



METABOLITES PROFILING AND THE ANTIBACTERIAL ACTIVITY OF ETHANOL LEAF EXTRACT OF *JATROPHA CURCAS*

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ABSTRACT

The current study was carried out to screen and assess the antibacterial activity of *Jatropha curcas* ethanol leaf extracts against some bacteria isolated from oral cavity of some students of Federal University Dutsin-Ma (FUDMA), Katsina State, Nigeria. A fresh leaf of the plant was collected, air dried, homogenized and extracted using 95% Ethanol. A total of twenty (20) oral cavity samples were collected using sterile swab sticks and process using microbiological standard techniques, the bacterial species was isolated using blood agar and confirmed using Gram's staining and biochemical tests. A preliminary phytochemical screening was performed using standard techniques. The existence of the phytochemical components of the *J. curcas* leaf ethanol extract was confirmed using (FTIR and GCMS). The ethanol leaf extract of *J. cactus* was tested for antibacterial activity using the agar well diffusion method. Phytochemical analysis gave positive results for alkaloids, Cardiac glycosides, Saponins, Tannins and Terpenoids. Octadecanoic acid 2,3-dihydroxypropyl ester (C₂₁H₄₂O₄) was found as prominent phytoconstituent in the extract which probably contributed to the antibacterial effect of the *J. curcas* leaf extract. *Streptococcus* Species and *S. aureus* was isolated from oral cavity as common pathogens. *J. curcas* leaf extract showed promising with an inhibitory zone of (20mm) at 25 mg/ml for *Streptococcus* species and (18mm) at 25 mg/ml for *Staphylococcus aureus*, respectively. In conclusion *J. curcas* ethanol leaf extract could be a good candidate for antibacterial agent development against both gram positive and negative strains of bacterial.

Keywords: Antibacterial activity, *Jatropha curcas*, FUDMA, *Streptococcus* species, *Staphylococcus aureus*

INTRODUCTION

In recent years, the exploration of natural sources for potential therapeutic agents has gained remarkable attention within the scientific community. Plants, in particular, have been a rich source of bioactive compounds with diverse pharmacological properties (Abd-Alla *et al.*, 2019). One such fascinating plant species under investigation is the *Jatropha curcas* (Bini da zugu (Hausa); Barbados nut (English)), belonging to the *Euphorbiaceae* family, is a perennial, monoecious shrub or small tree with a short tap root; bark pale brown, papery, peeling; slash exudes a copious watery latex, soapy to touch but soon becoming brittle and brownish when dry; branches glabrous, ascending, stout. Leaves alternate and palmate. Inflorescence a cyme formed terminally on branches and complex, possessing main and co-florescences with paracladia. The plant is monoecious and flowers are unisexual. Fruit an ellipsoid capsule, yellow, turning black. Seeds black, 2 per cell, ellipsoid, triangular-convex (Aliyu, 2006).

It has a long history of traditional medicinal use in various cultures (Jiménez-González *et al.*, 2023). Local communities have employed different parts of the plant for treating ailments ranging from skin infections to digestive disorders. In spite of its traditional reputation, the scientific exploration of *J. curcas* as a source of pharmacologically active compounds is relatively limited, necessitating further investigation. Metabolite profiling, is a strong analytical approach that involves the comprehensive identification and quantification of small molecules within a biological sample (Wolfender *et al.*, 2015). In medicinal plant research, metabolite profiling helps in much comprehension of the chemical constituents of plant extracts, revealing potential

bioactive compounds. The application of advanced analytical techniques, such as chromatography and mass spectrometry, facilitates the identification of metabolites, paving the way for the discovery of novel therapeutic agents (Farag *et al.*, 2012; Alonso *et al.*, 2015; Saurina, & Sentellas, 2017). The search for novel antibacterial agents has become imperative as a result of the risen antibiotic-resistant bacterial strains. The persistent threat of antibiotic resistance and the need for sustainable and effective healthcare solutions, the exploration of plant-derived compounds has emerged as a promising avenue for drug discovery (Ferri *et al.*, 2017; Huemer *et al.*, 2020). Plant extracts have shown promises as alternative sources of antibacterial compounds. Many plant species have demonstrated inhibitory effects against a wide range of bacteria, emphasizing the need to explore the antibacterial potential of *J. curcas* extracts. Understanding the specific mechanisms of action and the spectrum of antibacterial activity is also crucial for the development of effective therapeutic interventions. Preliminary studies indicate the presence of secondary metabolites such as alkaloids, flavonoids, terpenoids, and phenolic compounds, known for their pharmacological properties (Gurgel *et al.*, 2023). However, a comprehensive analysis of these metabolites and their correlation with antibacterial activity is needed. By employing advanced analytical techniques, this research aims to explore the chemical landscape of *J. cactus* and contribute valuable insights into natural product-based drug discovery. The study is therefore designed to specifically untie the metabolite profile of ethanol extract *J. curcas* and assessing the antibacterial activity against the isolates of human buccal cavities.

