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Full Length Research

Medicinal prospective of *Citrus limon* and *Citrus sinensis* peels essential oil by Gas Chromatography/ Mass Spectrometry (GC/MS) compositional analysis

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ABSTRACT: Citrus fruits are highly nutritious and medicinal fruits that are commonly cultivated throughout the tropics. This study was carried out to determine the bioactive compounds of the methanol peel extracts of *Citrus limon* and *Citrus sinensis*. The peels from fresh fruits of *C. limon* and *C. sinensis were* air-dried at room temperature and were pulverized into fine powder. The bioactive determinations were carried out by Gas Chromatography/Mass Spectrometry (GC/MS). The mass spectrum of the compounds found in the peels extract was matched with the National Institute of Standards and Technology (NIST) library. The results showed that four (4) and five (5) bioactive compounds were identified in *C. limon* and *C. sinensis* methanol peel extracts respectively. The *C. limon* peel extract contained 4-(methylsulfanyl) but-1-en-3-yne, Sulfanylphenylium, 1,3-thiazole and 3-methylidenecyclobutanecarbonitrile while *C. sinensis contained* Pyridine-2-carbaldehyde, 1-Methyl-1*H*-pyrrole-2-carbaldehyde, Glutamic acid, 2-Ethyl-5-methyl-1*H*-pyrrole and Pyrrolidin-2-one. The results of this study therefore unveiled that the *C. limon* and *C. sinensis peel extracts* contained variable bioactive compounds with medicinal value and could be useful raw materials for pharmaceutical industries.

Keywords: Citrus peels, methanol extract, medicinal phytochemicals, environmental wastes.

INTRODUCTION

The postulation that, for all need in all continents, there is a plant, is not only remarkably a hyperbolical statement but appears to be in affirmation in all ramifications. For millennia, people have been treated with herbal or animal-derived medicines, via knowledge handed down through generations. Tamil et al. (2017) reported that about 80% of people living in developing countries rely on medicinal plants for treatment of various kinds of diseases currently because they are relatively cheap and effective. So, studies in the field of phytochemistry to find out the bioactive substances from medicinal plants have considerably increased. Presently, scores of modern methods are accessible for standardization of crude drugs. Gas Chromatography Mass Spectrum (GC/MS) has become firmly established to identify the active principles in natural products (Rohloff, 2015).

Citrus fruits are highly nutritious and medicinal fruit that are commonly cultivated throughout the tropics. It belongs to the genus Citrus, subgenus Papeda. Citrus fruits include orange, lemon, lime and grapefruits with tangerine and pomelo inclusive. Citrus fruits are mainly used by processing industries while the peels and pulps are

generally discarded as waste products (Uraku et al., 2018). The fruits constitute only 0.9% of total daily calories and 1.7% of daily carbohydrate intake while the peel wastes are highly perishable and seasonal. These peel wastes could be wealth for the farmers if the processing industries and monitoring agencies develop tactics to use them and create awareness in bringing the useful products from waste materials (Suja et al., 2017). The wastes of citrus fruits are more than the juice yield in the fruit weight. Thus, a very large amount of the lemon and orange wastes are formed yearly (Arora and Kaur, 2013, Gotmare and Gade, 2018). Due to high wastes from citrus fruits in the environment, there is often an attention in bringing them into useful products and lemon and orange wastes are not exempted. To that effect, apt method needs to be adopted in the conversion of citrus peels and pulps into value-added products (Gotmare and Gade, 2018). By so doing, the environmental pollution can be reduced. Citrus peels have been reported by Uraku and Igwenyi (2016) to be rich in nutrients and phytochemicals. Obviously, they can be efficiently used not only as drugs but also as food supplements. The citrus peels have been acclaimed by rural dwellers to serve as mosquito repellent and anticoagulant among others.

