Trop J Phytochem Pharm Sci, April 2023; 2(2):59-64 **ISSN 2955-1226 (Print)**
ISSN 2955-1234 (Electronic)

Tropical Journal of Phytochemistry & Pharmaceutical Sciences

Available online at https://www.tjpps.org

Original Research Article

Comparative Phytochemical Composition and Functional Group Detection of *Annona muricata* Linn Seeds and Leaves

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ABSTRACT

Annona muricata Linn (*A. muricata*) belongs to the Annonaceae family, it is well-known for its high nutritional index and therapeutic relevance in traditional medicine. Aim of the study: To determine and compare the bio-active compounds profiled from selected parts of the *A. muricata* plant. In addition, study findings is aimed at advancing pharmacological knowledge of selected plant parts of *A. muricata.* Gas chromatography-Mass spectrometry (GC-MS) and Fourier transform-infrared (FT-IR) spectroscopy were utilised to comparatively profile the phytochemical compositions of the ethanolic extracts of *A. muricata* seeds and leaves. From GC-MS analyses, nineteen phytochemical compounds were identified in the ethanolic extract of *A. muricata* seeds while eighteen phytochemical compounds were identified in the ethanolic extract of *A. muricata* leaves. Comparatively, cyanogenic glycosides, resveratrol and tannin were identified uniquely from *A. muricata* seeds, while the cardiac glycosides and anthocyanin were identified uniquely from *A. muricata* leaves. The FT-IR analyses confirmed the presence of C-Cl, C-O, C=C, N-H, SCN and -OH functional groups. However R_2CHOH and R-COO were identified uniquely in *A. muricata* seeds and leaves respectively. These findings will be relevant in plant pharmacology and the development of enhanced medical strategies.

Keywords: *Annona*, *muricata*, seeds, leaves, spectrometry, spectroscopy, phytochemicals

Received 16 March 2023
Revised 22 April 2023
Accepted 23 April 2023
Published online 01 May 2023

Introduction

In the development of innovative therapeutic regimens, plants have remained the central focus for their bioactive compounds. The discovery and identification of plant products have been crucial to the discovery of several pharmaceutical solutions.[1] Many medicinal plants around the world are basic and crucial for the provision of health and medical care worldwide.[2] *A. muricata* tree is from the Annonaceae family and the fruit is well known by its English name "sour-sop" in Nigeria, and by the name "graviola" or "guanabana" in North and South America.[1,3,4,5] *A. muricata* has shown various potentials in ethno-traditional medicine. Various plant parts of *A. muricata* have been reported to possess anti-diabetic, anti-parasitic, anti-helminthic, antipyretic and anti-inflammatory properties.[4,5,6,7,8] In addition, this unique plant is well speculated as a potential anti-cancer regimen.[3]

Gas chromatography-Mass spectrometry (GC-MS) couples the separation ability of Gas chromatography (GC) and the sensitivity of Mass spectrometry (MS). The GC-MS possesses the capability to efficiently separate heterogeneous compounds and the detection of the separated molecules.[9] In the GC-MS, the mass analyser separates the ions in the heterogeneous compound based on their respective mass to charge ratio, while the detector determines the mass to charge values and records the relative abundance.

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Citation: Onuoha C, Nwachi EC, Nwanya EU, Osuagwu OL, Nsofor WN, Nzebude CP, Ujowundu FN, Chukwudoruo CS. Comparative Phytochemical Composition and Functional Group Detection of *Annona muricata* Linn Seeds and Leaves. Trop J Phytochem Pharm. Sci. 2023; 2(2): 59-64. http://www.doi.org/10.26538/tjpps/v2i2.3

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With the detection of individual compounds through the production of mass spectrum and retention time parameters, the GC-MS is advantageous and mostly employed in the quantitative determination of heterogeneous biological compounds.[9,10]

Fourier transform-Infrared spectroscopy (FT-IR) technique identifies substances through the extent of absorption of mid-infrared (IR) radiation, the interaction with electromagnetic fields in the IR region and their characteristic molecular vibrations.[11,12] With the exposure of matter to IR, absorption of IR cause molecules to vibrate, The extent of vibration provides compositional information of the matter.[12]

Annona muricata possesses numerous phytochemicals and bioactive compounds with notable pharmacological effects.[3] With current advances in analytical technologies, GC-MS and FT-IR can be utilised to identify and determine phytochemical contents in biological compounds. The objective of this study was to comparatively evaluate the phytochemical profiles obtained from GC-MS and FT-IR techniques, from ethanolic extracts of seeds and leaves of *Annona muricata*. Data obtained should provide enhanced insight into the investigated medicinal plant.