MATERIALS AND METHODS

Plant Collection identification and Processing

The *J. curcas* leaf was collected in clean polythene bag and transported to the Department of Plant Science and Biotechnology for authentication and confirmation of the taxonomic identity by a botanist. Voucher specimen with

reference number FUDMA/023/030/Vsp was deposited in the Department. The plant material was then air dried at room temperature 25°C for two weeks and pulverized into fine powder using clean mortar and pestle. The powdered *J. curcas* leaf was kept in dried clean plastic container.



Plate 1: The Leaf Part of *Jatropha curcas* Plant

Extraction and Organoleptic Properties

Extraction of Plant Material

Cold maceration method was adopted for the extraction, where, 100 grams of the *J. curcas* leaf plant material was macerated in 300ml of ethanol for 72 hours with frequent agitation using electric shaker. The mixture was filtrated first using muslin cloth followed by re-filtration using Whatman no.1 filter paper. The crude extract concentrated using rotary evaporator under a reduced pressure at 40°C and percentage yield was calculated using the formulae (Londonkar & NK, 2013; Upadhye et al., 2014).

Calculation of the percentage (%) yield of extract

$$= \frac{W_2}{W_1} \times 100$$

Where, w₂ = weight of extracted sample (g), w₁ = weight of powdered sample (g) before extraction

Phytochemical Screening of *J. curcas* leaf Extract

The ethanol crude extract was subjected to phytochemical screening for the presence of flavanoid, tannins, sterols, alkaloids, saponins and cardiac glycosides (Saulawa and Muhammad, 2017).

Bacterial Isolate Collection Identification and Characterization

A total of 20 Buccal mucosa swabs were randomly collected from volunteer students of Federal University Dutsin-ma, for the isolation and identification of bacteria. The collected swabs were inoculated into freshly prepared Blood agar and mannitol salt agar sterile plate. The inoculated plates were then incubated at 37 °C for 24 hours. Morphologically distinct colonies were sub-cultured on freshly prepared appropriate growth media for identification and characterization. The bacterial isolates were identified based on colonial morphology, Gram reaction and biochemical tests such as catalase, oxidase, urease, coagulase, indole, methyl red, Voges-Proskauer and Simon citrate tests were used as described by Cheesbrough (2006) and Gladys Chepkoech. (2019).

Standardization of Inoculum

A 0.5 McFarland standard was prepared by mixing the solutions of 0.05mL of 1% BaCl₂ and 9.95mL of 1% H₂SO₄. Overnight broth culture from the isolated cultures were prepared and separately inoculated into test tubes containing 10ml of normal saline until its turbidity matched that of

prepared 0.5 McFarland standard. These were used as standardized inocula.

Evaluation of Antibacterial activity of *J. curcas* against bacterial isolates of oral cavity

Different concentrations of *J. curcas* leaf extract was prepared by dissolving 1g of the crude extract in 2mL of DMSO to obtain a concentration of 500mg/mL. Serial double dilution was prepared by transferring 1mL of the stock into a sterile test-tube containing 1mL of DMSO to obtained 250mg/mL and this was subsequently continued to obtain 125mg/mL and then 62.5mg/mL. The sterile prepared plates of Mueller-Hinton agar were inoculated each with the standardized inoculum using radiant streaking. Sterile cock-borer was used to aseptically punch wells on the agar plates. A 0.1ml of each concentration was separately placed in the wells and labelled accordingly varied concentrations were then placed on the wells and then labelled accordingly. The preparations were incubated at 37°C for 24hours.

