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QUANTITATIVE DETERMINATION OF PHYTOCHEMICAL CONSTITUENTS OF FRACTIONS OBTAINED FROM *Ficus asperifolia* LEAVES MIQ (MORACEAE) AND THE CHARACTERIZATION OF COMPOUNDS IDENTIFIED IN THE RESIDUAL AQUEOUS FRACTION

***[1]Abdullahi Ibrahim Doma, [2]Umar Hauwa Yahaya, [3]Umar Bello Suleiman, [1]Abdullahi Rabiu Abubakar, [1]Adamu Yusuf Maitama, [1]Abdullahi Rahana Ameera, [1]Abdullahi Hamza Yaro**

[1]Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Bayero University, Kano. Postcode: 700233. P.M.B: 3452, Kano, Nigeria.
[2]Department of Chemistry. Federal College of Education (T) Gombe
[3]Department of Pure and Applied Chemistry, Faculty of Sciences, Bayero University, Kano.

*Corresponding authors' email: ibdomaa@yahoo.com Phone: +2348037019253

ABSTRACT

Ficus asperifolia (Miq), family Moraceae is popularly known as sand-paper tree that is found in marshy areas around river banks. In Nigeria, it is called *kawusa* by Hausa tribe, *ipin* by Yoruba tribe and *asesa* or *amerenwa* by Igbo tribe. This research aims to quantify secondary metabolites present in the crude methanol extract and fractions and to characterize the identified compounds in the residual aqueous fraction of *Ficus asperifolia* leaves. The powdered fruit was extracted using 6L of 70% methanol. The crude extract was dissolved in water and fractionated using chloroform, ethylacetate, and n-buthanol. Phytochemical screening was conducted to determine the chemical composition of crude methanol leaf extract of *Ficus asperifolia* and its fractions. The phytochemical screening conducted revealed the presence of saponins, tannins, flavonoids, alkaloids, steroids and cardiac glycosides. Quantitative analysis of total alkaloids, flavoniods, saponins, and cardiac glycosides was also carried out. The crude extract fractionated produced 16.5% of chloroform, 6.8% of ethylacetate, 5.9% of n-butanol and 70.8% of residual aqueous fractions. The extract was further characterized using the available spectroscopic techniques such as FT-IR, UV, and GC-MS respectively.

Keywords: Fractionation, Phytochemical, Quantitative, Metabolites, *Ficus asperifolia*

INTRODUCTION

Medicinal plants have been recognized and used throughout human history. They are the number one source of biologically active compounds and many documented medicinal plants have been scientifically proven to have therapeutic applications (Faustino *et al.* 2010). Research on medicinal plants has led to the discovery of novel lead compounds for potential development as drugs (Adebayo *et al*. 2015; Seo *et al*. 2018).

Ficus asperifolia is a variable plant that can be a scrambling shrub, or average-size tree, terrestrial or epiphyte which can reach up to 20 m in height (Ojo and Akintayo, 2014). It is widely distributed across Africa. Its presence has been reported in Senegal, Cameron, Sudan, Central and East African countries (Nkafamiya *et al.* 2010; Omoniwa and Luka, 2012). The common names of the plant include; English *Sandpaper tree*, Nupe/Hausa *Kawusa*, Yoruba *Ipin*, and Igbo *Anmerenwa* or *Asesa* (Burkill, 1997). Ethnomedicinally, the latex, leaves, bark and roots of the plant are generally used to treat headache, menstrual pain, inflammation of the gums, diabetes mellitus, hypertension, dysentery, liver problems, urinary and respiratory tract infections as well as tumors (Burkill, 1997; Watcho *et al*. 2009). The plant has been ethnomedicinally employed as an analgesic, anti-tumor, diuretic, abortifacient, antimalarial and menstrual cycle pain reliever (Adjanohoun, 1996; Arbonnier, 2004; Odiba *et al*. 2012).

Previous work done by Omoniwa and Luka (2012) on the aqueous stem extract of *Ficus asperifolia* revealed that it possesses hypoglycemic properties in diabetic rats. Nkafamiya et al. (2010) also reported that the crude fiber of the leaves of *Ficus asperifolia* has higher protein and mineral contents than some Nigerian vegetables. Other reported studies of *Ficus asperifolia* are the antibacterial effect of the aqueous bark extract (Nwanko and Ukaegbu-obi, 2014), uterotonic activities of the aqueous fruit extract (Pierre *et al*. 2009) and antioxidant activities of aqueous leaf extract (Ojo and Akintayo, 2014). Other scientific reports have revealed that the plant possesses gastroprotective (Raji *et al*. 2011), uterotonic (Watcho *et al*. 2011), hypolipidaemic (Omoniwa *et al.*, 2013) and antimicrobial properties (Lawal *et al*. 2016). In addition, the leaves have good antioxidant properties (Ojo *et al.*, 2016). Recent studies have indicated that the methanol leaf extract of *Ficus asperifolia* possesses good anti-inflammatory and analgesic activities (Abdullahi *et al.*, 2020). Preliminary phytochemical screening provides basic information about the different classes of secondary metabolites present in a plant and the medicinal importance of such plants (Shabbir *et al.*, 2013; Imafidon *et al*., 2018). The preliminary phytochemical screening of the leaf extracts of *Ficus asperifolia* revealed the presence of saponins, flavonoids tannins, alkaloids, terpenoids, steroids and cardiac glycosides which might be responsible singly or in complement for the observed biological activity of the extracts. This finding is in tandem with the reported work of Omaniwa and Luka, (2012) and Abdullahi et al. (2020) in the case of *Ficus asperifolia*. Secondary metabolites such as alkaloids, flavonoids, tannins, saponins, terpenoids, cardiac glycosides and steroids are responsible for plants' biological activities (Edewor-Kuponiyi, 2013; Rungsung *et al*. 2015). Phytochemicals constitute an integral part of medicinal plants and are responsible for their numerous bioactivities. Numerous plants have a wide variety of phytochemicals as their bioactive principles and are reported to possess antiplasmodial activity (Alshawsh *et al*. 2007; Matur *et al.* 2009). Although some studies have tracked antiplasmodial activity of plants to their alkaloids, flavonoids and terpenoids contents (Akuodor *et al.*, 2010; Philip, 2020; Tajjuddeen *et al.*, 2021), there are reported studies indicating antiplasmodial activity with saponins (Akanbi *et al.*, 2018; Nafiu *et al.*, 2021)