Previous study by Choike et al. (2017) on ethanol extraction and GC-MS analysis of *C. sinensis* peel oil reported fifteen compounds. Also, Kamaliroosta et al. (2016) identified seven compounds in the orange peel essential oil and eighteen compounds in sweet lemon peel. More so, Larijani (2004) appraised the essential oils of orange and sour lemon. The results revealed fourteen, twenty-two and twenty-one compounds in the *C. sinensis (Thomson*), *C. sinensis (Valencia*) and *C. limon* peel essential oil respectively. Mirza and Bahernik (2006) also assessed the chemical composition of the essential oil of orange peel by GC/MS and identified twenty-one compounds. The essential oils of three varieties of *C. sinensis* in Kenya (washington, valencia and salutiana – navel) were evaluated by GC/MS and 56, 72 and 73 compounds were identified in salutianat, valencia and washington respectively (Njoroge et al., 2005).

To the best of our knowledge, of all the studies done on GC/MS analysis of these peels, no research has been done on GC/MS analysis of *C. limon* and *C. sinensis* peels from Nigeria. Thus, this research was designed to determine the phytochemicals or bioactive components of *C. limon* and *C. sinensis* peels. The bioactive compounds may be postulated to be responsible for some of the ethnomedicinal potentials of citrus peels.

MATERIALS AND METHODS

Collection and identification of plant material

Fresh fruits of *Citrus limon* and *Citrus sinensis* were purchased at Abakpa Main Market in Abakiliki, Ebonyi State, Nigeria. The plant samples were identified and authenticated by Taxonomist, Dr. (Mrs) C. V. Nnamami at the Department of Biological Sciences, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria.

Preparation of plant extract

The peels of *C. limon* and *C. sinensis* fruits were obtained by using a sharp knife and washed thoroughly with distilled water to remove dirt and debris. The peels were sliced into smaller pieces before they were air-dried for two weeks at room temperature (28±3ºC). The dried peels were pulverized into fine powder using electric blenders (CORONA-REF. 121, Landers and Qlink blender, Model No. OBL-15L40). The powdered materials were stored in air tight polyethene bags protected from direct sunlight until required for use.

Five (5) grams of the powdered peels were extracted with 100 ml of 98% methanol overnight in a stopped bottle and with occasional stirring at room temperature (28±3ºC). The samples were first sieved using muslin cloth and then filtered using Whatman No.1 filter paper. This process was repeated three times to ensure that the pulverized peels are properly extracted. The filtrates were concentrated under reduced pressure at 40ºC for 45 minutes in a rotary vacuum evaporator and then lyophilized to get a brown solid extract. The yield of the extracts was expressed in terms of the percentage of the dry weight of initial plant materialused. The dry extracts obtained were kept in a refrigerator at 4ºC until required for use.

Gas chromatography/Mass spectrometry (GC/MS)

GC/MS analysis was carried out on a GC-MS (Model: QP2010 PLUS Shimadzu, Japan) comprising a AOC-20i auto-sampler and chromatograph interfaced to a mass spectrometer (GC/MS). The instrument was equipped with a VF 5 ms fused silica capillary column of 30 m length, 0.25 mm diameter and 0.25 µm film thickness. The temperatures employed were column oven temperature 80ºC and injection temperature 250ºC at a pressure of 108.0 kPa with total flow and column flow of 6.20 ml/min and 1.58 ml/min respectively. The linear velocity was 46.3 cm/sec and a purge flow of 3.0 ml/min. The GC program ion source and interface temperature were 200.00ºC and 250.00ºC respectively with solvent cut time of 2.50 min. The MS program starting time was 3.00 min which ended at 30.00 min. with event time of 0.50 sec, scan speed of 1666 µl/sec, scan range 40-800u and an injection volume of 1 µl of the plant extract (split ratio 10:1). The total running time of GC/MS was 30 min. The relative percentage of the extract was expressed as percentage with peak area normalization.

