GC-MS and FTIR Analysis of Aqueous Extract of Citrullus lanatus (Water Melon) Rind

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Abstract

Over the years, the Citrulluslanatus rind has been considered as waste and disposed ignoring the fact that it can be utilized as a value-added product and has many health benefits. The watermelon rind constitutes nearly a third of the watermelon weight. The evaluation of Citrulluslanatus (watermelon) rind as a possible raw material for a pharmaceutical and as a value added product of the fruit rind and this will encourage cultivation, utilization and possible application of watermelon rind in the treatment of various diseases. The aim of this research work is to determine the functional group using FTIR and chemical components of aqueous extract of watermelon rind using Gas Chromatography–Mass Spectrometry (GC–MS). Dried powder of Citrulluslanatus (watermelon) rind extracts was used for FTIR analysis. 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, was prepared to obtain a translucent sample disc. The powdered of Citrulluslanatus was loaded in FTIR analysis (Shimadzu, IR Affinity 1, Japan), having a Scan range from 400 to 4000 cm–1. While The phyto-constituents of Citrulluslanatus (watermelon) aqueous rind extract were determined by Gas Chromatography (Agilent 6890 series) coupled with HP-5MS column mass spectrometer at column temperature of 30°C and was heated to 300°C at 10°C for 5min. The helium was used as carrier gas at a flow of 1.0ml/min. The identification of the constituents of water melon rind aqueous extract was performed by matching their mass spectra and retention indices with those obtained from authentic samples and/or NIST/Wiley spectra libraries, using different types of search (PBM/NIST/AMDIS) and available literature data. The FTIR analysis Citrulluslanatus aqueous rind extract shows different absorption band which indicated the characteristic peak of different functional groups such as OH, C=O C-O, H-N, C-H etc which are associated with phenols, ketones, flavonoids, aldehydes, amides, alkenes etc. while the Gas Chromatography-Mass spectrometry analysis of aqueous extract of watermelon rind reveals the presence of different fatty-acid, methyl ester and volatile organic compounds this includes Hexadecanoic acid,2,hydroxy-1-(hydroxymethyl)ethyl ester, Eicosane,2methyl-, 1-Hexadecanesulfonic acid,3,5-dichloro-2,6-dimethyl-4-pyridyl ester, 9,12-Octadecadienoic acid(Z,Z)-2,3-dihydroxypropyl ester, Estra-1,3,5(10)-tri-en-17β-ol etc. The finding showed that Citrulluslanatus aqueous rind extract have thirty-one (31) bioactive constituents of pharmacological important identified by GC-MS and FTIR analysis. These compounds from Citrulluslanatus rind can be used in pharmaceutical industry for development of novel lead drugs for management of diseases.

Keywords: Citrulluslanatus, Rind, Phytoconstituents, GC-MS, FTIR.

INTRODUCTION

The plant kingdom has proven to be the most useful in the treatment of diseases and have provides an important source of material to pharmaceuticals industries worldwide. The most important of these bioactive constituents of plants are steroids, terpenoids, carotenoids, flavonoids, alkaloids, tannins and glycosides. Plants in all facet of life have served a valuable starting material for drug development (Ajibesin, 2011). Antibiotics or antimicrobial substances like saponins, glycosides, flavonoids and alkaloids etc are found to be distributed in plants, yet the bioactivity of these compounds are yet to be explained.
these compounds were not well established due to the lack of knowledge and techniques.

Watermelon is used amazingly for its nutritional and medicinal values because of its high water content which contained sugar and energy booster and hydrate body during dehydration, especially during the hot seasons (Ayoola et al., 2011). Watermelon’s Seeds, the dried seeds (dark flat) of the fruit are used as snacks when salted and roasted in China, Israel, etc. In Africa, the seeds are made into coarse flour or oil may be extracted from them and use for domestic consumption, and the juice can be extracted from watermelon to produced wine. The imperfect fruit of the Watermelon are used as Livestock feed, while the immature fruits can be prepared and used as summer squash (Stray, 1998).

