Ngr. J. Multi. vol-2, issue-8 (Aug-2026) pp-454-463

ISSN: 3108-2750 (E), 3139-8065 (P)

Nexus Global Research Journal of Multidisciplinary

Journal Homepage: https://nexusglobalresearch.com/journal/ngrjm/

Research Article

Gas chromatography-mass spectrometry and Fourier Transform Infrared Analysis of bioactive compounds present in Methanol Leaf Extract of Jatropha curcas L.

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ABSTRACT:
Background: Medicinal Plants and herbs played tremendous roles in human healthcare and their products are used widely as raw materials in many pharmaceutical industries. Jatropha curcas had been identified to be one of medicinal plants with numerous medical applications. The present study was carried out to investigate and characterize the chemical components of the Methanol Leaf Extract of J. curcas. A GCMS was employed to identify the bioactive compounds while FTIR analysis was performed to identify the functional groups presented in the extract. **Method**: The whole plant of J. curcas was collected and identified. The leaf of the plant was collected cleaned, air dried to a constant weight under shield and crushed into coarse powder. Two hundred grams (200 g) of the powdered leaf was weighed and macerated in 2 L of 70 % methanol for 72h, filtered, concentrated and analyzed. GC-MS analysis was carried out using Agilent Technologies GC system with GC-5977A/MS-597C model. The relative percentage amount of each component was calculated by comparing its average peak to the total areas. Interpretation on mass spectrum was done using Mass Hunter Library and the database of National Institute Standard and Technology search library. The FTIR analysis was performed using agilent cary 630 FTIR. The peaks were viewed and the wave numbers and transmittance were recorded. **Result:** The GCMS analysis revealed the presence of 50 peaks of bioactive compounds. The result of the FTIR spectroscopy showed strong and broad spectrum between 3700 and 3100 cm-1 and characteristic spectra were also produced at 2109.7 1580.4, 1401.5, and 1047.4 cm-1 indicating that the major functional groups or bonds present included OH, NH, C≡C, C=C and C-O, **Conclusion:** The therapeutics and pharmacological properties of the plant could be attributed to presence of these bioactive compounds in the plant

Keywords: Functional Groups, Herbal Medicines, Jatropha Curcas, Phytochemical Compounds

Citation: Arubi, P. O., Kwanashie, H. O., Maiha, B. B., Ejiofor, J. I., & Eru, E. U. (2026). Gas chromatography-mass spectrometry and Fourier transform infrared analysis of bioactive compounds present in methanol leaf extract of Jatropha curcas L. Nexus Global Research Journal of Multidisciplinary, 2(8), 454–463.

INTRODUCTION:

Medicinal Plants and Herbs are greatly acknowledged for playing pivotal roles in the provision of medication for human health. They had been used for prevention and treatment of various human ailments from time immemorial (Reddy Prasad, 2012). They form key module of traditional medicines and are also sources of raw material for the pharmaceutical industries. Hongal et al., (2024) in their work stated that more than 80% of the world populations rely on traditional medicines for their primary healthcare requirement and that about 21,000 plants were known to be used as sources of medication worldwide.

Jatropha curcas is a perennial, drought resistant multipurpose shrub widely distributed in the wild or semi cultivated areas in central and South America, Africa, India and south East Asia with life span of over 50 years (Kamal et al., 2011). Taxonomically, it belongs to the kingdom Plantae, order Malpighiales and family Euphorbiaceace (Ale et al., 2024). In Nigeria the plant is known as Binidazugu and Lapalapa in Hausa and Yoruba languages respectively (Adebusuyi et al., 2021). It is also known as Olulu idu in Ibo language, Gyedan in Tiv, Ochigbede in Idoma and Omangba in Igede (Arubi et al., 2024). Under favorable condition the plant can grow to a height of 5 meters and a trunk diameter of 20 cm in three years (Lustosa Sobrinho et al.,

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Received: 01 Aug 2026 | **Accepted:** 17 Aug 2026 | **Published:** 22 Aug 2026
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2022). The plant was reported to contain various chemicals compounds including apigenin, vitexin and isovitexin in leaves, taraxerol, sitosterol and α-amyrin in stem bark while the seeds were also reported to contain Lectin, phorbolesters, phorbol acetate and lipase (Namrata Jaspal et al., 2023). Other chemical compositions of the plant were listed to include stigmasterol, campesterol alkaloids, steroids sapogenins, triterpene alcohol and 1-triacontanol (Bossou Annick Flore ArIette Doboué et al., 2020). The plant has made a tremendous impart in ethnomedical practices in different forms to treat various ailments including fever, mouth infections, jaundice, joint rheumatism, edema, cough, hemorrhoids, dyspepsia, diarrhea, gonorrhea, diabetes, convulsion, hair loss, toothache, ring worms and gout (Sarabia, JBF et al., 2022).