Procedure for FTIR Spectroscopic Analysis

FTIR analysis was done with the Buck Scientific M530 USA. A deuterated triglycine sulphate detector and potassium bromide beam splitter was used. Gramme A1 programme collected and altered spectra. A 1.0 g sample was well mixed with 0.5 ml of nujol and put over the salt pellet. Fourier Transform Infrared (FTIR) spectra were taken from 4,000 to 6,000 cm⁻¹ during measurement. Co-addition with 32 scans and 4 cm⁻¹ resolution merged these spectra. Transmitter values were used to display FTIR spectra.

GC-MS Analyses of the Phyto-constituents

GC-MS analysis of bioactive compounds from the extract was done using the dried solid extract, which was dissolved in methanol and analyzed using GC-MS model Qp2010 plus SHIMADZU equipped with detector and slit injection system. The electron ionization method used in GC-MS spectroscopic detection was 70-eV electrons. The initial temperature was maintained at 60°C for 3 minutes and was gradually increased to 250°C. 1.6µl of solution was injected for analysis the sample injection temperature was maintained at 250°C throughout the experimental period. An adequate solvent diluted one microliter of the produced 1% extracts was split less administered into the system. Each extract's chemical component % was calculated using the chromatogram peak area.

RESULTS AND DISCUSSION

Phytochemical profile of ethanol leaf extract of *J. curcas*

The phytochemical profile of *J. curcas* leaf extract as presented in Table 1, indicated a total of six phyto-chemicals screened for, but only five were identified in the extract, which include; Alkaloids, Saponins, Tannins, Terpenoids and cardiac glycosides, while flavonoids were not found. This is

similar to the finding of Doe, & Smith. (2022) where all the same phytochemicals were identified, however, flavonoid was found in large quantity while steroid was in trace amount which could be associated with the geographic location and soil composition where the plants were grown (Doe & Smith, 2022).

Table 1: Phytochemical profile of the ethanol leaf extract *J. curcas*

Plant extract	Phytochemical Profile					
	Alkaloids	Flavonoids	Cardiac glycosides	Saponins	Tannins	Terpenoids
<i>J. curcas</i> leaf extract	+	-	+	+	+	+

KEY: + indicates the presence of phytochemicals

- Indicates absence of phytochemicals

Table 2: Bacterial contaminants of buccal cavity of some students in Federal University Dutsin-Ma

Sampling Site	Number of Samples Collected	Cultural Characteristics	Suspected Bacteria	Gram Reaction	Biochemical Test Results	Confirmed Bacteria
FUDMA Take-off Campus	20	MSA (20+)	<i>Staphylococcus aureus</i>	20+ Gram-positive rods	Catalase (+), Coagulase (+), MR (+), Citrate (+), VP (+), Oxidase (-)	<i>Staphylococcus aureus</i>
		BA	<i>Streptococcus species</i>	3 Gram-positive rods	Catalase (-), Oxidase (-)	<i>Streptococcus species</i>

Key: + = Positive, - = Negative MSA = Mannitol Salt Agar BA = Blood Agar MR = Methyl Red VP = Voges-Proskauer, N= Total Number of samples collected, N+ = Total Number of the isolate.

Identification of bacteria associated with cavity of some students in Federal university Dutsin-Ma

The Table 2 provides information on the sampling site, number of samples collected, Cultural characteristics, suspected bacteria, gram reaction, biochemical test and confirmed bacterial contaminants isolated from the buccal cavity of the students at the Federal University Dutsin-Ma Take-off Campus. The results indicated that of the total of 20 samples were collected from the buccal cavity of students based on the cultured conducted using Mannitol Salt Agar (MSA) and Blood Agar (BA), particularly selective. *Staphylococcus aureus* was detected in all the 20 samples, exhibiting Gram-positive rod morphology and positive reactions to various tests, but negative for the oxidase test. *S. aureus* was identified on Blood Agar (BA), with three isolates showing Gram-positive rod morphology. *S. species* are known for its role in skin and soft tissue infections, and often isolated from mucosal surfaces, including the oral cavity. Studies by Lee et al. (2020) reported *S. aureus* colonization in healthy individuals' buccal cavities, increasing the risk in an immunocompromised condition. *Streptococcus* species, particularly *S. mitis* and *S. salivarius*, are part of the normal flora of the human mouth which according to Haffajee et al. (2019), play roles in oral health and biofilm formation. Similarly, their catalase-negative nature and their ability to grow on blood agar, was also observed. The results aligned with the existing literature on the prevalence of *S. aureus* and *Streptococcus* species in the oral cavity, suggesting that FUDMA students' buccal microbiota shares similarities with patterns observed in other populations. The presence of these bacteria, while often benign, may pose infection risks if introduced into compromised systems.

