Phytochemical composition, Fourier Transform Infrared Spectroscopy (FTIR) and Gas Chromatography-Mass Spectrometry (GCMS) Analysis of Aqueous-Methanol Fraction of *Harungana madagascariensis* Leaves

Asadu Chidimma Lilian [1*], Eze Peace Chidozie [2], Ubenyi Stanley Mary [3]
[1-3] Department of Medical Biochemistry, Faculty of Basic Medical sciences, ESUT, College of Medicine, Parklane, Nigeria

* Corresponding Author: **Asadu Chidimma Lilian**

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Abstract
Harungana madagascariensis is a plant abundant in several phytochemicals that can be extracted, purified, and packaged for the aim of promoting optimum health of humans. This medicinal plant is dominant in Africa was analyzed to identify its Phytochemical constituents and functional groups using Gas Chromatography-Mass Spectrometry (GC-MS) and Fourier Transform Infrared Spectroscopy (FTIR). The aqueous-methanol fraction was obtained using standard extraction methods. The fraction was subjected to phytochemical analysis, which revealed the presence of diverse secondary metabolites including alkaloids, flavonoids, tannins, saponins and terpenoids, phenolic compounds, which are known for their antimicrobial, antioxidant and anti-inflammatory properties.
The fourier transform infrared (FTIR) spectral performed revealed the presence of characteristic functional groups such as OH stretch in phenol and alcohol, -CH stretch in alkenes, C=O -C-O stretch in esters, the presence of these functional groups provides strong evidence of the therapeutic potential of the plant.
The Gas Chromatography-Mass Spectrometry (GC-MS) profiling elucidates the presence of key metabolites and fatty acid derivatives such as Tri-hydroxy-phenylalanine, Quinolinone, 9-octadecenamide, n-Hexadecanoic acid, Linoleic acid ethyl ester, 9, 12- octadecanoic acid, Butanoic acid, 2-methyl-, methyl ester, hexadecanoic acid and Propanoic acid. These compounds are known to possess diverse pharmacological properties. The combined use of these spectrometric techniques provides complementary insight into the biochemical make up of *H. madagascariensis* leaves. The findings support the ethnomedicinal use of the plant and offer a scientific basis for its therapeutic potential. Further isolation and structural elucidation of the key metabolites are recommended to explore their pharmacological efficacy and potential for drug development.

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1. Introduction

Harungana madagascariensis leaves, commonly known as the dragon's blood tree or orange-milk tree, is a tropical plant widely used in traditional African medicine. Traditionally the leaves, in particular, have been used to treat a variety of ailments including skin infections (Tsioutsiou *et al*., 2022), inflammation and inflammatory related disorders [2], malaria [3] and gastrointestinal issues [4]. Phytochemicals is naturally occurring compounds found in plants that often have health benefits. They are responsible for the medicinal properties of many plants used in traditional healing and pharmaceutical industries. These bioactive compounds include flavonoids, alkaloids, tannins, phenolics, saponin, steroids etc. They help plants protect themselves against diseases, pest and environmental stress while also offering therapeutic effects when used by humans [5]. The screening of plant extract is

a new approach to find therapeutically active compounds in various plant species.

Fourier Transform Infrared Spectroscopy (FTIR) a technique used to identify the chemical bonds and functional groups in a plant's extract. It works by measuring how infrared light is absorbed by the sample, producing spectrum that reflects the types of chemical bonds present based on characteristic vibrational frequencies. This method helps in characterizing the molecular makeup of the plant extract, offering insight into the phytochemicals responsible for its medicinal effects [6].

Gas Chromatography on the other hand separates the compounds in the plant extract, while mass spectrometry provides detailed structural information about each compound based on their mass-to-charge ratio. GCMS is widely used to detect essential bioactive compounds such as alkaloids, acids and esters, which can have antimicrobial, antioxidant, anticancer, and other health-promoting activities [7]. Advanced analytical techniques are essential to identify and validate the presence of these bioactive compounds, providing a scientific knowledge for the plant's ethno medicinal applications.

