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GC-MS ANALYSIS OF CONSTITUENTS OF THE ESSENTIAL OIL FROM THE LEAF AND FRUIT OF NIGERIAN CULTIVAR OF *Annona muricata*

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ABSTRACT

Annona muricata (soursop) has a long history of ethnomedicinal properties, which is associated with essential oils present in the plant. The bioactive compounds in the oils from the leaf and fruit of Nigerian variations of the plant are thus provided in this study. GC-MS was used to analyse and identify the chemical compounds present in the essential oil from *A. muricata* leaf and fruit. The major chemical compounds in the leaves were caryophyllene (26.04%), (1S-cis)-(δ-cadinene) (12.62%) and spathulenol (8.49 %). Others that were obtained in less quantities included α-cadinene (5.64%), tau-cadinol (3.84%), α-caryophyllene (3.49%) and phytol (2.83%). In the fruit oil, the compounds identified were *n*-hexadecanoic acid (24.82%), *E*-nerolidol (15.30%) and caryophylene (32.30%). The presence of the compounds in the essential oil may provide scientific basis for the uses of the plant in the management of cancer and other parasitic infections.

Key words: *Annona muricata*, Soursop, Essential oil, Medicinal.

INTRODUCTION

The plant, *Annona muricata*, popularly known as soursop, is an evergreen in the *Annonaceae* family cultivated in the tropical and subtropical climates around the world, including Malaysia, America, Australia and Africa (Gajalakshmi *et al.,* 2012). Fruits of this plant measure 15–20 cm in diameter, are succulent, heart-shaped and green in colour. The fruit pulp is white and creamy with some sweetness. The fresh fruit may contain 55–170 black seeds, and turns brownish when dried (Awan *et al.,* 1980). Several ethno-medical insights provided by local ethnic groups have offered a platform for researchers to transform ethnobotanical information into evidence-based knowledge through scientific studies (Agu *et al.*, 2017; Adedeji *et al.*, 2018).

Annona species like *A. squamosa* and *A. reticulata* are used in traditional medicine to manage some ailments (Abdul Wahab *et al.*, 2018). Although the bark, roots, and leaves of *A. muricata* have been used traditionally for medicinal purposes, it is the leaves that are most often used for a variety of ethnomedicinal purposes (Abdul Wahab *et al.,* 2018). *In vitro* and *in silico* studies were reported by (Dewi *et al.,* 2021), the crude extract of the plant have been used in chemotherapy as antioxidant (Gavamukulya *et al.,* 2017), anticancer (Najmuddin *et al.* 2016; Omara *et al.*, 2020), antimicrobial (Mathew *et al.,* 2016) and antimalarial (Somsak *et al.,* 2016). *A. muricata* is used ethnobotanically as sedative, astringent, insecticide, pesticide, vermifuge and analgesic (Ishola *et al.*, 2014). Several parts of the plants have been found useful as anti-diabetic (Adeyemi *et al.*, 2009; del Campo-Rayas *et al.,* 2022).

The active compounds in *Annona* plants are acetogenins, alkaloids, phenols and other compounds (Mutakin *et al.*, 2022). The ethnomedicinal qualities of the plant have been attributed to some of these compounds (Coria-Téllez *et al.*, 2018). In addition, the presence of essential oils (EOs) accounts for the fragrances of many Annonaceae plants. Essential oils are characterised as mixtures of volatile molecules, aromatic, oily liquids that may be extracted from a range of plant parts, including seeds, leaves, flowers, buds, roots, and fruits. EO has a variety of purposes in nature, including attracting insects and allowing plants to communicate allelopathically (Cascaes *et al.,* 2021). Importantly, scientific research has supported the antibacterial, antioxidant and anti-inflammatory effects of EOs (Owolabi *et al.,* 2013; Gyesi *et al.*, 2019). However, it is noted that the compounds present in essential oils differ based on regional variances. In Nigeria, there have been few investigations into the chemical composition of *A. muricata*. Consequently, this study has been designed to assess the chemically relevant profile of the EO

found in the leaf and fruit of the Nigerian cultivar.

EXPERIMENTAL

Collecting and Processing the plant

Fresh leaves and fruits of *A. muricata* were obtained from sub-urban area in Lagos, Nigeria. The plant was identified and authentication was done at the herbarium of the Botany Department, Faculty of Science, University of Lagos. The voucher number LUH 6290 was allocated. The plant materials were pulverized.

Extraction of Essential oil

Distilled water (3 L) was added to 100 g of pulverized sample of *A. muricata* placed in a 5 L round-bottomed flask. The essential oil was extracted by the hydro-distillation and collected into *n*-hexane. The extraction was carried out for 4 h and the essential oil was collected into a glass vial after the extraction. Anhydrous sodium sulphate (Na_2SO_4) was used to eliminate the water from the extract. The colourless essential oil has a pleasant scent.