Materials and Methods

Plant materials

Fully mature *Annona muricata* fruits were harvested and *A. muricata* leaves were collected from trees in Nsukka, Enugu state, Nigeria in January, 2021. The fruits and leaves were identified and authenticated by Dr. Hyginus C. Ogbuehi of the Department of Crop Science and Biotechnology, Imo State University, Owerri, Nigeria where the identified plant has a taxonomic serial number of 18098.

All chemicals and reagents were obtained from certified suppliers and were of analytical grade. 50 g of pulverised *A. muricata* seeds and leaves were extracted with absolute ethanol. The extracts were subsequently analysed using GC-MS and FT-IR.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

1 µL of reconstituted ethanol extract of the investigated samples was evaluated as test samples by GC-MS, while an experimental blank was conducted with 1 µL of absolute ethanol.

The GC-MS analyses of the ethanolic extract of *A. muricata* seeds and leaves were performed with the BUCK M910 Gas Chromatography analyser equipped with a flame ionisation detector. A RESTEK-15 meter column (with dimension: 15 m by 250 µm by 0.15 µm) was used. The injector temperature was 280°C with a splitless injection of 2 µL of sample and a linear velocity of 30 cm/s. The carrier gas, Helium 5.0 Pa has a flow rate of 40 mL/min. The oven control initial temperature was at 200°C, which subsequently was heated to 330°C at a rate of 3°C per minute, and the temperature was kept constant for 5 minutes. The detector operated at a temperature of 320°C. Phytochemicals were determined through the comparison of mass spectra generated from analyte and reference sample.[9,10] The concentration of the different phytochemicals is expressed in µg/g, µg/mL or ppm. Identified compounds were matched with the National Institute Standard and Technology (NIST) database.

FT-IR

The Fourier transform-infrared (FT-IR) spectroscopy analyses of ethanol extract of *A. muricata* seeds and leaves were performed with the BUCK scientific FTIR M530 (USA). 1.0 g of the pulverised *A. muricata* seeds and leaves were weighed out. 0.5 ml of Nujol was added to the weighed sample and mixed properly and placed on the salt pellet. The FT-IR instrument is equipped with a deuterated triglycine sulphate detector and a potassium bromide-filled beam splitter. The FT-IR spectroscope has a scan range of 600 - 4,000 cm^{-1} and was co-added at 32 scans with a resolution of 4 cm^{-1}. The Gram A1 software was used to obtain and manipulate spectra. FT-IR spectra were displayed as transmitter values.

Results and Discussion

Medicinal plants are extremely vital as they possess chemical compounds that can be used to produce potentially useful chemotherapeutic interventions for several emerging diseases and disorders.[13] There is a need for life scientists to explore plants in tropical Africa, to obtain more knowledge on the pharmacological contents embedded therein, as numerous plants in tropical Africa have largely not been evaluated for their pharmacological properties. This study is largely focused on the comparative assessment of the *Annona muricata* seeds and leaves and the evaluation of the phytochemical disparities.

Extracts of *annona muricata* seeds and leaves were subjected to Gas chromatography-mass spectrometry analysis (GC-MS) and Fourier transform - infrared spectroscopy (FT-IR) evaluation. GC-MS is a combined analytical instrument, which is used for the identification and quantification of unknown organic compounds in a complex mixture. Interpretation of findings is carried out by matching the spectra obtained with reference spectra. On the other hand, FT-IR spectroscopy is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds.

The information obtained from GC-MS analysis includes: identified compounds, retention time, peak area, peak area (%), and external (concentration) are presented in Table 1. With GC-MS analysis, nineteen compounds were identified in the ethanolic extract of *A. muricata* seeds while eighteen compounds were identified in the ethanolic extract of *A. muricata* leaves. Compounds identified in the ethanolic extract of *A. muricata* seeds are proanthocyanidin, naringin, ephedrine, naringenin, sparteine, phenol, flavanones, steroids, epicatechin, kaempferol, phytate, flavone, cyanogenic glycoside, sapogenin, catechin, ribalinidine, tannin, flavan-3-ol and resveratrol (Table 1). Compounds identified in the ethanolic extract of *A. muricata* leaves are proanthocyanidin, naringin, cardiac glycosides, ephedrine, anthocyanin, naringenin, sparteine, phenol, flavanones, steroids, epicatechin, kaempferol, phytate, flavone, sapogenin, catechin, ribalinidine and flavan-3-ol (Table 1).