Identification of phytocompounds

Interpretation on the mass spectrum was conducted using the database of National Institute Standard and

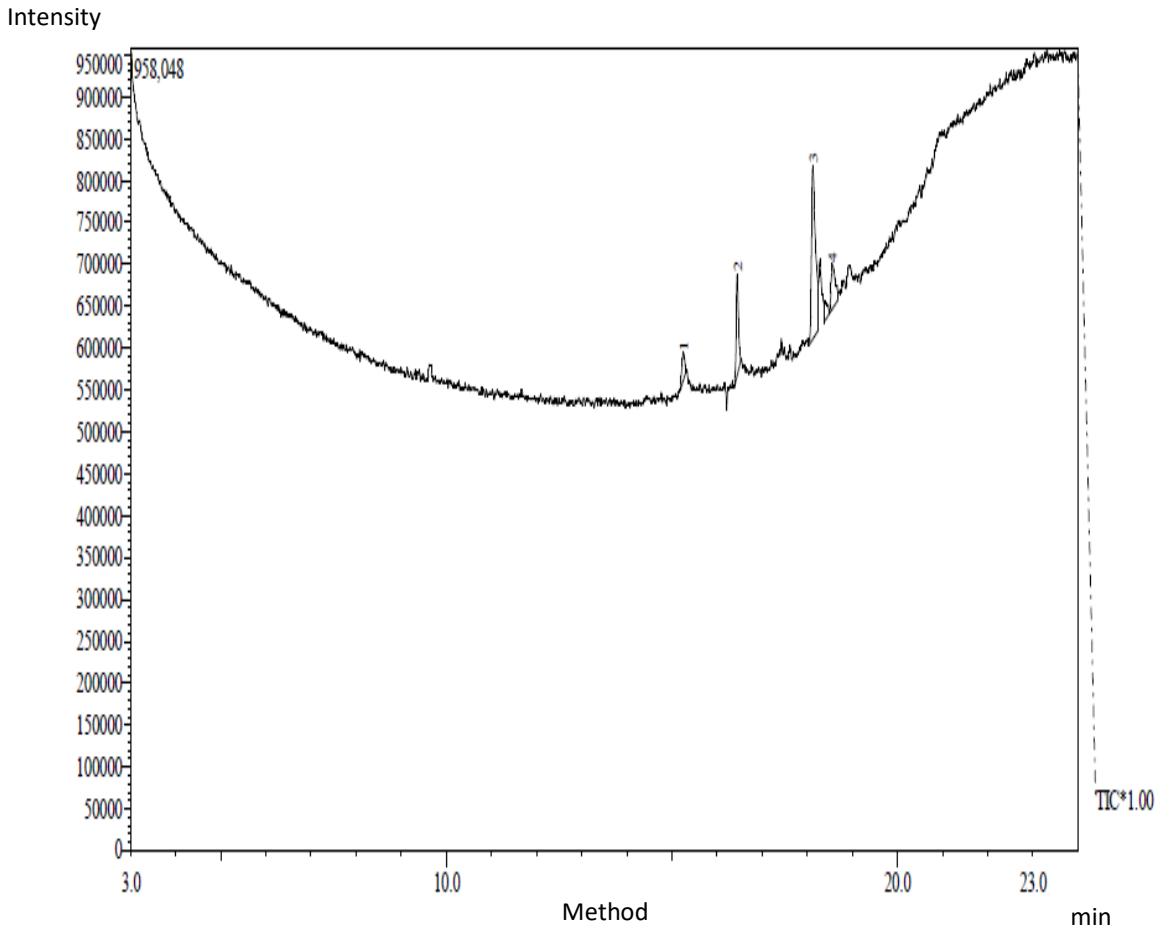


Figure 1. Chromatogram of methanol peel extract of *C. limon* essential oil.

Technology (NIST) having more than 62,000 patterns. The fragmentation pattern spectra of the unknown components were compared with those of known components stored in the NIST library (NIST Ver. 3.2 of 2010). The compound bioactivity prediction was based on Duke's Phytochemical and Ethnobotanical Databases (2014). The relative percentage amount of each phytocomponent was calculated by comparing its average peak area to the totalarea. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULTS

Chromatogram of methanol extract of essential oil peel

The chromatogram of essential oil of *Citrus limon* and *Citrus sinensis* peel reveled four and five peaks respectively (Figures 1 and 2).

Identified compounds

The identified compounds in methanol peel extracts of *C. limon* and *C. sinensis* essential oil with retention time, peak area (%), molecular weight and formular are depicted in Tables 1 and 2.