J. curcas in its natural

Gas chromatography-mass spectrometry (GC-MS):

GC-MS is a hyphenated analytical technique used in various human endeavors. It combines the separation properties of gas-liquid chromatography with the detection feature of mass spectrometry to identify different substances within a test sample. It is a highly effective multipurpose technique applied in drug detection, petrochemical and hydrocarbons analysis, geochemical research, pollutants and pesticides detection in vegetables, pharmaceutical and controlled drug analysis, forensic and anti-doping investigation, clinical toxicology and to identify the poison and venoms, academic research and identification of unknown samples (Chauhan *et al.,* 2014, Tadikonda Rama Rao *et al.,* 2023). Gas chromatography separates the components of a mixture and mass spectroscopy characterizes each of the components individually. GC-MS provides two separate dimensions of information about the components in the sample, GC retention time and electro ionization (EI) mass spectra. GC retention time is related to specific chemical properties of the molecules in question (such as volatility, polarity, presence of specific functional groups) while molecular weight (derived from the mass spectrum) is indicative of atomic composition (Gohike and Mclafferty, 1993). The advantages of using GC-MS for compound analysis include its ability to separate complex mixture, quantify analytes and determine trace levels of organic contamination (Hites 2016)

Fourier-transform infrared (FTIR):

FTIR is a technique used to obtain an infrared spectrum of absorption or emission of a sample. The techniques provide complementary data regarding a molecular structure of compounds. It is an effective analytical instrument for detecting functional groups and bonding information such as hydroxyl (OH), aromatic primary amines (C-N), amines (N-H), carboxyl (C=O), Aromatic Bromo compounds (C-Br), aldehyde, esters, ether, and bonding information such as alkanes (C-C), alkenes (C=C) and alkynes (C≡C) of bioactive compounds which can be used in different pharmaceutical products (Laldinfeli Ralte *et al.,* 2022). It releases peaks and spectra that act as

fingerprint of specific molecular structure and chemical bonding.

The present study was carried out using GCMS and FTIR spectroscopic analytical processes to determine and characterize the functional groups and chemical compounds composition of methanol leaf extract of *Jatropha curcas*

METHOD:

2.1 Collection the authentication of plant materials

The whole plant of *J. curcas (Euphorbiaceae)* was collected from Area C residential Quarters of Samaru Campus, Ahmadu Bello University (ABU), Zaria Nigeria in August 2025. The plant was identified and authenticated at the Herbarium unit of the Department of Botany, Faculty of Science, ABU, Zaria Nigeria by Musa Sanusi by comparing with the existing specimen voucher number 1911 and deposited for future reference.

2.2 Extraction of Plant Material

The leaf of the plant was cleaned using flowing distilled water and air dried to a constant weight under shield. They were then crushed into coarse powder using mortar and pestle. Two hundred grams (200 g) of the powdered leaf was weighed and macerated in 2 L of 70 % methanol for 72h. The resultant mixture was filtered using muslin cloth followed by whatman's filter paper number one. The pooled filtrate was concentrated to dryness over a water bath at 50 ^{o}C then stored in an air tight container and put inside a desicator until required for use. The percentage yield of the extract was calculated using the formula:

$$\% \text{ Yield} = \frac{\text{Weight of extract (g)}}{\text{Weight of the powdered plant material (g)}} \text{ X } 100$$

The extract solution was prepared fresh prior to use in each study.