Table 1: Phytochemical constituents identified in ethanolic extracts of *A. muricata* seeds and leaves by GC-MS analysis.

S.N	Compound	Plant parts	Retention time	Peak area	Peak Area (%)	External
1.	Proanthocyanidin	seeds	0.116	3681.8254	3.2	4.3463 ppm
		leaves	0.253	4097.1544	2.4	4.8365 ppm
2.	Naringin	seeds	2.223	6793.2211	5.8	5.9642 ug/ml
		leaves	2.390	12411.2552	7.4	10.8966 ug/ml
3.	Cardiac glycoside*	leaves	4.120	6535.5572	3.9	4.7061 ug/ml
4.	Ephedrine	seeds	6.893	4491.1913	3.8	0.9860 ug/ml
		leaves	6.016	18197.8156	10.8	3.9951 ug/ml
5.	Anthocyanin*	leaves	7.470	8449.9404	5.0	9.4837 ug/ml
6.	Naringenin	seeds	10.593	4339.0384	3.7	10.7378 ug/ml
		leaves	10.366	19612.9266	11.7	48.5359 ug/ml
7.	Sparteine	seeds	13.300	4918.6080	4.2	1.1068 ug/ml
		leaves	12.970	6248.6418	3.7	1.4061 ug/ml
8.	Phenol	seeds	15.783	12794.3857	10.9	28.7514 ppm
		leaves	15.460	4973.5534	2.9	11.1765 ppm
9.	Flavonones	seeds	19.516	12631.1433	10.8	2.8359 ppm
		leaves	17.966	11346.0970	6.8	2.5474 ppm
10.	Steroids	seeds	22.293	4749.7578	4.1	2.7583 ppm
		leaves	20.313	12767.3954	7.6	7.4143 ppm
11.	Epicatechin	seeds	26.000	6833.3794	5.9	5.9507 ug/g
		leaves	22.730	9578.7656	5.7	8.3414 ug/g
12.	Kaempferol	seeds	28.566	5744.9478	4.9	6.4478 ug/ml

	leaved	leaves	25.650	10085.22.01	6.0	11.3190 ug/ml
13.	Phytate	seeds	29.493	4459.3978	3.8	0.8028 ug/ml
		leaves	27.536	11533.7678	6.9	2.0763 ug/ml
14.	Flavone	seeds	33.753	3207.2732	2.7	2.1124 ug/ml
		leaves	29.860	5482.1428	3.3	3.6106 ug/ml
15.	Cyanogenic glycoside*	seeds	34.206	5932.5289	5.1	8.6103 ug/ml
16.	Sapogenin	seeds	37.260	6525.2532	5.6	5.5377 ug/ml
		leaves	34.600	6053.9620	3.6	5.1377 ug/ml
17.	Catechin	seeds	38.326	9393.7324	8.0	10.3115 ug/ml
		leaves	36.876	6994.1568	4.2	7.6774 ug/ml
18.	Ribalinidine	seeds	39.586	4412.4024	3.8	1.3239 ug/ml
		leaves	39.200	10237.5643	6.1	3.0716 ug/ml
19.	Tannin*	seeds	40.930	3451.5680	2.9	2.0044 ug/ml
20.	Flavan-3-ol	seeds	42.086	6000.1311	5.1	0.5143 ppm
		leaves	42.276	3511.2838	2.1	0.3010 ppm
21.	Resveratrol*	seeds	42.943	6524.2634	5.6	4.2970 ppm

(*) characterises phytochemicals that were uniquely identified in an *A. muricata* plant parts.