Phytochemicals in methanol extract of *C. limon* and *C. sinensis* peel

The proposed phytochemicals in methanol peel extracts of *C. limon* and *C. sinensis* with their name of compound, molecular structure and bioactivity are shown in Tables 3 and 4.

DISCUSSION

The gas chromatogram and mass spectra of methanol peel extracts of *Citrus limon* and *Citrus sinensis* were presented in Figures 1 and 2. Thus, four and five peaks were depicted respectively and these suggested presence of four and five compounds in the methanol peel extracts of *C. limon* and *C. sinensis* essential oil. The projected compounds, retention time (RT), peak area percentage,

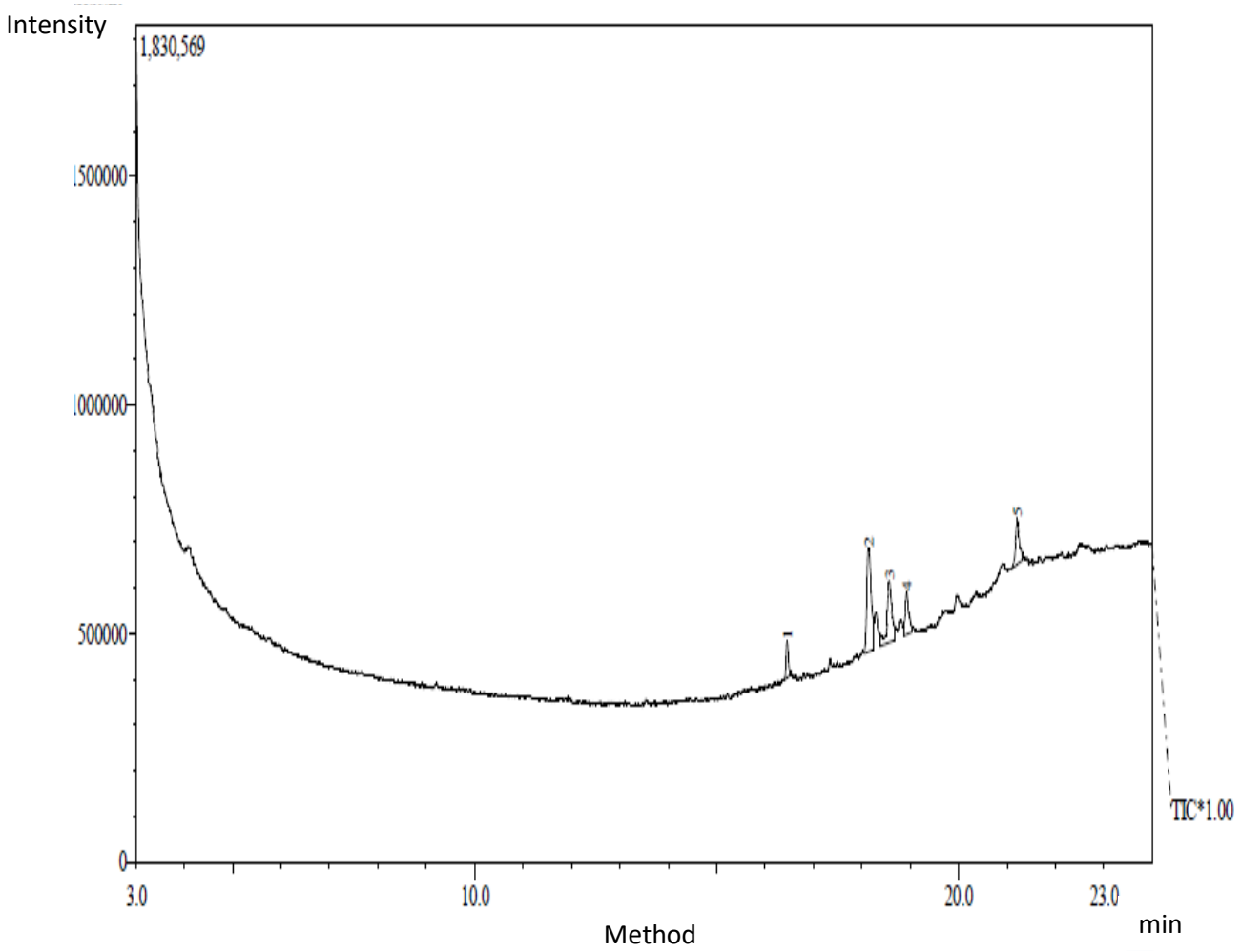


Figure 2. Chromatogram of methanol peel extract of *C. sinensis* essential oil.