The present study aims to investigate the bioactive composition of *Harungana madagascariensis* leaves using FTIR and GC-MS techniques. By combining these analytical techniques, it gives insights in the major compounds and functional groups present in the fraction, thereby supporting and providing a basis for pharmacological studies.

2. Materials and Methods

Plant Collection and Identification

The fresh leaves were collected from Adada River in Nsukka, Enugu State. It was identified and authenticated by Mr. Alfred Ozioko of Bioresource Development and Conservation Programme (BDCP) Research Centre, Nsukka, Enugu State Nigeria.

2.1. Methods

2.1.1. Preparation of Plant Extract

Harungana madagsacariensis leaves were air-dried at room temperature and pulverized into powder for extraction. The pulverized plant (2000 g) was macerated in 80% methanol and allowed to stand for 72 h at room temperature. The mixture was filtered with Whatman No. 1 filter paper and the filtrate was concentrated using a rotary evaporator to get a semi-solid extract. Solvent partitioning of the crude aqueous-methanol extract was done by using the protocol designed by [8]and modified version of [9]. Fractionation was carried out using n-hexane, ethyl-acetate, and aqueous-methanol. Crude extract (20 g) was weighed and dissolved in 250 ml of 20% aqueous-methanol (v/v) to form a stock solution. Then, 250 ml of n hexane was added to the solution and poured into a separating funnel. The mixture was allowed to stand for 20 min for proper separation, and the upper part was collected in a beaker. The aqueous-methanol part was washed repeatedly with n hexane, after which the different n hexane fractions were collected. The above procedure was repeated using ethyl-acetate. At the end, ethyl-acetate fractions were collected and concentrated. The fractions were concentrated using rotary evaporator. While aqueous-methanol fraction was used for Structural elucidation after subjecting the different fractions to a biological activity guided study.

2.2. Phytochemical Screening

Phytochemical screening of aqueous-methanol extract of *Harungana madagascariensis* leaves were carried out using the procedures as described by [10, 11].

2.3. Procedure for column chromatography of aqueous-methanol fraction of *Harungana madagascariensis* leaves

Fractionation was done using column chromatography. The fraction was spotted on thin layer chromatographic plate precoated with silical-gel (Merck, silica gel 60 F_{254}). The plates were developed in a chromatographic tank with different organic solvents. The solvent system which gave the highest separation was used for further purification of the fraction.100 g of silica gel of mesh size 60-120were mixed with 250 ml of eluent Butanol: Acetic acid: Water (70:20:10) and stirred for few minutes. The column was packed with the silica gel using a funnel. The sample was introduced by pouring on the wall of the column and subsequently eluted at 20 ml volume interval. In all 40 of 15 ml each were collected. They were spotted on TLC plate and their R_f value calculated. The ones with similar retention factor (R_f) value were pooled together. In all two sub-fractions were obtained.

2.4. Procedure for FTIR of aqueous-methanol fraction of *Harungana madagascariensis* leaves

The FTIR spectra of the fraction were carried out using FTIR-8400S spectrophotometer (Shidmazu model) available at the National Research institute for Chemical Technology (NARICT) Laboratory Zaria. The fraction was used in a form of a thin film, held in between two potassium bromide discs. The liquid paste was dropped on each disc and they spread into a thin film. The disc was then mounted in the FTIR spectrometer in the range 1.20 x 1013-1.20 x 1014 Hz) within electromagnetic spectrum if infrared section. Absorption is written in terms of wavenumbers (cm^{-1}).