2.3 Determination of Chemical compounds: GC-MS analysis

GC-MS analysis of methanol leaf extract of *J. curcas* was performed as described by Devasahayam Leema Rose Mary *et al.,* (2022) and Ibrahim Chikowe *et al* (2024) using Agilent Technologies GC system with GC-5977A/MS-597C model (Agilent Technologies, Santa Clara, CA, USA) equipped with HP-5MS column (30 m in length x250 μm in thickness of film). Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min. The injector temperature was maintained at 250 ^{o}C, the ion-source temperature was 200 ^{o}C, and the oven temperature was programmed from110 ^{o}C (with an increase of 10 ^{o}C/min) to 200 ^{o}C, then (with an increase of 5 ^{o}C/min) to 280 ^{o}C. Mass spectra were taken at 70 eV and a scan interval of 0.5 s. The total GC-MS running time was 36 minutes. The relative percentage amount of each component was calculated by comparing its average peak to the total areas. The mass-detector used in the analysis was Turbo-mass Gold-PerkinElmer, and the software adapted to handle mass spectra and chromatograms was a Turbo-mass version 5.2. Interpretation on mass spectrum GC-MS was done using Mass Hunter Library and the database of National Institute Standard and Technology (NIST) search library. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library (Azhar Abbas *et al.,* 2023; Hafiz Muhammad Saeed *et al.,* 2023)

2.4 Fourier-transform infrared (FTIR) procedure

The FTIR analysis was performed as described by (Coates 1998) and Made Pharmawati and Luh Putu Wrasiati (2020) using agilent cary 630 FTIR. On turning on the power, a warm up time of 15 minutes was allowed. The power of the attached system was also turned on for initialization on MicroLab PC window. The sampling operation was initiated and the method transmittance was selected. The crystal was cleaned with organic solvent and 15 mg of the sample was encapsulated into pellet and loaded in with a resolution of 4 cm and a scanning time of 2-3 s. The blue line was checked from red to green region for sample alignment. Sample identity was then put in for coding. The peaks were viewed and the wave numbers and transmittance were recorded.

2.5 Statistical analysis

All graphs were plotted using origin 2023 and Microsoft excel 2021

RESULTS AND DISCUSSION:

(GC-MS)

Plants and herbs are reliable sources of raw material for pharmaceutical industries containing considerable quantities of bioactive substance that are used for the production of various drugs. They are applied in traditional medicines in the treatment of various diseases including smallpox, hepatitis, meningitis poliomyelitis (Ibrahim Babangida Abubakar *et al.,* 2021). The yield and percentage yield of methanol leaf extract of *J .curcas* were 18.4 g and 9.2% respectively.

Gas chromatography-mass spectrometry

GC-MS analytical procedure has been widely used to identify Phyto-compounds of clinical importance. It was used to determine the chemical composition of methanol leaf extract of *Jatropha curcas.* The result of the study showed that there were a total of 50 peaks. The peaks were identified by comparing their mass spectral fragmentation pattern and retention time with those of the known compounds described in the Mass Hunter/NIST14 Library and these correspond to a total of 150 possible chemical compounds (Sermakkani .and Thangapandian 2012, Rizka Angrainy *et al*., 2024) The 50 possible compounds with highest qualities are presented (Table 1). Prominent among them are 11-Bromoundecanoic acid, cis-1-Methyl-2-(2'-propenyl) cyclopropane, Cyclopentaneundecanoic acid, D-Mannoheptulose, 11-(2-Cyclopenten-1-yl) undecanoic acid, (+)-,4-Chloro-3-n-butyltetrahydropyrane, and Dimethyl Sulfoxide. The Various therapeutics and pharmacological properties of the plant could be attributed to the presence of these bioactive compounds in the plant.