From this study, cyanogenic glycosides, resveratrol and tannin were identified uniquely in the phytochemical profile of *A. muricata* seeds, while the cardiac glycosides and anthocyanin were identified uniquely in the phytochemical profile of *A. muricata* leaves (Table 1). The pharmacological importance of either the seeds or leaves of *A. muricata* may depend on the unique phytochemicals in either plant parts. Phytochemicals identified solely in the *A. muricata* seeds are cyanogenic glycosides, resveratrol and tannin. There are about 25 types of cyanogenic glycosides found in edible plants. Cyanogenic glycosides are secondary metabolites that are synthesised in some plants, which when enzymatically hydrolysed by β-glucosidase, releases toxic hydrogen cyanide (HCN, also known as prussic acid).[14,15] Amygdalin (a cyanogenic glycoside) has been purported to treat cancers but its toxicity negates its potential. Acute toxicity assessments of some cardiac glycosides have indicated that intravenous administrations may be more advantageous than oral administrations, due to the inherent toxicity of cyanogenic glycosides via the oral cavity and gut.[15,16] Resveratrol (3,5,4′-trihydroxy-trans-stilbene; $C_{14}H_{12}O_3$) belongs to the stilbenoid polyphenol group and is widely associated with grape seed and skin. Resveratrol, also regarded as phytoalexin is a protective substance produced by plant tissues in response to pathogens.[17] Resveratrol has also been studied widely and has been identified to possess potential in cancer prevention and therapy. Resveratrol, a polyphenol is capable of inducing apoptosis in cancer cells and inhibiting all carcinogenic processes.[17,18] Tannin ($C_{42}H_{32}O_{26}$) is a water-soluble polyphenol that is made up of ten galloyl or trihydroxylphenyl units surrounding a glucose center. It has been reported to be effective in the inhibition of cancer initiation, promotion and progression.[19] Tannin was found to inhibit proliferation, induce cell cycle arrest and apoptosis of breast and prostate cancer cells.[19,20,21]

Cardiac glycosides and anthocyanins respectively were identified uniquely in the leaves of *A. muricata* plants. These phytochemicals have been identified to possess some potential that needs to be elucidated. Cardiac glycosides refer to naturally-occurring cardioactive steroids that are bound to sugar moieties. Despite their toxicity at elevated doses, this phytochemical plays a unique role in the treatment of congestive heart failure and atrial arrhythmia.[22,23,24] Cardiac glycoside increases the contractile force of the cardiac muscles by binding and inhibiting the ubiquitous membrane protein, Na^+/K^+-ATPase, which leads to increased intracellular Na^+ and Ca^+ concentrations and decreased intracellular K^+ concentrations.[23] In addition, it was reported that cardiac glycosides possess anti-cancer potentials at doses that were not toxic to humans.[25] Furthermore, anthocyanins are uniquely present in *A. muricata* leaves. Anthocyanins belong to the flavonoid family and are plant pigments with antioxidant properties.[26] The antioxidant capability of anthocyanins protects plants from biotic and abiotic stress, this antioxidative potential makes anthocyanins-rich foods beneficial in promoting favourable health status.[27]

FT-IR spectroscopy is a reliable and sensitive method that detects active biomolecular compositions. The functional groups of active components present in the ethanolic extracts of *A. muricata* were identified with the FT-IR technique. Identified peak values in the region of infra-red radiation indicated specific functional groups. From FT-IR analyses, spectra of *A. muricata* seeds and leaves are shown in Figures 1 and 2. Peak values identified from the *A. muricata* seeds and leaves are shown in Tables 2 and 3 respectively. Functional groups were identified from the ethanolic extract of *A. muricata* seeds and leaves through FT-IR analysis.

From the FT-IR spectra of *A. muricata* seeds, the peak value around 734.7372 cm^{-1} was assigned to C-CI stretching vibration of a chloro-compound. The absorbance around 918.2517 cm^{-1}, 1083.473 cm^{-1} and 1292.243 cm^{-1} were assigned to C-O stretching vibration of an ether compound. The peak around 1382.754 cm^{-1} was assigned to C=C stretching vibration of an ethene compound. The medium band around 1615.537 cm^{-1} was assigned to N-H stretching vibration of a 1° amine. The value around 1921.941 cm^{-1} and 2974.040 cm^{-1} was assigned to SCN stretching vibration of a thiocyanate compound respectively. The peak around 2047.190 cm^{-1} was assigned to C-O stretching vibration of a carboxylic acid, while the wavelength around 2253.869 cm^{-1} was assigned to the C-O anti-symmetric stretch of a carbonyl compound. The absorbance around 2471.218 cm^{-1} corresponds to the CN anti-symmetric stretch of a nitrile compound. The weak band around 2678.850 cm^{-1} and 2856.412 cm^{-1} were assigned to CH stretching vibration of a methylene compound. The broad band around 3119.463 cm^{-1}, 3365.444 cm^{-1}, 3570.053 cm^{-1} and 3812.227 cm^{-1} were assigned to OH stretching vibration of 1°, 2° and 3° alcoholic compounds respectively (Table 2).