2.5. Procedure for GC-MS of aqueous-methanol fraction of *Harungana madagascariensis* leaves

Chemical composition of active fraction was determined by GC/MS-QP-2010 plus Ultra using a DB-5 MS fused silica capillary column (30 × 0.25 m internal diameter, film thickness 0.25 µm). For GC-MS detection an electron ionization system with ionization energy of 70 eV was used. Carrier gas was Helium gas, flow rate was 1.2 mL/min. Injector and MS transfer line temperature were set at 260ºC and 270ºC at a rate of 8ºC/min to 280ºC; hold time was 10 minutes. Samples were completely dissolved in absolute ethanol and 0.3 µL was injected through auto-sampler in the spilt mode, split ratio was 1:100. Relative percentage of each constituent was expressed as percentages by peak area normalization. Each component was identified based on its column retention time relative to computer-based matching of mass spectra with those of standards (NIST and Wiley libraries for GC-MS system).

3. Results

3.1. Phytochemical screening of aqueous-methanol extract of *Harungana Madagascariensis* leaves revealed the presence of flavonoids, Phenols, Alkaloids, Tannin, Glycosides and Steroids as shown in Table 1.

Table 1: Qualitative and quantitative phytochemical analysis of and aqueous-methanol fraction

Phytochemicals	Relative Abundance	Mg/100g ±SD
Flavonoids	+++	1911.7±3.6
Phenolics	+++	4806.5±1.1
Alkaloids	+	48.93±1.1
Tannin	++	909.9±0.9
Glycosides	++	477.82±2.0
Steroid	+	91.03±1.1
Terpenoids	++	274.38±2.7

Key: + low concentration, ++ Moderate concentration, +++ High concentration

3.2. FTIR peak values and functional groups identified in aqueous-methanol fraction of *H. madagascariensis* leaf

The FTIR spectrum of aqueous-methanol fraction in Table 2 and Fig 1 revealed the presence of different functional groups as follow: -NH_2 group in amines (3500–3300cm-^{1}), OH stretch in phenol and alcohol (3280.09 and 3459.66 cm-^{1}), -CH stretch in alkenes, carboxylic acid and alcohol (2906.52-2766.69 cm-^{1}), -CH_3 group stretching vibration in aliphatic compounds (2261.24 and 2206.67cm-^{1}), -C-O stretch in esters (1056.62 cm-^{1}).

Table 2: FTIR peak values and functional groups identified in aqueous-methanol fraction of *H. madagascariensis* leaf

Wave number (cm^{-1})	Functional group	Possible compounds present
3000-2800	C-H Stretch	Alkane
3500-3200	O-H Stretch	Alcohols and Phenols
3500-3300	NH_2	Amines
2270-2100	C≡C Stretch	Alkynes
730.360	C-H bend	Aromatic compound
1000-650	=C-H	Alkenes and Aromatic compounds
1300-1000	C-O-C and C-OH	Ethers, alcohols, sugars
1410.7	N=N stretching	Azides
1609.913	C=C, C=N, NH	Aliphatics, Aromatics, Amines
2450.843	S-H stretching	Thiols
3075.777	C-H stretch	Alkane
1870-1650	C=O	Aldehyde and Carboxylic acids

