibitory activity against mycobacterium tuberculosis (Suresh *et al.*,2010). 9,12,-Octadecadienoic acid(Z,Z)-, acid which is also the bioactive component with highest percentage in lime also has its own benefits: 9,12,-Octadecadienoic acid(Z,Z)-, acid has antiarthritic and anti-inflammatory property (Lalitharani et al., 2009). Sudha *et al.*, (2013) stated that 9,12,-Octadecadienoic acid(Z,Z)- has functions such as Hypocholesterolemic, Anticoronary, Nematicide, Hepatoprotective, Hypocholesterolemic etc. The bioactive components with the highest percentage in the investigated citrus peel have a lot of medicinal and synthetic values.

V. 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 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.

Competing Interests

Authors have declared that no competing interest exists.

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