Gas Chromatography

The GC was fitted with the capillary column of 30 m x 250 µm x 0.25 µm film in dimensions and packed with HP-5MS 5% (phenyl methyl siloxane). Helium was the carrier gas at a flow rate of 1 mL/min. The extract was injected into the gas chromatography to separate the constituents of the essential oil. The temperature program for the analysis was column initially set at 70 °C and held for 3 min and then increased gradually to 250 °C at 4 °C/min and held for 4 min. The total time used per sample run was 52 min. The MS had a Chem-station control for programming and data processing. Ionization energy of 70 eV was used to document the mass spectra. The MS library was used to identify the molecular ions that were found. The results were obtained and recorded.

RESULTS

The chromatogram (TIC) obtained at the end of 52 min from the GC-MS of the leaf essential oils is in Figure 1. The GC–MS analysis of the leaf oil revealed 12 compounds with the most abundant being caryophyllene (26.04%) at 16.49 min retention time (R_t) as presented in Table 1.

On the other hand, three compounds caryophyllene (32.30%), *n*-hexadecanoic acid (24.80%) and (*E*)- nerolidol were identified in the essential oil from the fruit (Table 2). Figure 2 illustrates the GC-MS chromatogram of the essential oil from the fruit. Clearly, the compounds identified from the two oils differed significantly. Fruit oil (32.30%) had more caryophylene than the leaf oil (26.04%). Phytol was present in the leaf but not in the fruit.

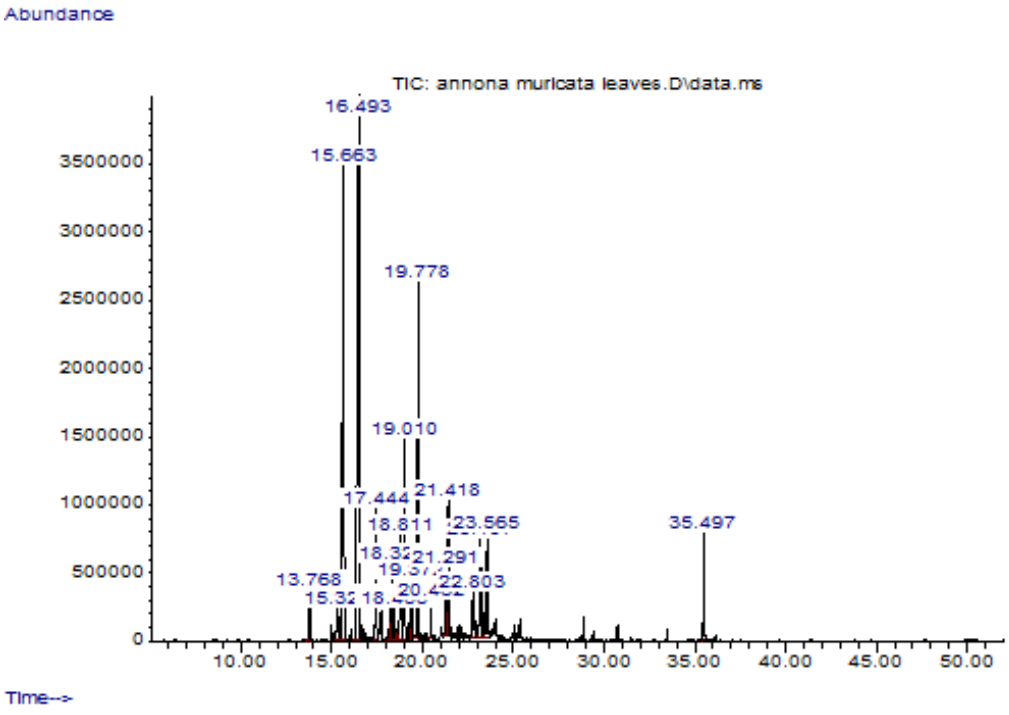

Figure 1: Chromatogram of essential oil from *A. muricata* leaf.