Antibacterial Effect of *J. curcas* against bacteria isolated from Buccal Cavity of some Federal university Dutsin-ma students

The Table 3 presents the antibacterial activity of *J. curcas* leaf extract, with the highest concentration of the extract showing 16mm in 100g/ml of the extract, while at the lowest concentration of the extract is at 8mm in isolate 2 at 25g/ml of the extract. The study conducted by Doe and Smith (2022) and other related works (Amadi et al. (2021) and Chen & Wang. (2020) primarily examined the antibacterial activity of *J. cactus* leaf ethanol extract, demonstrating significant efficacy against *E. coli* and *S. aureus*. The current study found that *J. curcas* ethanol leaf extract effectively inhibited *S. aureus* and *Streptococcus* species in students' buccal cavities, with the highest zone of inhibition at 100 mg/ml, and moderate at 25 mg/ml. The study further highlighted the significant activity against *S. aureus* and *Streptococcus* species, with zones of inhibition increasing as concentration increases, indicating a concentration-dependent antibacterial effect. Amadi et al. (2021) found *J. curcas* leaf extract to exhibit antimicrobial properties against multiple strains, including *S. aureus*. However, Chen and Wang emphasized the bioactive compounds, such as alkaloids and flavonoids were responsible for the antibacterial properties of *J. curcas*. This aligns with the findings from the current research, where the ethanol extract exhibited substantial inhibition against both *S. aureus* and *Streptococcus species*. It is likely that the active compounds noted by Chen and Wang. (2020) contributed to the observed effects highlighted in the current study.

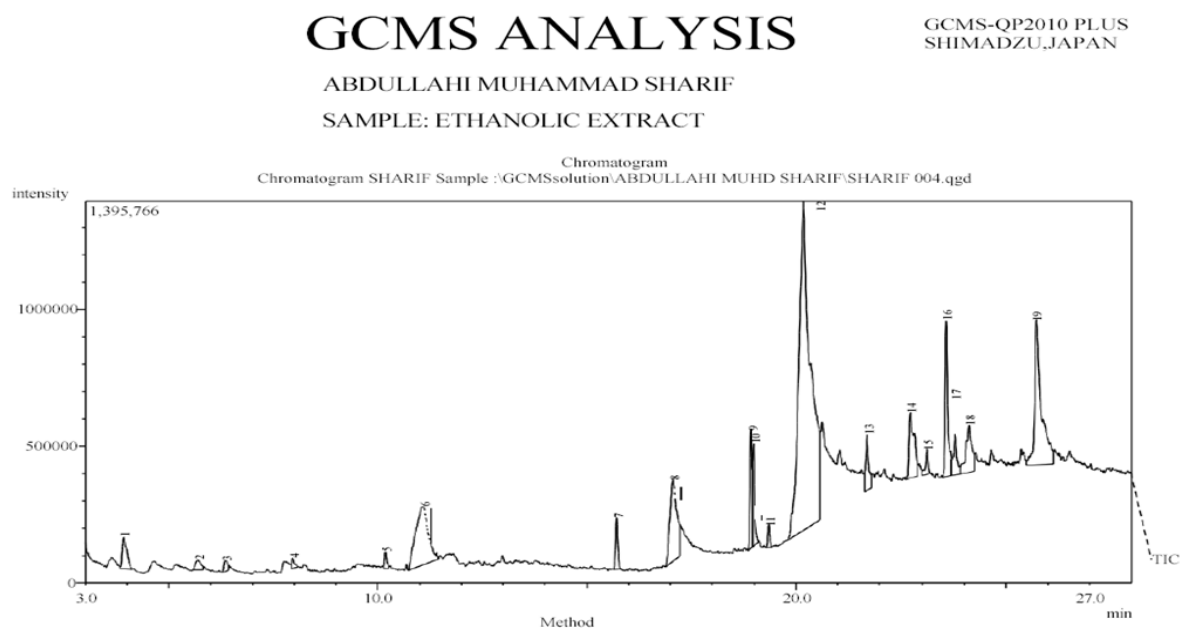
Table 3: The antibacterial Effect of the ethanol leaf extract of *J. curcas* against bacteria isolated from Buccal Cavity of some Students