From the FT-IR spectra of *A. muricata* leaves, the absorbance around 1308.555 cm^{-1} and 1449.428 cm^{-1} was assigned to C=C stretching vibration of an ethene compound respectively. The peak around 1638.810 cm^{-1} was assigned to NH stretching vibration of a 1° amine compound. The value 1835.289 cm^{-1} was assigned to C-O stretching vibration of a cyclic ester. The absorbance around 2034.741 cm^{-1} and

ISSN 2955-1226 (Print)
ISSN 2955-1234 (Electronic)

2186.774 cm^{-1} were assigned to C-O stretching vibration of a carboxylic acid compound respectively. The peak around 2453.935 cm^{-1} was assigned to CN anti-symmetric vibration of a nitrile compound. The weak band around 2710.781cm-1 was assigned to C-H stretching vibration of methylene. The peak at 2982.939 cm^{-1} was due to SCN stretching vibration of a thiocyanate compound. The wavelength around 3137.522 cm^{-1} and 3805.483 cm^{-1} was assigned to OH stretching vibration of 1° and 3° phenolic compounds respectively (Table 3).
FT-IR spectroscopy recorded similar functional groups in both investigated plant parts. However, the functional groups R_2CHOH and R-COO were recorded to be present distinctly in *A. muricata* seeds and *A. muricata* leaves respectively.

Conclusion

This study was conducted to establish the bioactive composition of *A. muricata* seeds and leaves through the use of GC-MS and FT-IR. Compounds identified in ethanolic extract of *A. muricata* seeds and leaves are proanthocyanidin, naringin, ephedrine, naringenin, sparteine, phenol, flavanones, steroids, epicatechin, kaempferol, phytate, flavone, sapogenin, catechin, ribalinidine and flavan-3-ol. Uniquely, cyanogenic glycoside, tannin and resveratrol were found in ethanolic extract of *A. muricata* seeds, while cardiac glycoside and anthocyanin were found in ethanolic extract of *A. muricata* leaves. Identified phyto-constituents of *A. muricata* seeds and leaves would be greatly utilised in virtual *in silico* studies, relevant for the development of novel drugs and extensive medicinal plant research.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