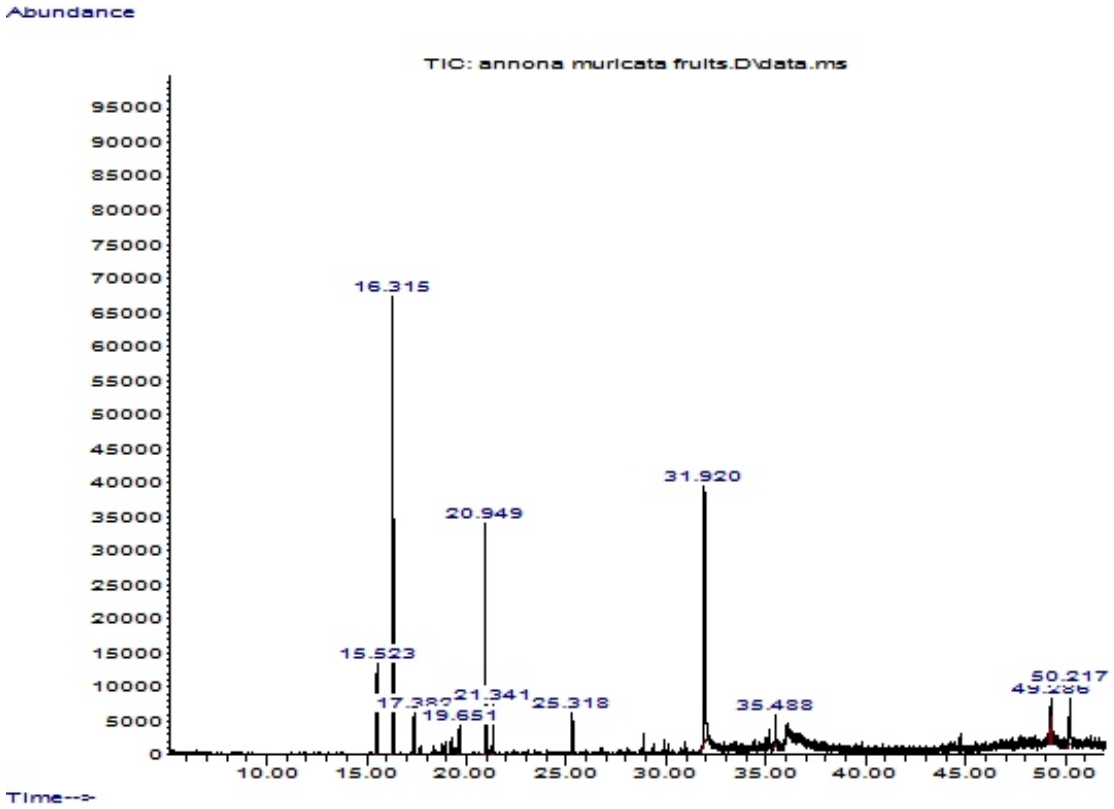

Figure 2: Chromatogram of essential oil from *A. muricata* fruit.

Table 1: Compounds analysed from essential oil of *A. muricata* leaf by GC-MS.

S/N	Name of compound	Class of Bioactive Compound	R_t (min.)	Abundance (%)
1	Phytol	Diterpene alcohol	35.50	2.83
2	tau -Cadinol	Sesquiterpenoid alcohol	23.19	3.84
3	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene,[1ar-(1a.α,4a.α,7.β.,7b.α)] (spathulenol)	Tricyclic Sesquiterpene alcohol	21.29	8.49
4	1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl),(1S-cis) Naphthalene (δ-cadinene)	Sesquiterpene	19.78	12.62
5	1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-Naphthalene (β-cadinene)	Sesquiterpene	19.37	1.77
6	1,2,4a,5,6,8a-hexahyd ro-4,7-dimethyl-1-(1-methylethyl)- , (1.α4a.α.,8a.α)-Naphthalene (α-cadinene)	Sesquiterpene	19.01	5.64
7	Bicyclogermacrene	Sesquiterpene	18.81	3.58
8	1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R(2.α,4a.α,8a.β)]-Naphthalene (α-selinene)	Sesquiterpene	18.46	0.77
9	1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-, [3aS-(3a.α,3b.β,4.β,7.β,7aS*)]- (β cubenene)	Sesquiterpene	18.32	1.87
10	α-Caryophyllene	Sesquiterpene	17.44	3.47
11	Caryophyllene	Sesquiterpene	16.49	26.04
12	1-methyl-4-(1- methylethyl)-1,3-Cyclohexadiene (α-terpenene)	Monoterpene	13.77	1.58

Table 2: Compounds analysed from essential oil of *A. muricata* fruit by GC-MS.

S/N	Name of compound	Class of Bioactive Compound	Rt (min)	Abundance (%)
1	*n*-Hexadecanoic acid (palmitic acid)	Saturated fatty acid	31.92	24.82
2	3,7,11-Trimethyl, (E)- 1,6,10-dodecatrien -3-ol (nerolidol)	Sesquiterpene alcohol	20.95	15.30
3	Caryophyllene	Sesquiterpenes	16.32	32.30