and glycosides (Yun *et al.,* 2021) respectively. This research was aimed at determining and quantifying some of these phytochemicals for possible identification of compounds in them. Chromatography and spectroscopy have become more effective and reliable tools used for phytochemical analysis (Patle *et al*., 2020; Mabasa *et al*., 2021). Fourier transform infrared (FTIR) spectroscopy is used to characterize and identify functional groups (Saxena *et al*., 2012). Ultraviolet-visible spectrophotometry (UV-Vis) is related to photon spectroscopy in the UV-visible region (Saxena *et al.* 2012; Johnson and Fatima, 2018). This technique uses light that is in the visible ranges of the electromagnetic spectrum (Saxena *et al*. 2012; Johnson and Fatima, 2018). The colour of the chemicals involved affects the absorption, and molecules undergo electron transition in these ranges (Saxena *et al*. 2012). Analysis of complex media using ultraviolet-visible (UV Vis) spectroscopy is a disadvantage due to limitations by inherent difficulties when it comes to assigning peaks to any constituents in the system (Akambi *et al*., 2018; Nafiu *et al*., 2021). Therefore, the UV-Vis findings must be supplemented with other analytical techniques, such as GC-MS or LC-MS, for appropriate phyto compound profiling and constituent identification.

MATERIALS AND METHODS

Preparation of plant material

The fresh leaves of *Ficus asperifolia* Miq were collected from Toro district, Toro Local Government Area of Bauchi State, Nigeria. The plants were identified and authenticated at the herbarium unit of the Department of Biological Sciences, Bayero University, Kano, Nigeria. A voucher specimen number BUKHAN 0106 was collected for *Ficus asperifolia*.

Extraction

Fresh leaves of *Ficus asperifolia* were collected from the plant, rinsed with clean water and shade-dried. The plant materials were then pulverized into a fine powder using a porcelain mortar and pestle and sieved. Powdered plant material weighing 2kg was macerated with 7L of 70% v/v methanol at room temperature for 7 days with occasional agitation of the mixture. At the end of the extraction, the crude methanol extract was filtered using What man's filter paper (1mm mesh size) and then concentrated in a water bath maintained at 45^{o}C until greenish-black residues were obtained and stored in a desiccator.

Fractionation of crude extract

The methanol crude extract of *Ficus asperifolia* leaf was subjected to liquid-liquid partitioning to separate the extract into different fractions. The extract was reconstituted with 300 ml of distilled water. The reconstituted extract was placed in a separating funnel and 300 ml of chloroform was added sequentially as a 1:1 (v/v) solution and shaken (Deng *et al*. 2007). The sample was left to stand for 30 minutes in the separating funnel until a fine separation line appeared indicating the supernatant from the sediment before desorption. The process was repeated at least thrice until chloroform fraction was exhaustively collected in a container and concentrated in a water bath maintained at 45^{o}C. The same process was sequentially repeated using ethyl acetate (EAF), n-butanol (NBF) as well as the residual aqueous (RAF) fraction.

Phytochemical Screening

The chemical composition of crude methanol extract and fractions was determined using a phytochemical screening Test as shown below by Trease and Evans, (2009).

Saponins

Frothing Test: In this test, 3g of a powdered crude extract of *Ficus asperifolia* was mixed with 5 ml of distilled water in a test tube and shaken vigorously. The frothing produced was mixed with a few drops of olive oil and mixed briskly. The formation of foam indicated the presence of saponins.

Tannins

Ferric Chloride Test: To 1ml solution of *Ficus asperifolia* crude extract, CHF, EAF, NBF, and RAF in a test tube, 1% gelatin solution containing ferric chloride was added and shaken. The formation of the bluish-black colour showed the presence of phenols.

Flavonoids

Shinoda's Test: 1ml of *Ficus asperifolia* extract, CHF, EAF, NBF, and RAF solution were transferred into a test tube, then few drops of concentrated HCl acid were added, followed by 0.5 mg of magnesium ribbon and shaken. Emergence of pink coloration indicates the presence of flavonoids.

Alkaloids

Dragendoff's Test: In this test, 1 ml of *Ficus asperifolia* extract, CHF, EAF, NBF, and RAF were added to 1ml of potassium bismuth iodide solution (Dragendoff's reagent) and shaken. An orange-red precipitate formed indicating the presence of alkaloids. Wagner's Test: 1ml of plant extract, HF, CHF, EAF, NBF, and RAF were added to 1ml of potassium iodide (Wagner's reagent) and shaken. The appearance of reddish-brown precipitate signified the existence of alkaloids.

Mayer's Test: 1ml of plant extract, CHF, EAF, NBF, and RAF were added to 1ml of potassium mercuric iodide (Mayer's reagent) and shaken. The emergence of whitish precipitate confirmed the presence of alkaloids.

Anthraquinones

Bontrager's Test: 10ml of benzene was added to 6g of the *Ficus asperifolia* crude extract, CHF, EAF, NBF, and RAF powdered sample in a conical flask and soaked for 10 minutes, then filtered. A 10 ml of 10% ammonia solution was added to the filtrate and shaken vigorously for 30 seconds. The appearance of pink colour in the ammonia phase showed the occurrence of anthraquinones.

Steroids

Libermann Bruchard's Test: 5ml aqueous plant crude extract, CHF, EAF, BF, and RAF were transferred into a test-tube. Then 2ml of chloroform and concentrated H_2SO_4 were added, appearance of red colour at the lower chloroform layer signified the presence of steroids.

Cardiac Glycosides

Keller Killiani's Test: 10 ml of *Ficus asperifolia* extract, CHF, EAF, NBF, and RAF solution were transferred into a test-tube. Then 4 ml of glacial acetic acid was added, followed by 1 drop of 2% FeCl3 solution and shaken. Then 1 ml of concentrated H_2SO_4 was added to the mixture. A brown ring formed between the two layers indicated the existence of cardiac glycosides.

Quantitative Analysis

Total Alkaloids

The sample of *Ficus asperifolia* crude extract, CHF, EAF, NBF, and RAF 5 g was taken into a 250 ml beaker. Then 200 ml of 10% acetic acid in ethanol was transferred, covered and allowed to stand for 4 hrs. The mixture was filtered and then

concentrated over water bath to ¼ of the initial volume. Later, a few drops of concentrated NH_4OH were added to the extract until a complete precipitate was formed. The whole mixture was allowed to settle and the precipitate was collected and washed with dilute NH_4OH and then filtered. The residue produced was dried and weighed as alkaloid (Harbone, 1973).