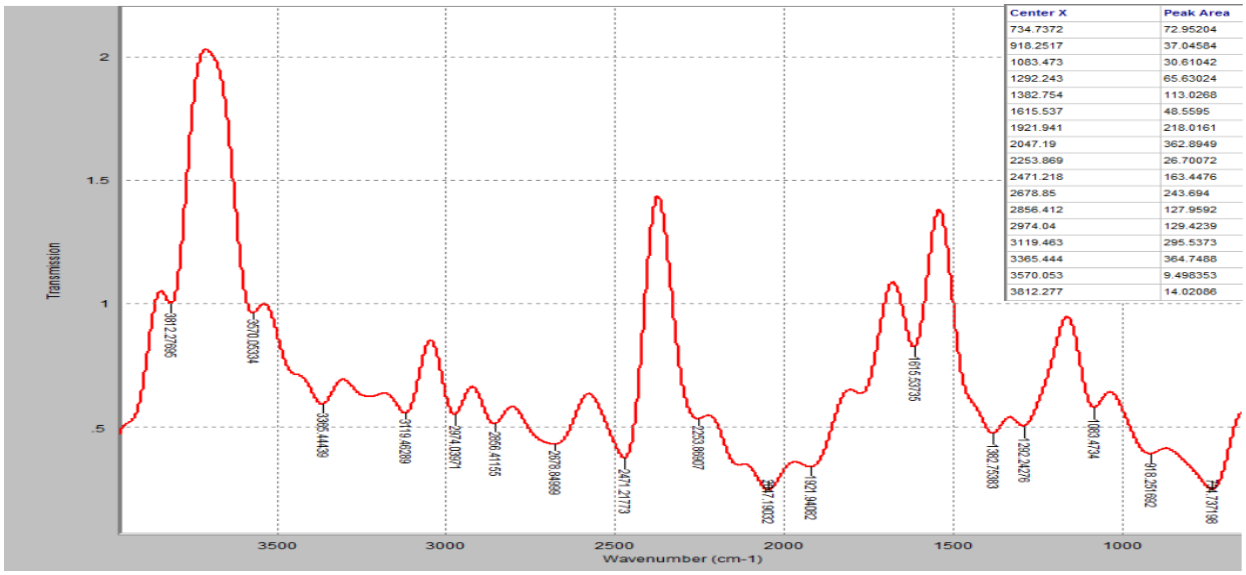

Figure 2: FT-IR spectrum of ethanolic extract of *A. muricata* seeds

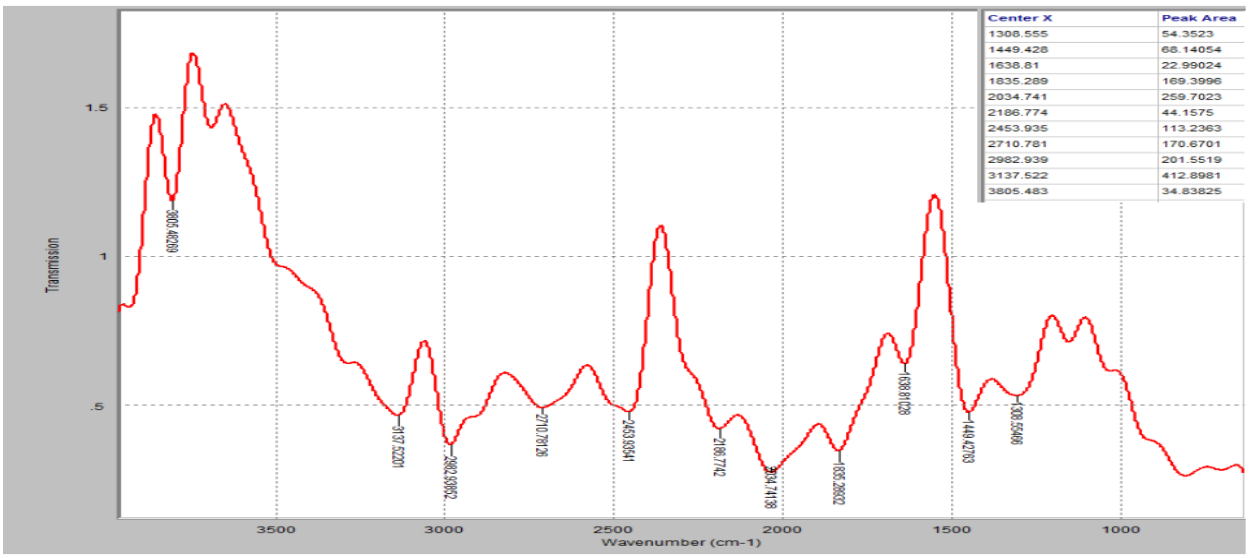

Figure 2: FT-IR spectrum of ethanolic extract of *A. muricata* leaves

Table 2: Functional groups identified in ethanolic extract of *Annona muricata* seeds via FT-IR analysis.

S.No.	Peak values (cm^{-1})	Functional group	Compounds
1.	734.7372	C-CI	Chloro Cl symmetric stretch
2.	918.2517	R-O-R	Ether CO symmetric stretch
3.	1083.473	R-O-R	Ether CO symmetric stretch
4.	1292.243	R-O-R	Ether CO symmetric stretch
5.	1382.754	$H_2C=CH_2$	Ethene CH anti-symmetric stretch
6.	1615.537	RNH_3	1° amine NH stretch
7.	1921.941	R-S-C≡N	Thiocyanate SCN antisymmetric stretch
8.	2047.190	RCOOH	Carboxylic acid CO stretch
9.	2253.869	RC=O	Carbonyl CO antisymmetric stretch
10.	2471.218	R-C≡N	Nitriles CN antisymmetric stretch
11.	2678.850	CH_2	Methylene CH stretch
12.	2856.412	CH_2	Methylene CH stretch
13.	2974.040	R-S-C≡N	Thiocyanate SCN antisymmetric stretch
14	3119.463	RCHOH	1° alcohol OH stretch
15.	3365.444	R_2CHOH	2° alcohol OH stretch
16.	3812.277	R_3CHOH	3° alcohol OH stretch

Table 1: Functional groups identified in ethanolic extract of *Annona muricata* leaves via FT-IR analysis

S.No.	Peak values (cm^{-1})	Functional group	Compounds
1.	1308.555	$H_2C=CH_2$	Ethene CH anti-symmetric stretch
2.	1449.428	$H_2C=CH_2$	Ethene CH anti-symmetric stretch
3.	1638.810	RNH_3	1° amine NH stretch
4.	1835.289	R-COO	Cyclic ester CO symmetric stretch
5.	2034.741	RCOOH	Carboxylic acid CO stretch
6.	2186.774	RCOOH	Carboxylic acid CO stretch
7.	2453.935	R-C≡N	Nitriles CN antisymmetric stretch
8.	2710.781	CH_2	Methylene CH stretch
9.	2982.939	R-S-C≡N	Thiocyanate SCN antisymmetric stretch
10.	3137.522	RCHOH	1° alcohol OH stretch
11.	3805.483	R_3CHOH	3° alcohol OH stretch

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