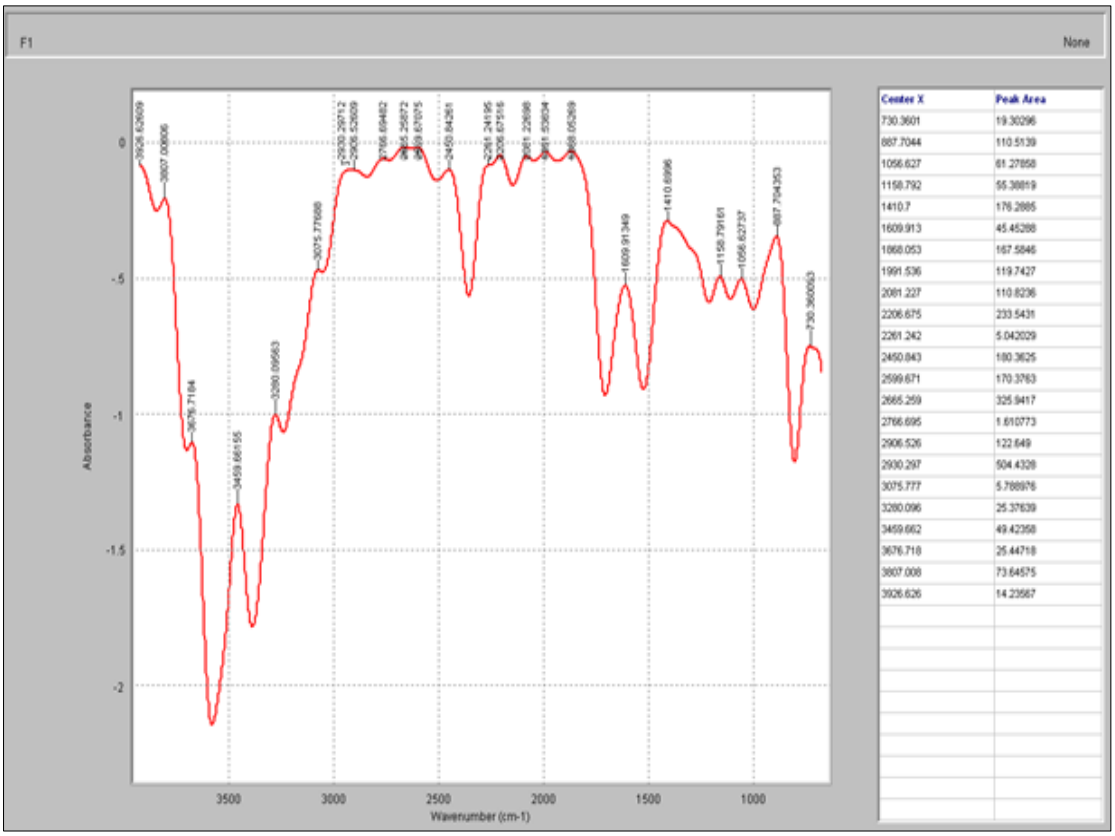

Fig 1: FTIR peak values and functional groups identified in aqueous-methanol fraction of *H. madagascariensis* leaf

3.3 Some Phytocompounds detected in aqueous-methanol leaf fraction of *H. Madagascariensis* by GC-MS analysis

The compounds present in aqueous-methanol fraction of *H. Madagascariensis* were identified by GC-MS analysis. Spectrogram showing the peak identities of the compound is presented in Fig 2. Active principles with their Retention Time (RT) and Area (%) are presented in Table 3. The following compounds were identified in the fraction. Bis (2-ethylhexyl) phthalates found to be responsible for the peak area (53.78%) followed by Trihydroxyphenylalanine (45.90%), 9-octadecenamide (14.55%), Quinolinone, (13.34%) and n-Hexadecanoic acid (12.73%).

Table 3: Some Phytocompounds detected in aqueous-methanol leaf extract of *H. Madagascariensis* by GC-MS analysis

Name of compound	Retention time	Area (%)
Bis (2-ethylhexyl) phthalate	43.175	53.78
Trihydroxyphenylalanine	43.307	45.90
9-octadecenamide	40.073	14.55
Quinolinone, hydrazine	22.914	13.34
n-Hexadecanoic acid	38.840	12.73
Propanoic acid	10.135	7.40
Butanoic acid, 2-methyl-, methyl ester	6.438	6.05
9, 12- octadecanoic acid	39.541	4.17
Hexadecanoic acid	40.066	3.86
Linoleic acid ethyl ester	39.929	2.20
10, octadecanoic acid	41.461	1.80