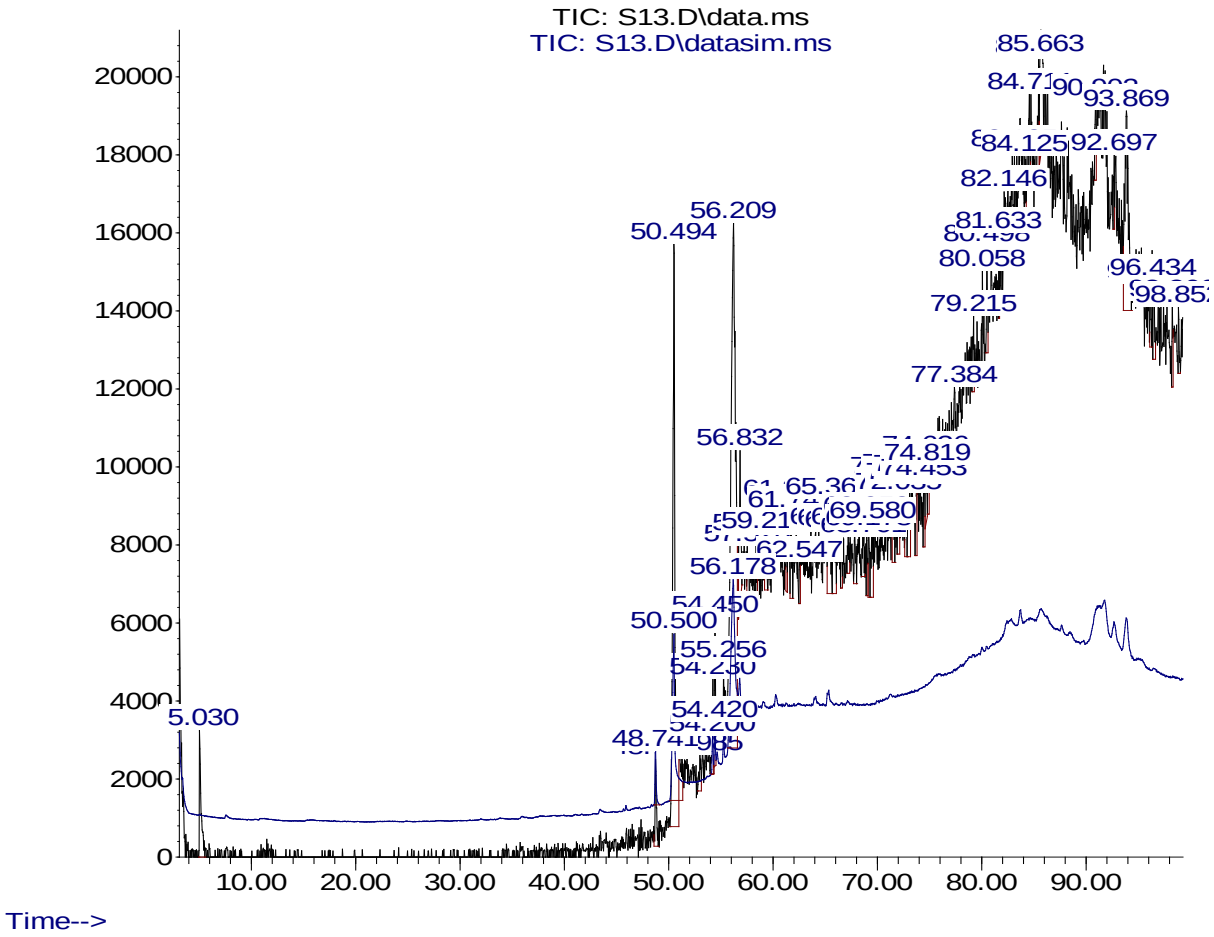