Watermelon was said to possess high level of antioxidants which decreases the risk of kidney stone and bone loss due to old age, and it is a powerful diuretic diet, has the availability of amino acids and Beta –Carotene which protect heart disease. Also rich in lycopene, which is a pigment that gives the red color that naturally, occurred in Citrulluslanatus which prevent ailments of prostate and oral cancer. It is a good source of vitamins such as A, B, C, and Thiamine.

The seeds of the watermelon are said to contain considerable amount of minerals such as calcium, iron, manganese, phosphorus, potassium, sodium, zinc, copper and magnesium which assist in the growth and development of the healthy body that take part in metabolic activities of all living organisms (Worthington-Roberts, 2008).

In some Asian and African countries, up to 80% of the population relies on traditional medicine for their primary health care needs. When adopted outside of its traditional culture, traditional medicine is often called complementary and alternative medicine.

The World Health Organization notes, that "inappropriate use of traditional medicines or practices can have negative or dangerous effects” and that "further research is needed to ascertain the efficacy and safety” of several of the practices and medicinal plants used by traditional medicine systems. Practices known as traditional medicines include Ayurveda, Siddha medicine, Unani, ancient Iranian medicine, Irani, Islamic medicine, traditional Vietnamese medicine, traditional Chinese medicine, traditional Korean medicine, traditional African medicine, and many other forms of healing practices(CHO, 2002).

2. METHODS
Collection and Identification

Citrulluslanatus (water melon) was collected from Wudil farm and was identified at Biology Department, Kano University of Science and Technology; the collected fruits were rinsed several times with distilled water before subjecting them to analysis. Fresh samples of the fruits were sliced with cleaned knife to separate the rind (exocarp) from the pulp (mesocarp). The seeds were carefully removed from the pulp. The rind was chopped into tiny cubes while the pulp was shredded and the rind was bench dried for twenty days and pulzerized into coarse powder and kept in polythene bags at room temperature for extraction.

Extraction of the water melon rind extract

Exactly 5 grams of the crushed watermelon rind was carefully weighed and transferred into a clean 50ml conical flask and diluted with 10ml of distilled water. The 50ml conical flask was then placed as a shaking water-bath and heated at a temperature of 60°C for ten minute to increase the value of the water soluble polyphenols in the watermelon rind extract. After the ten minute, the conical flask was removed and cool. The cold watermelon rind was then filtered using Whatman number 1 filter paper. A light-green colored filtrate was obtained as the watermelon rind aqueous extract. The aqueous extract was allowed to dry at a room temperature and it was dissolved in methanol and taken for GC-MS and FTIR analysis.

Fourier Transform Infrared (FTIR) Spectroscopy Analysis of Water Melon Rind Aqueous Extract.

Dried powder of Citrulluslanatus (water melon) aqueous rind extracts was used for FTIR analysis. 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, was prepared to obtain a translucent sample discs. The powdered of Citrulluslanatus was loaded in FTIR machine (Shimadzu, IR Affinity 1, Japan), having a Scan range from 400 to 4000 cm⁻1.

Gas Chromatography – Mass Spectroscopy (GC-MS) Analysis of Citrulluslanatus (Water Melon) Rind Aqueous Extract.

The phyto-constituents of Citrulluslanatus (water melon) rind extract were determined by Gas Chromatography (Agilent 6890 series) coupled with HP-5MS column mass spectrometer at column temperature of 30°C and was heated to 300°C at 10°C for 5min. The helium was used as carrier gas at a flow of 1.0ml/min. The identification of the constituents of water melon rind aqueous extract was performed by matching their mass spectra and retention indices with those obtained from authentic samples and/or NIST/Wiley spectra libraries, using different types of search (PBM/NIST/AMDIS) and available literature data (Kulkarni et al., 2015).