Total Flavonoids

In this test, powdered samples of *Ficus asperifolia* crude extract, CHF, EAF, NBF, and RAF 2.5g were mixed with 50 ml of 80% aqueous methanol in 250 ml beaker, and allowed to stand for 24 hours at room temperature. The supernatant layer was discarded, and the residue was re-extracted three times with 50ml of ethanol. The solution produced was filtered using Whatman filter paper number 42 (125mm). The filtrates were later evaporated to dryness over a water bath. The content was cooled in a desiccator and weighed until constant weight was obtained (Boham and Koupai-Abyazan, 1974). The percentage yield for flavonoid was calculated as follows: % Yield = Weight of flavonoid / Weight of sample ×100.

Total Saponins

Ficus asperifolia crude extract, CHF, EAF, NBF, and RAF 20 g each were placed in a conical flask and 100 ml of 20% aqueous ethanol was added. The mixture was heated in a water bath at 55 ºC for 4 hours with continuous stirring. It was filtered and the residue Re-extracted with another 200 ml of 20% ethanol. The combined extract was reduced to 40 ml over a water bath at about 90 ºC. The concentrate was transferred into a 250 ml separation funnel and 20 ml of diethyl ether was added and vigorously shaken. The aqueous layer was recovered while the ether layer was discarded. The aqueous layer was purified and 60 ml of n-butanol was added. The n-butanol extract was washed twice with 10 ml of 5 % aqueous NaCl. The remaining solution was evaporated and dried in the oven to a constant weight (Obadoni and Ochuko, 2002). The saponin content was calculated using the formula below: % Yield = Weight of saponin / Weight of sample ×100.

Total Cardiac Glycosides

In this test, 10% of *Ficus asperifolia* extract, CHF, EAF, BF, and RAF were mixed with 10 mL of freshly prepared Baljet's reagent (95 mL of 1% picric acid + 5 mL of 10% NaOH). The mixture was allowed to stand for 1 hour. This is followed by dilution with 20 mL distilled water and the absorbance was measured at 495 nm using a UV spectrophotometer (Solich *et al*., 1992). The percentage yield of cardiac glycosides was calculated as follows: Weight of sample (g): Absorbance of sample/Absorbance of standard x Concentration of standard. % Yield = Weight of saponin / Weight of sample ×100.

Identification Methods

FT-IR

Fourier Transform Infrared Spectroscopy (FTIR) About 1 mg of the dried powder of RAF, all the samples were taken into pellets in KBr and were allowed to pass through infrared radiation and absorbed at certain frequencies in FTIR spectroscope (Shimadzu, Japan), with a scan range from 400 to 4000 cm^{-1} and a resolution of 4 cm^{-1} The sample was identified using sodium chloride plates (Abubakar and Haque, 2020; Dhivya and Kalaichelvi, 2017). The infrared spectra showing absorbance against wavelength was obtained with respect to literature characteristic absorption bands, some deductions were made and samples were identified based on their functional group.

Ultraviolet Spectroscopy Measurement

About 1 mg of the dried powder of RAF was dissolved in n-butanol and centrifuged at 3000 rpm for 10 minutes, then filtered through the Whatmann No.1 filter paper. The sample was diluted to 1:10 n-butanol. The sample was scanned at wavelength ranging from 200 to 1100 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected (Abubakar and Haque, 2020; Dhivya and Kalaichelvi, 2017).

Gas Chromatography-Mass Spectrometry (GCMS)

The phytochemical analysis of the methanol leaf extract of *Ficus asforipolia* was conducted using Gas Chromatography-Mass Spectrometry (GC-MS) on an Agilent GCMS system. Separation of phytoconstituents was achieved on a 60-meter TRX 5-MS capillary column (30 m × 250 µm film) with a 2 µL sample injection. The temperature program started at 80 °C for 3 minutes and then increased at a rate of 10 °C per minute until reaching 280 °C, where it was held for 19 minutes. Helium was used as the carrier gas at a flow rate of 1.21 mL/min. The injector and source temperatures were set at 260 °C and 220 °C, respectively. The system utilized electron ionization with an energy of 70 eV and a multiplier voltage of 380 within the m/z range. The identification of individual phytoconstituents was accomplished by consulting the Institute of Standards and Technologies (NIST) libraries (Ahmad *et al*. 2020).

RESULTS AND DISCUSSION

Percentage Yield

The 2kg powdered *Ficus asperifolia* leaves macerated produced 216g of dried crude methanol extract giving a 10.8% yield.

Fractions Obtained

One hundred grams (100 g) of the crude extract was fractionated and the results are presented in Table 1:

Table 1: Percentage Yield

Extract	Percentage yield (%) w/w
CRF	(16.5)
EAF	(6.8)
NBF	(5.9)
RAF	(70.8)

CRF = chloroform fraction, EAF =ethyl acetate fraction, NBF = n-butanol fraction and RAF = residual aqueous fraction.

Phytochemical Constituents

The phytochemical constituents present in methanol leaf extract and fractions of *Ficus asperifolia* are saponins, tannins, flavonoids, alkaloids, steroids and cardiac glycosides. The result is shown in Table 2:

Table 2: Phytochemical Constituents of Methanol Leaf Extract and Fractions of *Ficus asperifolia*

Chemical constituents	Crude	CRF	EAF	NBF	RAF
Alkaloids	+	-	-	-	+
Anthraquinone	-	-	-	-	-
Steroid	+	+	+	+	+
Terpenoids	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+
Saponins	+	-	+	+	+
Tannins	+	-	+	+	+
Flavonoids	+	-	+	+	+

+ =present, **- =** absent, CRF = chloroform fraction, EAF =ethyl acetate fraction, NBF = n-butanol fraction and RAF = residual aqueous fraction.

Quantity of Preliminary Phytochemical Constituents

The quantitative analysis revealed that Crude extract contains 10 mg/g alkaloids, 10 mg/g flavonoids, 24.5 mg/g saponins, and 0.87 mg/g cardiac glycosides. CRF contains only cardiac glycosides 1.82 mg/kg. However, EAF contains alkaloids flavonoids 1.0 mg/g, saponins 1.0 mg/g and cardiac glycosides 2.11 mg/g. Furthermore, NBF contains flavonoids 21.0 mg/g, saponins 13.0 and cardiac glycosides 0.2 mg/g while RAF contains alkaloids 3.0 mg/g, flavonoids 8.0 mg/g, saponins 1.0 mg/g and cardiac glycosides 1.85 mg/g. The result of the quantitative analysis is shown in Table 3:

Table 3: Quantity of Phytochemicals in Extract and Fractions of *Ficus asperifolia*

CONSTITUENT	QUANTITY (mg/g)				
	Crude	CRF	EAF	NBF	RAF
Alkaloids	10.00	-	-	-	3.00
Flavonoids	10.00	-	1.00	21.00	8.00
Saponins	24.50	-	1.00	13.00	1.00
Cardiac Glycoside	0.87	1.82	2.11	0.20	1.85

CRF = Chloroform fraction, EAF =Ethyl acetate fraction, NBF = N butanol fraction and RAF = Residual aqueous fraction.