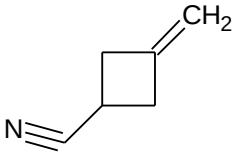
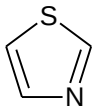
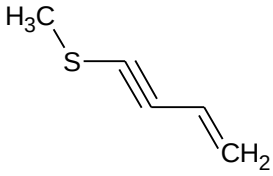
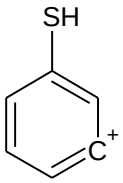
Table 1. Identified compounds in methanol peel extract of *C. limon* essential oil with retention time, peak area (%), molecular weight and formular by GC/MS analysis.

Peak No	Name of Compound	Retention time	Peak area %	Molecular weight	Molecular formula
1	3-methylidenecyclobutanecarbonitrile	15.258	6.48	93.12	C_6H_7N
2	1,3-thiazole	16.447	18.13	85.12	C_3H_3NS
3	4-(methylsulfanyl) but-1-en-3-yne	18.126	56.53	98.16	C_5H_6S
4	Sulfanylphenylium	18.561	18.86	109.16	C_6H_5S

Table 2. Identified Compounds in methanol peel extract of *C. sinensis* essential oil with retention time, peak area (%), molecular weight and formular by GC/MS analysis.

Peak No	Name of Compound	Retention time	Peak area %	Molecular weight	Molecular formula
1	Pyrrolidin-2-one	16.454	7.46	85.10	C_4H_7NO
2	Pyridine-2-carbaldehyde	18.144	40.16	107.11	C_6H_5NO
3	1-Methyl-1*H*-pyrrole-2-carbaldehyde	18.566	25.00	109.12	C_6H_7NO
4	2-Ethyl-5-methyl-1*H*-pyrrole	18.929	12.75	109.16	$C_7H_{11}N$
5	Glutamic acid	21.203	14.63	147.12	$C_5H_9NO_4$

Table 3. Proposed phytochemicals in methanol peel extract of *C. limon* essential oil *with* their Name of compound, Molecular structure and Bioactivity.

Peak No.	Name of compound	Molecular structure	Bioactivity
1	3-methylidenecyclobutanecarbonitrile		Nf
2	1,3-thiazole		Nf
3	4-(methylsulfanyl) but-1-en-3-yne		Decrease Endothilial Leukocyte Adhesion, Decrease Endothilial Platelet Adhesion, Encephalopathic, Endocrinactive, Endoanesthetic, Endocrinprotective, Endorphinogenic, Ergotamine-Enhancer, Enterotoxic, Enterotonic, Enterostimulant, Enterorelaxant, Enteromotility-Enhancer, Enterodepressant, Enterocontractant, Enkephalinogenic, Energizer, Fertility-Enhancing, Fibrinolysis Enzyme Activator, Memory-Enhancer, Stimulate PUFA Desaturase and Elongase Enzymes, Trypsin-Enhancer
4	Sulfanylphenylium		Nf

Nf: Not found. Source: Duke.s Phytochemical and Ethnobotanical Database (2014).

Table 4. Proposed phytochemicals in methanol peel extract of *C. sinensis with their Name* of compound, molecular structure and Bioactivity.