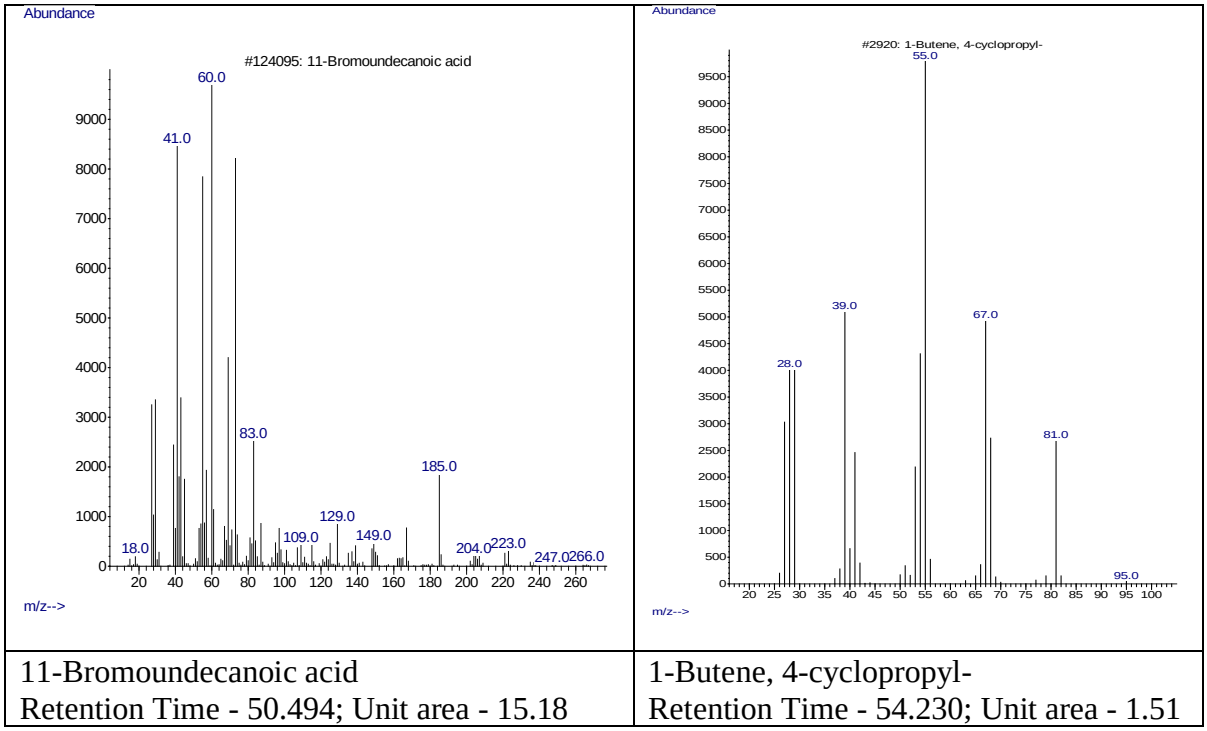