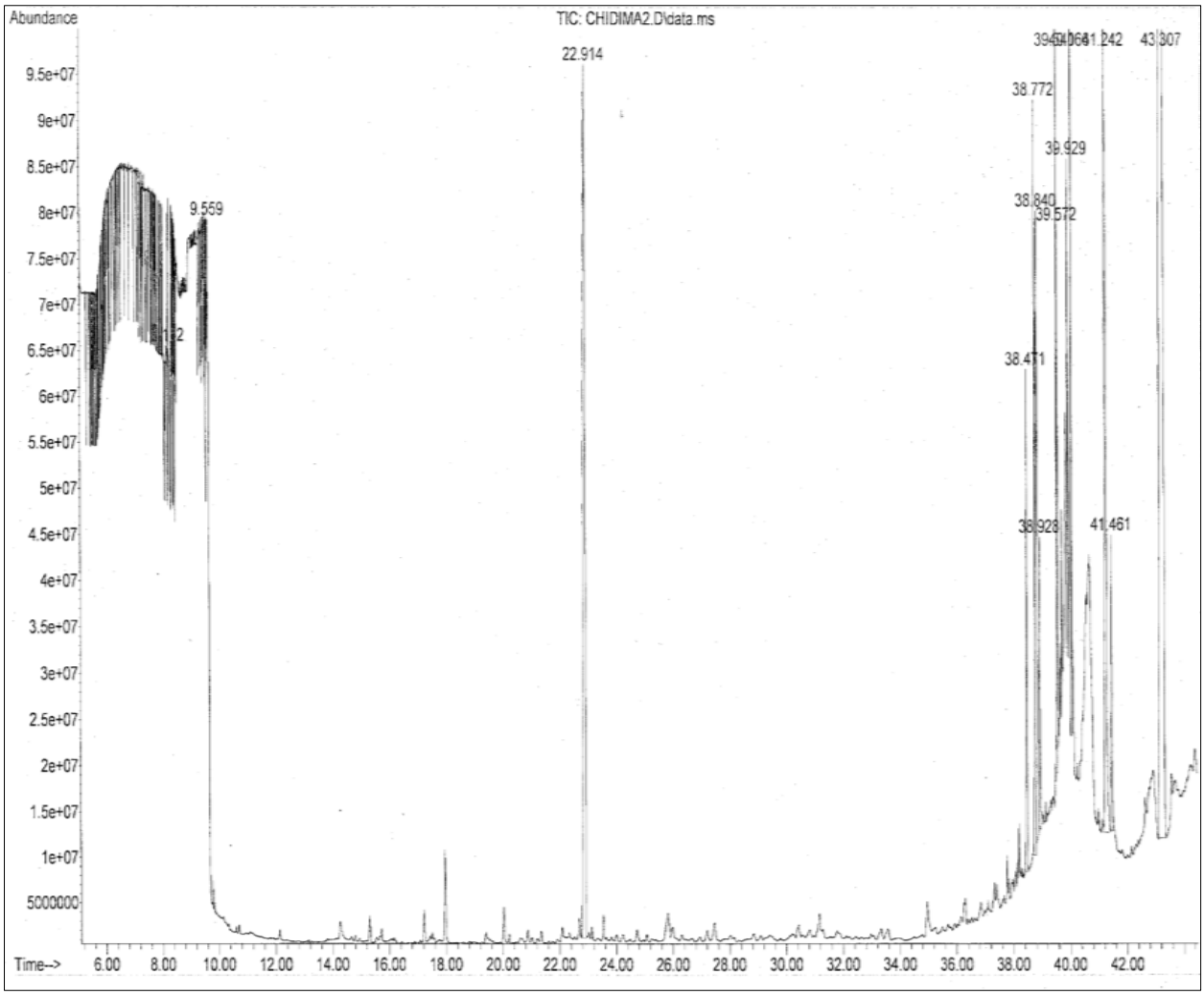

Fig 2: Some Phytocompounds detected in aqueous-methanol leaf extract of *H. Madagascariensis* by GC-MS analysis