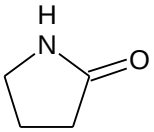
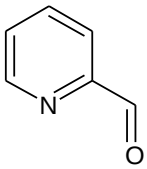
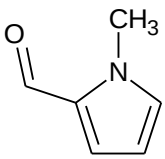
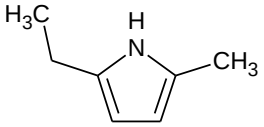
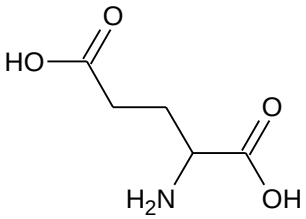
S/No	Name of Compound	Molecular structure	Bioactivity
1	Pyrrolidin-2-one		Nf
2	Pyridine-2-carbaldehyde		Nf
3	1-Methyl-1*H*-pyrrole-2-carbaldehyde		Catechol-O-Methyl-Transferase-Inhibitor, Methyl-Donor, Methyl-Guanidine-Inhibitor, 11B-HSD-Inhibitor, 17-beta-hydroxysteroid dehydrogenase-Inhibitor, 5-HETE-Inhibitor, 5-HT-Inhibitor, 8-HETE-Inhibitor, Anti-5-HT, Anti-HIV-Integrase, Antidote (Heavy Metals), Antidote (Hydrazine), Antidote (Hypoglycin-A), Aryl-Hydrocarbon-Hydroxylase-Inhibitor, Hallucinogen, Hallucinogenic, HDL-genic, Helicicide, Hemagglutinator

Table 4. Contd.

4	2-Ethyl-5-methyl-1*H*-pyrrole		Hypoxemic, improve Cerebral Hypoxia, Increase T-helper, Increase Tyrosine Hyroxylase Activity, Suppress HMG-CoA Reductase Activity, Testosterone-Hydroxylase-Inducer, Tyrosine-Hyroxylase-Activator
5	Glutamic acid		Urine-Acidifier, Urinary-Acidulant, Inhibit Production of Uric Acid, Increase Aromatic Amino Acid Decarboxylase Activity, Arachidonic-Acid-Inhibitor, Arachidonic acid-Inhibitor

Nf: Not found. Source: Duke.s Phytochemical and Ethnobotanical Database (2014).

molecular weight and molecular formula were chatted in Table 1 and 2. The phytochemicals present in the methanol peel extracts of *C. limon* with peak area percentage were 4-(methylsulfanyl) but-1-en-3-yne (56.53), Sulfanylphenylium (18.86), 1,3-thiazole (18.13) and 3-methylidenecyclobutanecarbonitrile (6.48) while that of *C. sinensis* were Pyridine-2-carbaldehyde, 1-Methyl-1*H*-pyrrole-2-carbaldehyde, Glutamic acid, 2-Ethyl-5-methyl-1*H*-pyrrole and Pyrrolidin-2-one with peak area percentages of 40.16, 25.00, 14.63, 12.75 and 7.46 respectively.

Tables 3 and 4 showed the nature, structures and biological activities of the phytochemicals found in the methanol peel extracts of *C. limon* and *C. sinensis* essential oil respectively. Out of four compounds found in *C. limon,* only 4-(methylsulfanyl) but-1-en-3-yne with peak area percentage of 56.53 had an identify biological activities (Table 3) while in *C. sinensis,* five compounds were recognized and only three compounds had eminent bioactive roles (Table 4). However, there is no information on biological activity of other compounds in methanol peel extracts of C*. limon* and *C. sinensis* essential oil.

The outcome of this study is incongruity with report of Kumar and Vijayalakshmi (2011) on GC/MS analysis of ethanol extract of *V. vinifera* seed. Also, the report of Choike et al. (2017) is not in consonance with the results obtained in this study. Studies carried out by Qiao et al. (2008), Das et al. (2014), Prasad et al. (2016), Rashmi et al. (2017), Kadhim et al. (2017), Uraku et al. (In press) are in divergence with the outcome of this study. The detergency of this study with the reports of other researchers is in both number and natures of phytoconstituents found. The discrepancy in these peel extracts could be due to differences in plant parts, solvents of extraction and geographical location.

Conclusion

The bioactive compounds identified by GC/MS analysis of the peels have demonstrated that peels are important raw materials in drug formation. This study unveiled that the essential oil of *C. limon* and *C. sinensis* peels contained phytchemicals that could be beneficial to man. This study will help researchers to covert waste to wealth.

CONFLICT OF INTEREST

No conflict of interest associated with this work.

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