3. RESULTS AND DISCUSSION

FT-IR analysis of aqueous extracts of Citrulluslanatus(watermelon) rind.

Citrulluslanatus aqueous rind extract shows characteristics absorption band at 3622, 3946, 3819,
3674, 3651 and 3525 cm⁻¹ which indicated the presence of OH band stretch and this reveals phenols while the peak band at 3867, 3905, and 3838 cm⁻¹ indicated the presence of C-H band stretch indicated for aldehyde. Also the band observed at 780,817 and 672 cm⁻¹ were for alkyl halides. The characteristics absorption band exhibited at 2113, 2206, 2530 and 1950 cm⁻¹ were for functional group C=O stretch which indicated the presence of aromatic ketone. Also, the band observes at 3353, 3320, 3264 and 3294 cm⁻¹ was for Amine due to N-H stretch and 1596 cm⁻¹ was for primary amine due to the N-H bend. The absorption band at 2151, 2050, 2020 and 2091 cm⁻¹ showed the presence of C-N stretch which indicates flavonoids and also band observed at 1242 and 1030 cm⁻¹ were for Ethers due to C-O bend. The absorption at 719 and 2921 cm⁻¹ indicated the presence of C-H bends which showed Alkenes and also peak at 1393 cm⁻¹ showed the presence of sulphonamide due to S=O stretch (Table 1/ Figure1).

### Table 1.0: FT-IR analysis of aqueous rind extracts of *Citrullus lanatus* (watermelon)

| S/N | Frequency (cm⁻¹) | Components Peaks | Functional Group |
|-----|------------------|------------------|------------------|
| 1   | 3946             | OH stretch       | Phenols          |
| 2   | 3819             | OH Stretch       | Phenols          |
| 3   | 3905             | C-H stretch      | Alkenes          |
| 4   | 3674             | OH Stretch       | Amide            |
| 5   | 3867             | C-H Stretch      | Aldehyde         |
| 6   | 3838             | C-H Stretch      | Aldehyde         |
| 7   | 3651             | OH Stretch       | Phenols          |
| 8   | 3525             | OH stretch       | Phenols          |
| 9   | 3622             | OH stretch       | Phenols          |
| 10  | 3353             | N-H stretch      | Amine            |
| 11  | 3320             | N-H stretch      | Amine            |
| 12  | 3264             | N-H stretch      | Amine            |
| 13  | 3294             | N-H stretch      | Amine            |
| 14  | 2921             | C-H stretch      | Alkenes          |
| 15  | 2530             | C=O stretch      | Ketones          |
| 16  | 2206             | C=O stretch      | Ketone           |
| 17  | 2113             | C=O Stretch      | Ketones          |
| 18  | 2091             | C-N stretch      | Flavonoid        |
| 19  | 2151             | C-N Stretch      | Flavonoid        |
| 20  | 2050             | C-N stretch      | Flavonoid        |
| 21  | 2020             | C-N stretch      | Flavonoid        |
| 22  | 1950             | C=O stretch      | Ketones          |
| 23  | 1900             | C=O stretch      | Ketones          |
| 24  | 719              | C-H Bend         | Alkenes          |
| 25  | 1596             | N-H bend         | Primary amine    |
| 26  | 1398             | S=O stretch      | Sulphonamide     |
| 27  | 1242             | C-Cl bend        | Ether            |
| 28  | 780              | C-Cl stretch     | Alkyl halide     |
| 29  | 817              | C-Cl bend        | Alkyl halide     |
| 30  | 672              | C-Cl or C-Br stretch | Alkyl halide |
| 31  | 1030             | C-O bend         | Ether            |
GC-MS analysis of aqueous Rind extract of *Citrilluslanatus*

The aqueous rind extracts of *Citrilluslanatus* revealed several peaks which represents different compounds as shown in the chromatogram by Gas Chromatography-Mass Spectrometry analysis in (figure 2). The peaks in the chromatogram were integrated and compared with the database of spectrum of known components stored in the Gas chromatography-mass spectrometry library. The Gas Chromatography-Mass spectrometry analysis of aqueous extract of watermelon rind reveals the presence of different fatty-acid, methyl ester and volatile organic compounds (table 2).