FT-IR Results

The result of FT-IR spectroscopic analysis of the extract shows the presence of OH-stretch at 3201 cm^{-1}, C=O at 1603 cm^{-1}, CH-bend at 1365 cm^{-1} and aromatic association at 2106 cm^{-1}, all absorption is within the expected functional group region (Table 4 and Figure I).

Table 4: FT-IR Spectroscopic Analysis of the Residual Aqueous Fraction of *Ficus asperifolia*

CHARACTERISTICS IR FREQUENCY RANGES	OBSERVED VALUE	CHARACTERISTICS VIBRATIONAL MODE
3200- 3550	3201	Strong broad band with hydroxyl group (OH)
2600-2550	2344	Weak band for S-H thiol group
1600-1300	1603	Strong sharp band for N-O bond
1550-1500	1521	Also stretching of N-O in nitro compounds
1450-1375	1439	Medium band due to OH bending in carboxylic acid.
1225-1200	1205	C-N stretching of an amine, C-O of an ester, or a vinyl ether
1124-1087	1100	Stretching of secondary alcohol
1070-1030	1048	C-O-C stretching of anhydride and S=O sulfoxide
980-960	965	C=C bending of alkene di substituted (trans) and mono substituted

UV spectroscopy results

The UV spectroscopy analysis carried out on the extract sample exposed the presence of multiple absorption bands at a range of (255–288nm) corresponding to the absorption region of flavones and their derivatives. Furthermore, absorption was found at the higher wavelength of 433.12nm which correspond to the absorption range of alkaloid secondary metabolites as shown in Figure II and III respectively.

Table 5: GC-MS Result showing most Prominent Compounds in the analysis of the residual aqueous fraction of *Ficus asperifolia*

RT	Area Pct	Library/ID	Ref	CAS	Qual
13.7351	1.2854	Butylated Hydroxytoluene	83558	000128-37-0	97
15.6924	1.576	Decanoic acid, silver(1+) salt	137505	013126-67-5	53
20.0363	0.9037	5-Octadecene, (E)-	113636	007206-21-5	74
22.9043	13.3174	Pentadecanoic acid, 14-methyl-, methyl ester	130841	005129-60-2	98
23.7298	5.0167	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	192206	000085-69-8	90

24.2596	8.2053	Hexadecanoic acid, ethyl ester	144309	000628-97-7	94
26.1839	23.4697	9,12-Octadecadienoic acid, methyl ester	153873	002462-85-3	99
26.2999	41.2087	9-Octadecenoic acid (Z)-, methyl ester	155750	000112-62-9	99
26.8108	5.8378	Methyl stearate	157883	000112-61-8	97
27.4238	0.8262	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, [1R-(1.alpha.,2.alpha.,5.alpha.)]-	17430	004863-59-6	58
27.5261	7.1531	(E)-9-Octadecenoic acid ethyl ester	169329	006114-18-7	95
28.0314	3.9915	Octadecanoic acid, ethyl ester	171407	000111-61-5	64
37.9637	-12.8639	Oleic Acid	142070	000112-80-1	59
38.0562	0.0726	Oleic Acid	142070	000112-80-	83

Discussion

Natural phenolic compounds as secondary metabolites are present in a broad range of plants, including those we consume as food crops. Some of these secondary metabolites have been suggested to act as primary antioxidants or free radical scavengers that can mitigate the oxidative damages induced by the parasites and in some disease pathogenesis. Others, such as some polyphenols and flavonoids, may be pro-oxidants in high ingested amounts (Halliwell, 2007; Asanga *et al*. 2017) this therefore suggest that better activity does not always depend on higher contents of these secondary metabolites but optimality to function either as anti or pro oxidants depending on what is targeted at a given period. These compounds are recognized for their antioxidant properties and their ability to mitigate chronic and degenerative diseases in humans, making them an essential component of a healthy diet. Antioxidant property of saponins have been reported by several studies towards preventing the generation can block of free radicals and are able to block protein synthesis in the parasites' apicoplast ribosome through structure-related interaction (Abdullelah and Zainal, 2007). However, it's important to note that under certain conditions such as high phenolic concentration, elevated pH levels, or in the presence of transition metal ions like Cu2+ or Fe3+, these antioxidants can exhibit pro-oxidant behavior (Rajashekar, 2023). This can lead to the generation of reactive oxygen species (ROS), including hydroxyl radicals, which can cause oxidative stress and cellular toxicity. While this may contribute to the development of various cancers and other pathogenic conditions, increased levels of ROS can also have beneficial effects by selectively eliminating malignant cells and enhancing food safety by eliminating foodborne pathogens (Rajashekar, 2023). Hence, the dual nature of phenolic compounds enables them to function both as antioxidants and pro-oxidants. Similarly, depending on the extent of their pro-oxidant activity, ROS can either trigger pathogenesis, exacerbate existing conditions, compromise immunity (thus increasing vulnerability to certain diseases like malaria), or serve as potential agents for eradicating malignant cells and foodborne pathogens (Rajashekar, 2023). Alkaloids are naturally occurring chemical compounds that contain mostly basic nitrogen atoms, in addition to carbon, hydrogen, oxygen and sometimes Sulphur, with some related compounds that have neutral and even weakly acidic properties (Neha Babbar, 2015). These groups are observed from the FTIR spectrum around 2344cm-1 (S-H), 1603cm-1 (N-O), and 1205 (C-N). Saponins are amphiphilic molecules of pharmaceutical interest and most of their biological activities are associated with their membranolytic properties. These molecules are secondary metabolites present in numerous plants. Structurally, all saponins correspond to the combination of a hydrophilic glycan, consisting of sugar chain(s), linked to a hydrophobic triterpenoidic or steroidic aglycone. Saponins present a high structural diversity and their structural characterization remains extremely challenging due to their structural diversity with extracts consisting of a huge number of congeners presenting only subtle structural differences. It can be better characterized using nuclear magnetic resonance spectroscopy (NMR) (Savarino *et al*. 2023).