Figure 1: GC-MS chromatogram of methanol leaf extract of *J. curcas*

Table 1: Compounds identified in the methanol leaf extract using Search library D:\MassHunter\Library\NIST14.L

Chrogr Peak	Retention Time	Area Unit	Possible compounds with highest qualities	Quality
1	5.030	2.33	Dimethyl Sulfoxide	7
2	48.735	1.24	1-Propanol,	9
3	50.494	15.18	11-Bromoundecanoic acid	45
4	52.985	0.66	Isobutylamine	3
5	54.230	1.51	1-Butene, 4-cyclopropyl-	10
6	54.450	1.38	Oxirane, 2,2'-(1,4-butanediyl)bis-	16
7	55.256	0.82	10-Azido-1-decanethiol	9
8	56.209	26.02	cis-1-Methyl-2-(2'-propenyl)cyclopropane	30
9	56.832	2.42	11-(2-Cyclopenten-1-yl)undecanoic acid, (+)-	33
10	57.528	0.62	Bicyclo[2.1.0]pentane	9
11	58.370	0.66	2-Hexene, 6-nitro-	12
12	59.213	0.67	11-(2-Cyclopenten-1-yl)undecanoic acid, (+)-	25
13	61.301	0.62	11-(2-Cyclopenten-1-yl)undecanoic acid, (+)-	25
14	61.741	1.12	Cyclohexanebutanoic acid	38
15	62.547	0.54	Phosphonousdibromide, cyclohexyl-	17
16	65.367	2.35	4-Chloro-3-n-butyltetrahydropyrane	38
17	65.770	1.54	Phosphonousdibromide, cyclohexyl-	17
18	66.503	0.62	11-(2-Cyclopenten-1-yl)undecanoic acid, (+)-	25
19	67.199	0.59	Oxirane, 2,2'-(1,4-butanediyl)bis-	10
20	67.822	0.81	Isoxazolidine, 4-ethyl-2,5-dimethy l-, trans-	25
21	68.701	0.79	11-(2-Cyclopenten-1-yl)undecanoic acid, (+)-	36
22	68.958	0.84	Oxirane, 2,2'-(1,4-butanediyl)bis-	7
23	69.178	0.79	1,2:4,5:9,10-Triepoxydecane	27
24	69.580	1.57	9-Octadecenal	50
25	71.559	1.15	Cyclopentaneundecanoic acid	42
26	72.035	0.90	Oxirane, 2,2'-(1,4-butanediyl)bis-	32
27	72.694	0.79	Oxirane, 2,2'-(1,4-butanediyl)bis-	10
28	73.061	1.55	1,2:4,5:9,10-Triepoxydecane	17
29	73.757	0.98	1-Octyn-3-ol	23
30	74.453	0.95	Cyclopentanethiol, 2-methyl-, cis-	33
31	74.636	0.81	Propanoic acid, 2-(aminooxy)-	32
32	77.819	0.68	alpha.-D-Glucopyranoside, methyl	38
33	77.384	0.98	7-Oxabicyclo[4.1.0]heptane, 3-oxiranyl-	17
34	79.215	0.98	11-(2-Cyclopenten-1-yl)undecanoic acid, (+)-	33
35	80.058	1.23	11-Bromoundecanoic acid	25
36	80.498	1.13	Chloroacetic acid, 6-chlorohexyl ester	17
37	81.633	0.55	11-Bromoundecanoic acid	43
38	82.146	0.78	1,5-Anhydro-l-rhamnitol	35
39	83.135	1.01	Morpholine, 4-methyl-, 4-oxide	35
40	84.125	1.16	Methyl 4,6-ethylidene-.alpha.-d-alactopyranoside	32
41	84.711	0.63	Sedoheptulosan	35
42	85.443	1.73	Methyl 4,6-ethylidene-. alpha.-d-alactopyranoside	27
43	85.663	3.63	D-Mannoheptulose	12
44	90.902	1.01	Methyl 4,6-ethylidene-.alpha.-d- alactopyranoside	27
45	92.697	0.70	11-(2-Cyclopenten-1-yl) undecanoic acid, (+)-	37
46	93.869	7.72	Cyclopentaneundecanoic acid	22
47	96.141	0.64	. alpha.-D-Galactopyranoside, methyl	32
48	96.434	1.15	11-(2-Cyclopenten-1-yl)undecanoic acid, (+)-	25
49	98.266	0.85	Methyl d-glycero-.beta.-d-gulo-heptoside	16
50	98.852	0.60	9-Oxononanoic acid	23



11-Bromoundecanoic acid Retention Time - 50.494; Unit area - 15.18	1-Butene, 4-cyclopropyl- Retention Time - 54.230; Unit area - 1.51

Figure 2: GC-MS Chromatograms of typical bioactive compounds present in methanol leaf extract of *J. curcas*

The FTIR spectroscopy is an effective analytical instrument for detecting functional groups and identifying chemical bonds in molecules. It provides a molecular fingerprint that can be used when comparing samples and, in most cases, it is combined with other analytical techniques in providing molecular structure of compounds. It was used to identify functional groups of bioactive compounds of methanol leaf extract of *J, curcas.* Absorption bands were observed between 600 and 4000 cm^{-1}. Asep Bayu Nandiyanto *et al.,* (2023) avowed that FTIR spectroscopy produces peaks and spectra which represent fingerprint of specific molecular structure and chemical bonding, although reference data are required. The FTIR spectroscopy of methanol leaf extract of *J, curcas* showed strong and broad spectrum between 3700 and 3100 cm^{-1} representing O-H and N-H groups which may be alcohol or phenol and amine or amides compound groups respectively (Sukamto and Rahmat 2023**).** Other characteristic spectra produced were at 1580.4, 1401.5, and 1047.4 cm^{-1} which might represent C=C stretching of aromatic rings, O-H bending of alcohol and C-O stretching of primary alcohol respectively. The prominent bands in alkynes correspond to the carbon- carbon triple bond and it was shown at 2109.7 cm $-^{1}$ with a weak band (John Coates 2000, Asep Bayu Nandiyanto *et al.,* 2019). The band is weak probably due to non-polarity and may be symmetrical alkynes. These functional groups are the integral parts of various secondary metabolites such as alkaloids, flavonoids, terpenoids, polyphenol and tannins which the extract might have contained and could guide in identifying different therapeutic application of the extract (Awadhesh Kumar and Y. Tunginba Singh, 2022).