**Table-2.0: GC-MS analysis of aqueous Rind extract of *Citrilluslanatus***

| Peak | Retention time | IUPAC Name | Molecular/ Structural Formular | Nature and pharmacological Uses |
|------|----------------|------------|--------------------------------|---------------------------------|
| 1.   | 10.9           | Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl) ethyl ester | C<sub>19</sub>H<sub>38</sub>O<sub>4</sub> | Palmitic acid ester : ,antioxidant, lubricant, pesticides, flavor, nematicide (Imis, 2016) |
| 2.   | 12.948         | Eicosane,2methyl- | C<sub>21</sub>H<sub>44</sub> | Acyclic alkene; present in petroleum product, generally flavoring agent used in food including condiments and seasonings, fragment and odour agents. (Kennedy *et al.*, 2004) |
| 3.   | 13.183         | 1-Hexadecanesulfonic acid,3,5-dichloro-2,6-dimethyl-4-pyridyl ester | C<sub>21</sub>H<sub>19</sub>Cl<sub>2</sub>NO<sub>3</sub>S | Anti-microbial, antioxidant. (Hamad 2015) |
| No. | Value | Compound Name | Chemical Structure | Description |
|-----|-------|---------------|--------------------|-------------|
| 4.  | 13.446| Octadecane, 3-ethyl-5-(2-ethylbutyl)- | ![Chemical Structure](image) | A s a volatile component and have anti-microbial effect (Thilina Gunathilaka, 2019) |
| 5.  | 14.098| 9-(2'2'-Dimethylpropanoilhydrazono)-3,6-dichloro-2,7-bis[2-(diethylamino)-ethoxy]fluorine. | ![Chemical Structure](image) | Anti-bacterial, anti-pyrotic analgesic effect and improve immunity. (Nat prod Rep, 2004 dec) |
| 6.  | 14.825| Hepatacosane | ![Chemical Structure](image) | As a volatile oil component and plant metabolites. Anti-microbial and cytotoxic properties. (Martin, 2015) |
| 7.  | 15.065| Tetracosane | ![Chemical Structure](image) | An alkene hydrocarbon; suitable solvent used in the synthesis Zns nano-particles. May be employed as the wax component to study the reduction in pour point and hydrocarbon solvent containing wax crystals on addition of polymer additive. |
| 8.  | 15.615| Octadecane, 3-ethyl-5-(2-ethylbutyl)- | ![Chemical Structure](image) | A cyclic alkene; A s a volatile component and have anti-microbial effect. (Thilina Gunathilaka, 2019). |
| 9.  | 16.341| 9-Octadecenoic acid(Z)-, phenylmethyl ester | ![Chemical Structure](image) | Anti-microbial activities. and, antibacterial activites (jeneciu et al., 2012) |
| 10. | 16.999| 4-Methyl-docosane | ![Chemical Structure](image) | Perfuming agent, anti-microbial |
| No. | Retention Time | Compound Description | Properties/Activities |
|-----|----------------|----------------------|-----------------------|
| 11  | 17.686         | Tetradecane, 2,6,10-  | Anti-microbial activities and cytotoxic properties |
|     |                | trimethyl              |                       |
| 12  | 18.355         | Heptacosane            | Anti-microbial and cytotoxic properties. As a volatile oil component and plant metabolites (Martin 2015) |
| 13  | 19.042         | Estra-1,3,5(10)-trien-17β-ol | Anti-microbial activities (P.R 2012) |
| 14  | 19.895         | 2-Hexadecanol         | Palmitic acid; Anti-microbial, anti-inflammatory, emulsifier and thickening agent (The Good scent company, 2018) |
| 15  | 22.338         | 7-methyl-Z-tetradecen-1-olacetate | Fatty acid; component of flavouring agent and perfumes. |