The prevailing compounds identified were Butylated Hydroxytoluene, Decanoic acid, silver (1+) salt, Pentadecanoic acid, 14-methyl-methyl ester, 1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester, Hexadecanoic acid, ethyl ester, 9,12-Octadecadienoic acid, methyl ester, Methyl stearate, Bicyclo [3,1,1]heptane, 2,6,6-trimethyl- [1R-(1,alpha,2alpha,5alpha.)]-(E)-9-Octadecenoic acid ethyl ester, Octadecanoic acid, ethyl ester and Oleic Acid. GC-MS allows precise identification of a sample from a complex mixture; it also gives a quantitative analysis of the compounds which is crucial for understanding plants' extract composition (Ahmad *et al*., 2020). The extract was found to be rich in ester and other polar phytochemical compounds which were similar with literature reported by Abubakar *et al.* (2020). Several reports have indicated that the leaves' extracts of this plant was used for many medicinal applications Abdullahi et al. (2023); Rungsung et al. (2015) and Edewor Kuponiyi, (2013). These established compounds were similarly reported by Romeh, (2013), who stated that chemical characterization of *Ficus asperifolia* Leaves indicated the detections of these predominant compounds as key components of *Ficus asperifolia* Leaves extract. Oleic acid from the leaves' extract can be used as an antioxidant, hypocholesterolemic,and nematicide as reported by Praveen *et al.* (2010).

In addition, Ogunlesi *et al.* (2009) reported that the secondary metabolite of *Ficus asperifolia* leaves is found to have effective, preventive, and therapeutic results against arthritis. The reactive oxygen species promoting substances like phytol constitute a promising innovative class of pharmaceuticals for the treatment of rheumatoid arthritis and possibly other chronic inflammatory diseases (Abdullahi *et al*. 2023). The result of FT-IR spectroscopic analysis of the extract shows the presence of OH-stretch at 3201 cm^{-1}, C=O at 1603 cm^{-1}, CH-bend at 1365 cm^{-1} and aromatic association at 2106cm^{-1}, all absorption is within the expected functional group region. These structural components found in the extract suggested the presence of alcohol, ester, carboxylic acid, and their derivatives. The absorption discovered from the FT-IR results agrees with that reported by Kumar and Pandey, (2013) and Panche et al. (2016).

The UV spectroscopy analysis carried out on the extract sample exposed the presence of multiples absorption bands at a range of (255–288 nm) corresponding to the absorption region of flavanols, and their derivatives. Similarly, absorption was found at a higher wavelength of 433.12 nm which corresponds to the absorption range of alkaloid secondary metabolites. Abdullahi *et al*. (2023) reported similar results. Our findings highlight the richness of bioactive compounds in *Ficus asperifolia* leaves, including

alkaloids, saponins, steroids, esters, acids, polyphenols, flavonoids, and other phytochemicals, which are known for their antioxidant, anti-inflammatory, anti-malarial and health-promoting properties. These compounds have the potential to contribute to the development of novel pharmaceuticals for various health applications.

CONCLUSION

The quantitative determination of phytochemical constituents present in fractions obtained from *Ficus asperifolia* leaves Miq (Moraceae) was conducted and compounds were successfully characterized through analytical and spectroscopic techniques which revealed the presence of flavonoids and its derivatives as the major constituent obtained from the *Ficus asperifolia* leaves' extract. Other secondary metabolite includes alkaloid, tannins, saponins and cardiac glycosides. Thus, secondary metabolite highlights the potential value of this plant in traditional medicine and modern drug development. These findings can serve as a foundation for further investigations and applications of this plant extract in the fields of pharmacology, ethnobotany, and natural product chemistry. *Ficus asperifolia* can be used as a source of natural antioxidants, antimicrobial and anticancer agents.

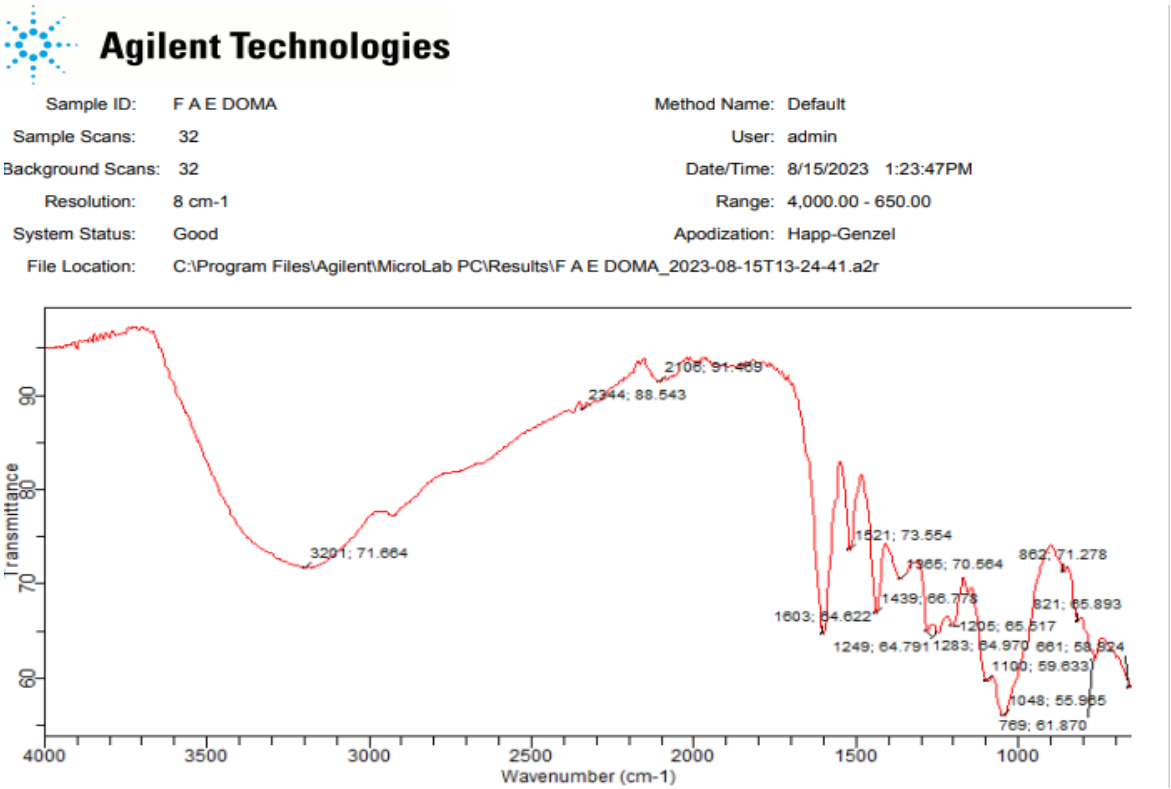

Figure 1: FT-IR Spectra showing absorption regions in the analysis of the residual aqueous fraction of *Ficus asperifolia*