4. Discussion

The quantitative and qualitative phytochemical analysis of the aqueous-methanol fraction of *H.madagascariensis* leaf indicated the abundance of many phytochemical constituents including; terpenoids, tannins, saponin, glycosides, flavonoids, phenols, among others. Phenolic compounds comprise one (Phenolic acids) or more (polyphenols) aromatic rings with attached hydroxyl groups in their structures. Their antioxidant capacities, Anti-inflammatory effects, antimicrobial activity, cardioprotective effects are related to these hydroxyl groups in their structure [12, 13]. Flavonoids have also shown to have anti-oxidative activity,

free radical scavenging capacity, hepato-protective, anti-inflammatory and anticancer activities [14]. Their activities are structure dependent. Functional hydroxyl groups of flavonoids mediate their antioxidant effects by scavenging free radicals and/or by chelating metal ions. The chelation of metal could be crucial in the prevention of radical generation which damage target biomolecules**.** The high flavonoids content obtained in this study supports its ethnomedicinal uses in treating infection and inflammation disorders as reported by [15] were it reported the use of the aqueous–methanol fraction in treatment of acute and chronic inflammatory infections. Tannins exhibit various medicinal properties; it reduces the permeability of mucosa to chemical irritation. Consequently, they reduce inflammation, exhibit astringent and protective action on the stomach mucosa and reduce excess acidity [16, 17]. Terpenoids also have been reported to relax cardiovascular smooth muscle by inhibition of Ca^{2+} influx in vascular smooth muscle or through quenching of reactive oxygen and nitrogen species and stimulation of nitric oxide (NO) synthesis [18, 19].The presence of these biologically active compounds suggests that the plant could serve as a potential source of drugs and its secondary metabolites could exert some biological activities.

The fourier transform infrared (FTIR) spectral result performed revealed the presence of functional groups. The broad peaks show the functional group as follows: $-NH_2$ group in amines, OH stretch in phenol and alcohol, -CH stretch in alkenes, carboxylic acid and alcohol, $-CH_3$ group stretching vibration in aliphatic compounds, -C-O stretch in esters. The presence of these functional groups in aqueous-methanol fraction of *H.madagascariensis* makes it a potential pharmacological source of new antibacterial, antioxidant and anti-inflammatory agents [20, 15].

The aqueous-methanol fraction of *H.madagascariensis* leaves revealed several peaks which represents different compounds. These identified phytoconstituents are presumed to be responsible for eliciting the pharmacological activity of this plant. The compounds includes: Trihydroxyphenylalanine, Quinolinone, 9-octadecenamide, n-Hexadecanoic acid, Linoleic acid ethyl ester, 9, 12-octadecanoic acid, Butanoic acid, 2-methyl-, methyl ester, hexadecanoic acid and Propanoic acid, interestingly, for some these compounds it have been reported to have antimicrobial, antioxidant and anti-inflammatory activities. Among the various compounds that are indicated to be present in the GC MS result, the following are known for their medicinal roles. Propanoic acid has been shown to enhance the colonic barrier function, exert immunosuppressive action, have anti-lipid activity and improve insulin sensitivity. These effects are beneficial in the prevention of obesity and type 2 diabetes [21, 22]. 9, 12 octadecanoicacid has been reported to have antihyperlipidemia and antiatherosclerosis [23, 24]. n-hexadecanoic acid has been reported to have anti-inflamatory activity [25]. Linoleic acid is a polyunsaturated omega-6 fatty acid, which is involved in many physiological functions, including hepatoprotective, antioxidant, and inflammation and wound healing [26, 27] Reported that linoleic acid enhanced wound closure in diabetic patients through the regulation of the inflammatory phase and angiogenesis. In addition, the hexadecanoic acid was shown to have antioxidant, antidiabetic and anti-cholesterol activities [28].

5. Conclusion

It could be concluded that the various bioactive compounds in the aqueous-methanol fraction could be responsible for the hypercholesterolemic, antimicrobial, antioxidant and anti-inflammatory activities of the plant. Therefore, management and prevention of oxidative stress and inflammatory disorders can be achieved through the use of *Harungana madagascariensis.* Further research is recommended to isolate and characterize the individual compounds identified for pharmacological evaluation.

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