| 16  | 22.967         | 9-Octadecenoic acid(Z)-phenylmethyl ester | Antibacterial activities (jeneciu et al., 2012) |
| 17  | 23.648         | Ethanol 2-(octadecyloxy) | Ant toxicity, anti-microbial (OH DH, et al., 1993) |
|   |   |   |   |   |
|---|---|---|---|---|
|   |   |   |   |   |
| 18. | 24.095 | Apidopermidin-17-ol,1-acetatyl-19,21-epoxy-15,16-dimethyl- | Anti-microbial activities. (Adelani-Akande et al., 2015) |
|   |   |   |   |   |
| 19. | 25.256 | Heptacosane | As a volatile oil component and plant metabolites. Anti-microbial and cytotoxic properties (Martins, 2015) |
|   |   |   |   |   |
| 20. | 25.846 | 2-hexadecanol | Palmitic acid; Antimicrobial, anti-inflammatory, emulifier and thickening agent. (The Good scent company, 2018) |
|   |   |   |   |   |
| 21. | 26.664 | i-propyl 9-tetradecenoate | Perfuming agent, binding agents, polar emollient used in cosmetics, antimicrobial, antioxidants, stimulants (Kadhim et al., 2016) |
|   |   |   |   |   |
| 22. | 26.978 | Octadecane,3-ethyl-5-(2-ethylbutyl) | As a volatile component and have anti-microbial effect |
|   |   |   |   |   |
| 23. | 27.19 | 9,12-Octadecadienoic acid(Z,Z)-2,3-dihydroxypropyl ester | Volatile organic compounds, has anti-microbial activities |
|   |   |   |   |   |
| 24. | 27.751 | 2,6-di-t-butyloctahydroazulene-3a,8-diol | As a component in insect repellents |
| No. | Retention Time (min) | Compound Description | Properties |
|-----|---------------------|----------------------|------------|
| 25. | 28.043              | Davcarpidan-1-methanol, acetate (ester) | Fatty acid; occurs naturally in plants and animals derivatives and its used in cosmetic products, thickeners and hardeners. |
| 26. | 28.523              | 4-methoxycarbonylmethyldodec-3-enedioic acid, dimethyl ester | Anti-fungal, antioxidants (Saenjum et al., 2012) |
| 27. | 29.216              | 9,12,15-Octadecadienoic acid-2,3-bis(trimethylsilyl)oxypropyl ester (Z,Z,Z) | Antibacterial activities, anti-inflammatory, cancer prevention, anti-acne (Hadi Hameed, 2015) |
| 28. | 29.565              | 9,12,15-Octadecatrienoic acid, acid, 2,3-bis(trimethylsilyloxypropyl ester (Z,Z,Z) | Antibacterial activities, anti-inflammatory, cancer prevention, anti-acne (Hadi Hameed, 2015) |
| 29. | 29.839              | 9,12,15-Octadecatrienoic acid, acid, 2,3-bis(trimethylsilyloxypropyl ester (Z,Z,Z) | Antibacterial activities, anti-inflammatory, cancer prevention, anti-acne (Hadi Hameed, 2015) |
| 30. | 30.555              | Hexasiloxane 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl | Volatile organic compounds, has antimicrobial activities (Adekunle, 2009) |
Fig-2: GC-MS Chromatogram of aqueous Rind extract of Citrilluslanatus

4. CONCLUSION
The finding showed that Citrilluslanatus aqueous rind extract have thirty-one (31) bioactive constituents of pharmacological important identified by GC-MS and FTIR analysis. These compounds from Citrilluslanatus rind can be used in pharmaceutical industry for design and develop of novel drugs for the treatment of diseases.

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6. ETHICAL APPROVAL
As per international standard written ethical permission has been collected and preserved by the author(s).

7. COMPETING INTERESTS
Authors have declared that no competing interests exist.

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