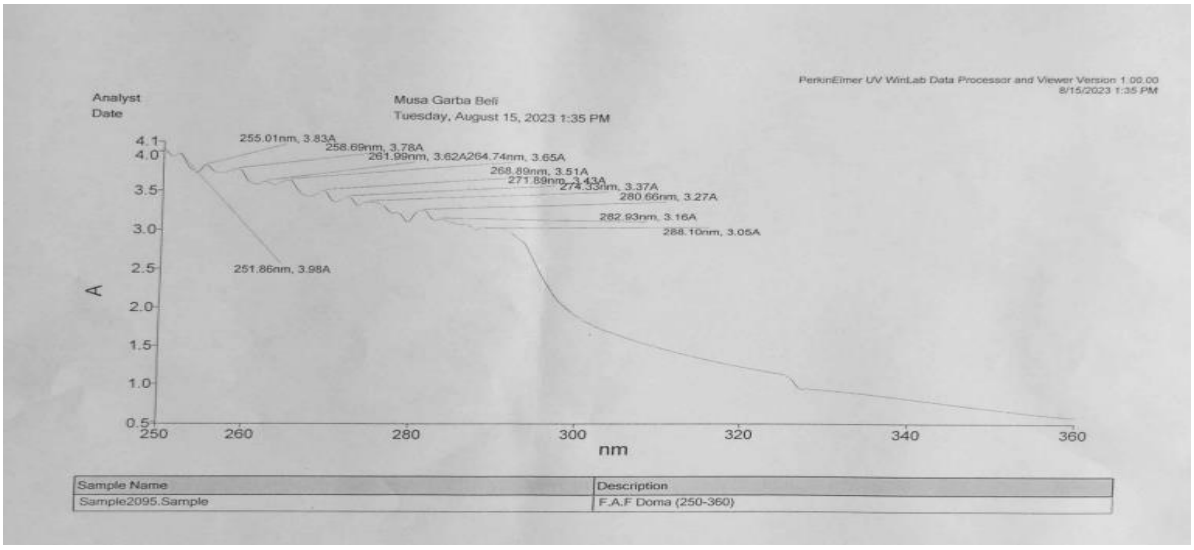

Figure 2: UV Spectra showing absorption regions in the analysis of the residual aqueous fraction of *Ficus asperifolia*

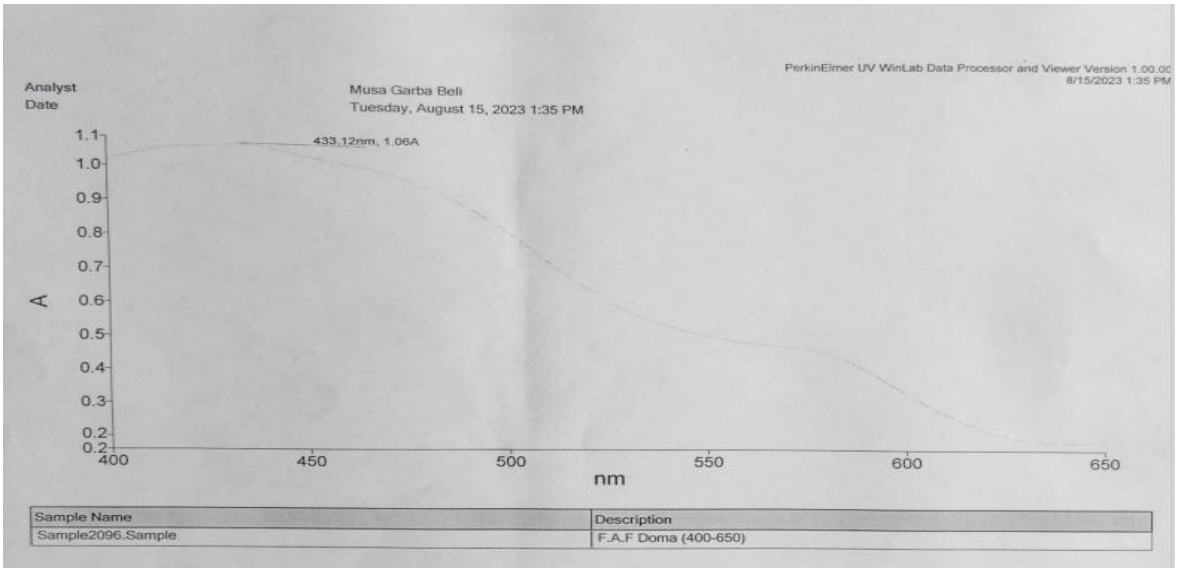

Figure 3: UV Spectra showing absorption regions in the analysis of the residual aqueous fraction of *Ficus asperifolia*

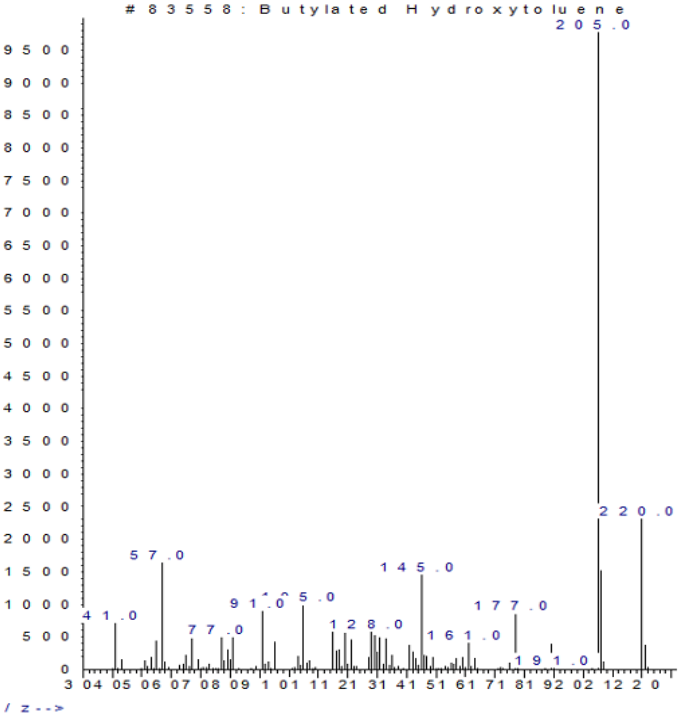

Figure 4: GCMS Peak area presentations showing most prominent compound in the analysis of the residual aqueous fraction of *Ficus asperifolia*