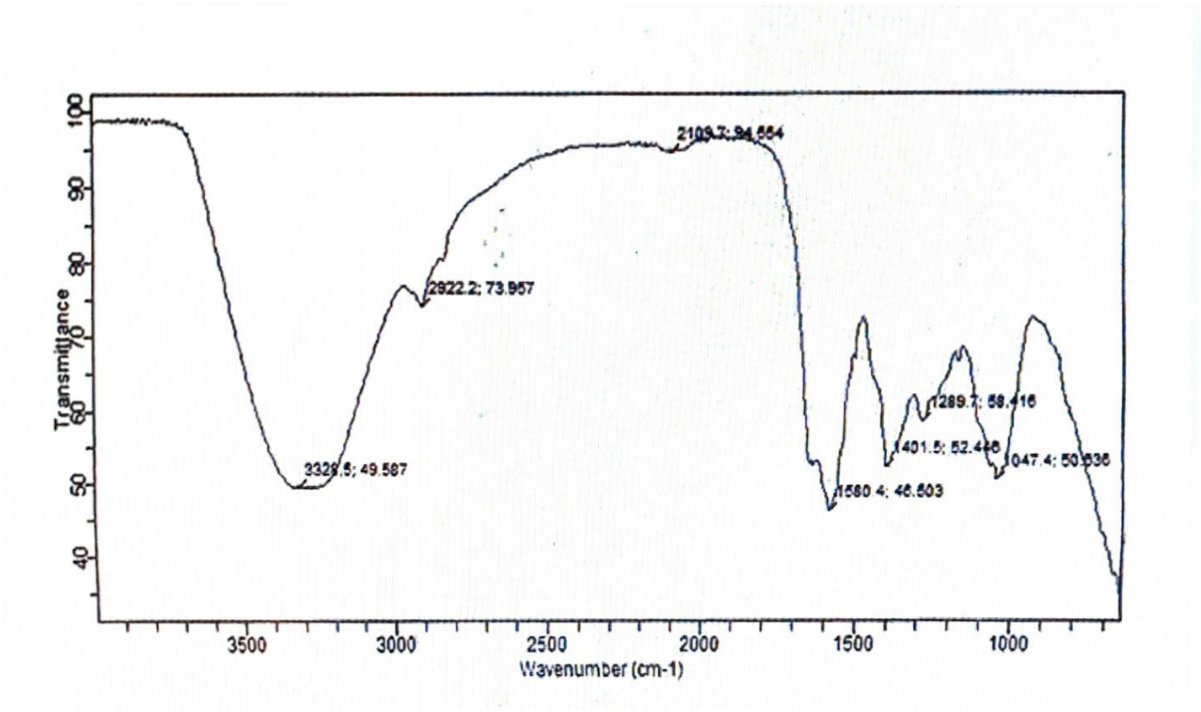


Figure 3: FTIR **spectrum of methanol leaf extract of *J. curcas* using agilent cary 630 FTIR**

Table 2: FTIR Wave number and Transmittance spectral peak values and the major functional groups of methanol leaf extract of *J. curcas*

Wave number (cm -1)	Transmittance	Group	Compound Class
3328.5	49.587	O-H stretching, N-H stretching	alcohol, phenol, amine, amides
2822.2	73.957	N-H stretching	amine salt
2109.7	94.554	C≡C stretching	Alkynes
1580.4	46.503	C=C stretching	aromatic rings
1401.5	52.446	O-H bending	Alcohol
1289.7	58.416	C-O stretching	aromatic ester
1047.4	50.636	C-O stretching	primary alcohol

Jatropha curcas was reported to have anticancer and antimicrobial activities against *Shigella sp., Staphylococcus aureus, Salmonella sp., Candida albicans* and *Trichophyton rubrum* which could be attributed to some of these phyto-compounds (Zeinab M. Saleh, *et al.,* 2023, Adeiza, *et al.,* 2024).

CONCLUSION

The study identified different bioactive compounds and functional groups in MLE of *J. curcas* using GCMS and FTIR analysis respectively. The bioactive compounds are thought to be responsible for various therapeutic and pharmacological properties of the plant leaves. The presence of these bioactive compounds justifies the use of the plant in the treatment of various ailments by traditional medical practitioners

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