DISCUSSION

In this study, caryophyllene is present in abundance in the essential oil of both the fruit and leaf of *A. muricata* and α -caryophyllene is also present. A study from Benin reported that the main constituent of the essential oil from the leaf of *A. muricata* was caryophyllene (13.6%) (Kossouoh *et al.,* 2007). Although the values obtained were lower than those reported in this study, their findings were consistent with this report. Owolabi *et al.* (2013) also reported that the main compound of leaf oil from the plant in Badagry, Nigeria was (*E*)-caryophyllene (38.9%). Caryophyllene is a sesquiterpene present in the essential oils from a variety of plants and is thought to have antibacterial, anticarcinogenic, anti-inflammatory, antioxidant and local anesthetic properties (Legault and Pichette, 2007). Its anti-cancer activity has been extensively researched, with several writers concluding that the compounds have considerable potential at inhibiting the growth and proliferation of a variety of cancer cells (Dahham *et al.,* 2015; Fidyt *et al*., 2016; Lei *et al*., 2021). The presence of caryophyllene in the essential oil enables anti-inflammatory and anti-cancer properties which may help to inhibit the growth of fibroids (Francomano *et al.,* 2019). On the contrary, the leaf essential oil of Ghanaian variant of *A. muricata* was basically *δ*-cadinene (22.58%) while Sitosterols was identified abundant component of the fruit essential oils (Gyesi *et al.,* 2019).

Hexadecanoic acid (24.82 %) was the second most abundant bioactive component from the fruit. This supports the findings of other studies (Gyesi *et al*., 2019). *n*-Hexadecanoic acid is a saturated fatty acid with antibacterial and antifungal properties, as well as the ability to alter immunological responses directly on T cells (Aparna *et al*., 2012; Shaaban *et al.,* 2021). Using structural and kinetics studies, Aparna *et al.* (2012) also proposed that *n*-hexadecanoic acid may have anti-inflammatory properties as an inhibitor of phospholipase A2, an important agent in the formation of potent inflammatory mediator.

The presence of phytol in the leaf may support anti-oxidant and anti-inflammatory properties. Phytol is a precursor of vitamins E and K and is also a cholesterol lowering agent, it is a diterpene alcohol (Santos *et al.,* 2013). The presence of some naphthalene derivatives like selinene, cardinene which have antioxidant, antimicrobial and anti-inflammatory properties may enhance the activity of the plant. The antioxidant properties enable the prevention of oxidation of free radicals in the body, the antimicrobial properties prevents uterine fibroids from being septic and the anti-inflammatory property may prevent swelling of the fibroid. The fruit is used consistently to manage fibroid in Nigeria. Another constituent of the essential oil is α-terpene, α -terpene has anti-viral properties. It contains anti-tumorous agents that inhibit the growth of tumours in the body. Nerolidol is also present in the fruit of *A. muricata,* it has antioxidant, antimicrobial (Viera *et al.,* 2010), anti-parasitic, insecticidal, anti-ulcer, skin enhancer, anti-tumor, anti-nociceptive and anti-inflammatory properties as reported by Chan *et al.* (2016).

The constituents present in the plant *A. muricata* mostly terpenes, support its use in herbal medicine as antibacterial, anti-viral, anti-fungal, anti-tumour, anthelmintic, analgesic, hypotensive, anti-inflammatory, immune enhancing, anti-carcinogenic, wound-healing, anti-malarial, anti-convulsing, anti-anxiety, anti-diarrhoea, anti-parasitic effects (Moghadamtousi *et al*., 2015).

In summary, the pharmacological property of medicinal plant is as a result of their chemical components. Terpenes and terpenoids are constituents of EOs and they have many

therapeutic properties. The oxygenated constituents may possess antioxidant properties which will interact the reactive oxygen species (ROS) in ailments and may scavenge the ROS and this will result in the relief of the patient (Adewole and Ojewole, 2009).

CONCLUSION

The chemical constituents from leaf and fruit of *A. muricata* as identified by GC-MS are the agents that give the plant the pharmacological properties like; antioxidant, anti-inflammatory, anti microbial, anti- allergic, anti-fungal, anti-carcinogenic, anti-depressing with other pharmacological activities (Patel and Patel, 2016). The main constituents in *A. muricata* leaf and fruit being caryophyllene and *n*-hexadecanoic acid in the fruit. The constituents present in the leaf and fruit of *A. muricata* have properties that are useful in the physiological management of tumors such as fibroid, cancer and other ailments that produce ROS that may support the use of the plant in the management of these diseases. The results may therefore be scientific support for the use of *A. muricata* in the management of tumours and fibroids. Caryophylene and its derivatives may be synthesised or isolated from *A. muricata* for the use in the management of fibroid and other related ailments.

CONFLICT OF INTEREST

All the authors do not have any actual or potential conflict of interest in any form whether financial, personal or other forms with other people or organizations within three years of beginning the work that could influence or be perceived to influence, the work.

AUTHOR CONTRIBUTIONS ARE AS FOLLOWS

[EO] conceived the idea
[EO] designed it
[EO, OO] did the experimental
[EO, OO] did the interpretation of the data and results
[EO, OO] the paper was written by the two authors.

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