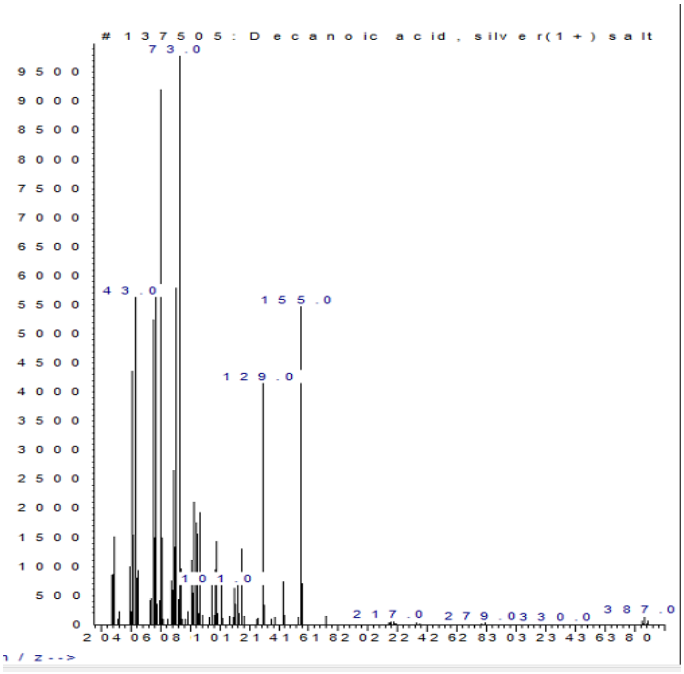

Figure 5: GCMS Peak area presentations showing most prominent compound in the analysis of the residual aqueous fraction of *Ficus asperifolia*

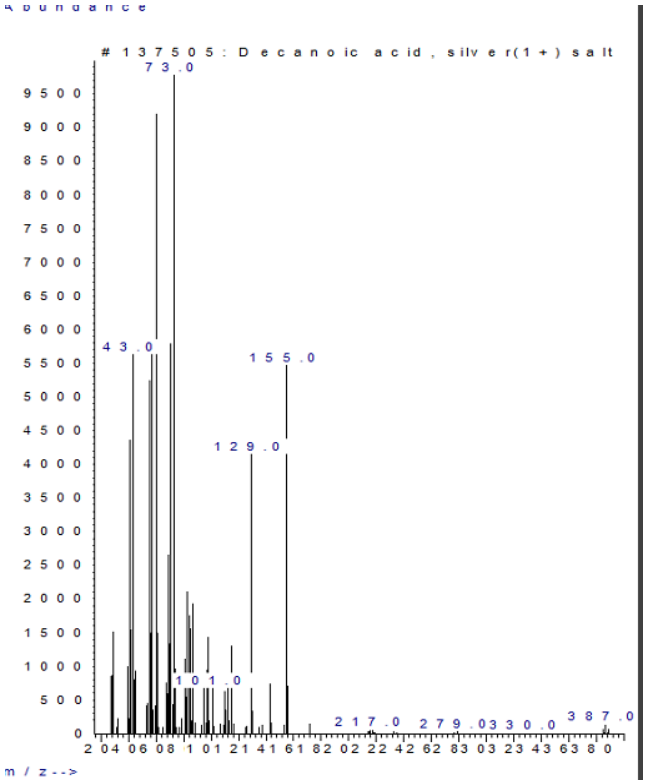

Figure 6: GCMS Peak area presentations showing most prominent compound in the analysis of the residual aqueous fraction of *Ficus asperifolia*

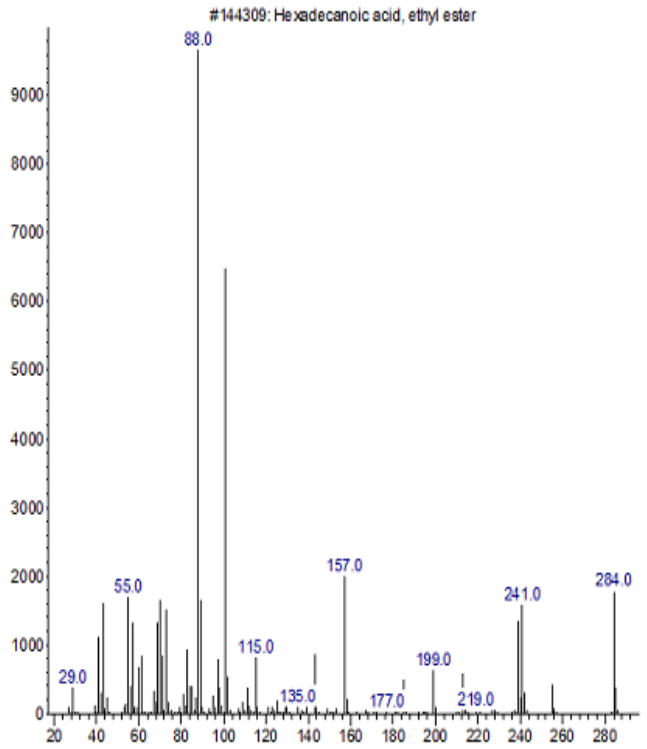

Figure 7: GCMS Peak area presentations showing most prominent compound in the analysis of the residual aqueous fraction of *Ficus asperifolia*

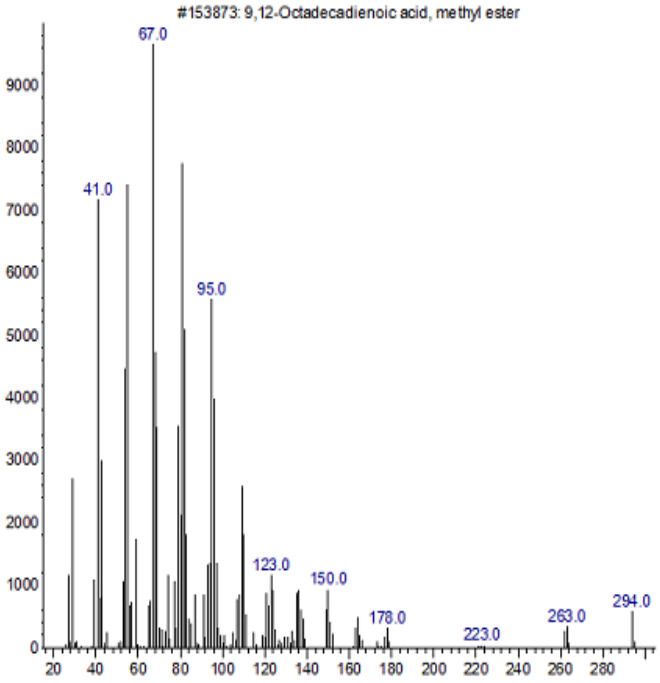

Figure 8: GCMS Peak area presentations showing most prominent compound in the analysis of the residual aqueous fraction of *Ficus asperifolia*

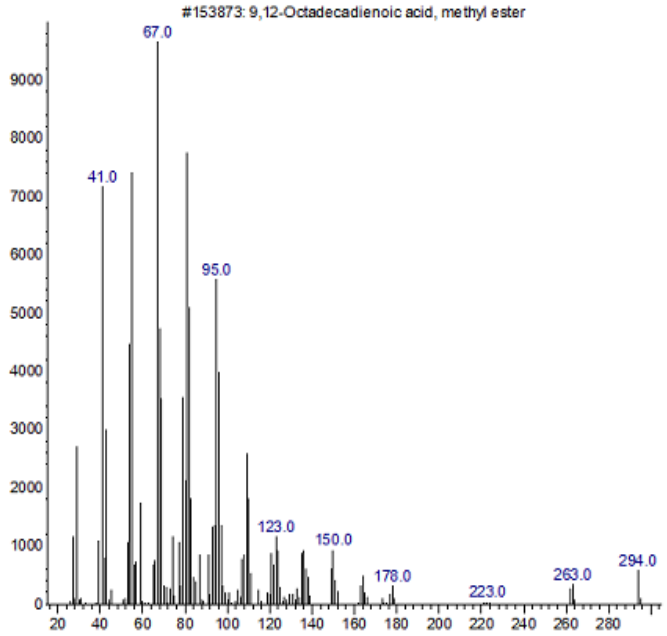

Figure 9: GCMS Peak area presentations showing most prominent compound in the analysis of the residual aqueous fraction of *Ficus asperifolia*

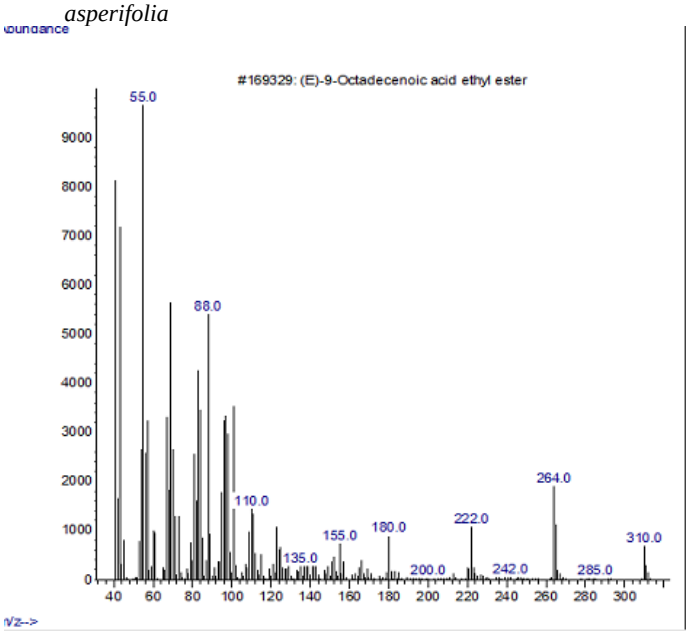

Figure 10: GCMS Peak area presentations showing most prominent compound in the analysis of the residual aqueous fraction of *Ficus asperifolia*

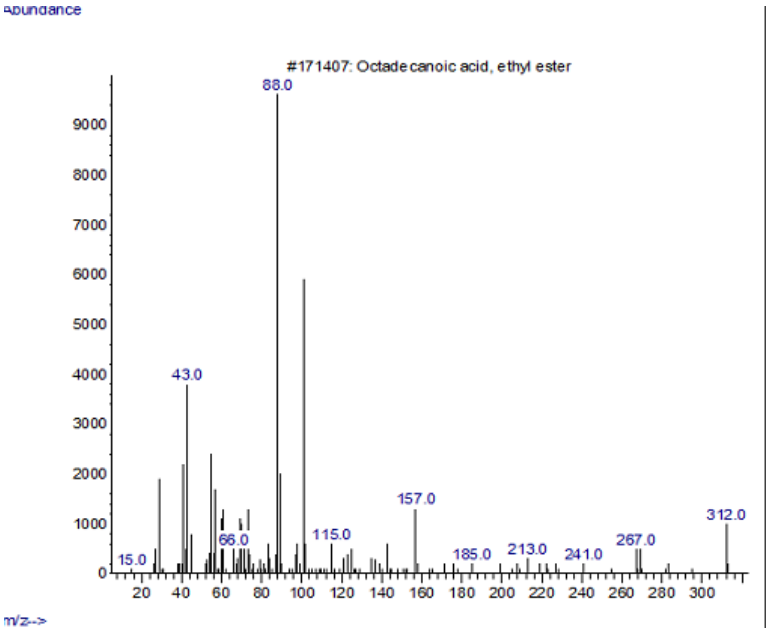

Figure 11: GCMS Peak area presentations showing most prominent compound in the analysis of the residual aqueous fraction of *Ficus asperifolia*

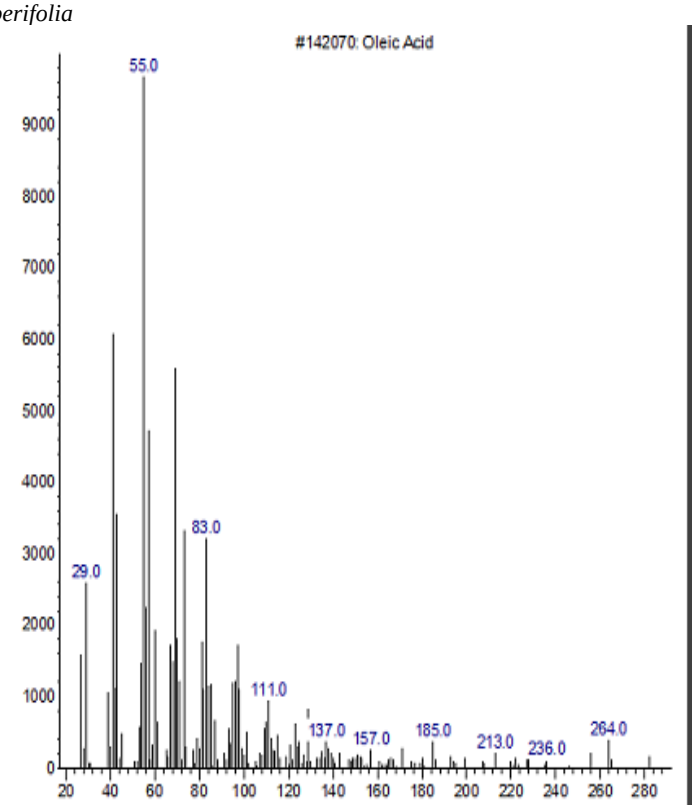

Figure 12: GCMS Peak area presentations showing most prominent compound in the analysis of the residual aqueous fraction of *Ficus asperifolia*

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