Concentration (mg/ml)	Zones of inhibition (mm) against bacterial isolates at varied concentration (mg/ml)	
	<i>S. aureus</i> (mm)	<i>Streptococcus</i> species (mm)
100	16	18
50	10	13
25	18	20
12.5	11	21

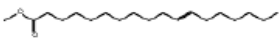

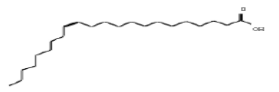

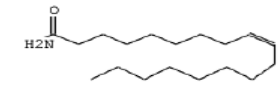
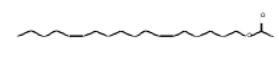
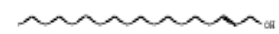
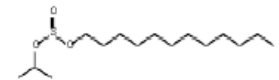
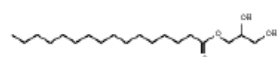
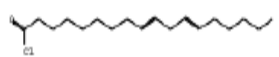
Identification of chemical constituents

The identification of Phyto-constituents derived from the extract was performed through the analysis of their gas chromatography (GC) retention time on an HP-5MS column,

and comparing their spectra with software data base of established standards from GC-MS systems, as presented in Figure 2 and Table 4.

Figure 2: The GCMS spectral of the Ethanol extract of *J. curcas***Table 4: The metabolite characteristic GCMS spectral of Ethanol extract indicating Retention Time, Base Peak, Phyto-constituent Molecular weight, Molecular formular Similarity Index (SI) and percentage Composition**

	Retention time	Base peak value	Pyto-constituent	Structure of phyto-constituent	Molecular weight	Molecular formular	SI %	Area %
1	3.917	55.05	3-Methyl-1-hexene		98	C7H14	87	1.75
2	5.700	43.05	Pyrimidine-2,4(1H,3H)-dione, 5-amino-6-nitroso-		156	C4H4N4O3	82	0.64
3	6.358	43.05	1,3-Dioxane, 4-methyl-		102	C5H10O2	84	0.45
4	7.967	79.05	3-Chloro-2-hydroxypropanoic acid		124	C3H5ClO3	78	0.30
5	10.183	43.05	1-Dodecanol		186	C12H26O	87	0.40
6	11.092	57.05	1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)		134	C6H14O3	78	8.76
7	15.708	74.05	Tridecanoic acid, methyl ester		228	C14H28O2	91	1.25
8	17.050	43.05	n-Hexadecanoic acid		256	C16H32O2	88	6.75
9	18.925	41.05	9,12-Octadecadienoic acid, methyl ester, (E,E)-		294	C19H34O2	90	2.88

10	18.983	41.05	11-Octadecenoic acid, methyl ester		296	C19H36O2	92	2.35
11	19.342	74.05	Cyclopentaneundecanoic acid, methyl ester		268	C17H32O2	81	0.49
12	20.183	55.05	13-Docosenoic acid, (Z)-		338	C22H42O2	90	45.64
13	21.700	55.05	15-Hydroxypentadecanoic acid		258	C15H30O3	70	1.94
14	22.708	59.05	9-Octadecenamide, (Z)-		281	C18H35NO	87	3.94
15	23.117	43.05	Z,Z-6,13-Octadecadien-1-ol acetate		308	C20H36O2	86	0.84
16	23.567	55.05	E-2-Octadecadecen-1-ol		268	C18H36O	84	4.92
17	23.783	43.05	Sulfurous acid, dodecyl 2-propyl ester		292	C15H32O3S	80	1.73
18	24.108	43.05	Hexadecanoic acid, 2,3-dihydroxypropyl ester,		330	C19H38O4	83	3.44
19	25.717	41.05	9,12-Octadecadienoyl chloride, (Z,Z)-		298	C18H31ClO	81	11.53

The GCMS analysis of ethanol extract presented in Table 4 identifies a range of 19 bioactive compounds showing 19 spectral of peaks in Figure 2. The findings reveal alkenes, fatty acids, esters, alcohols, amides, and chlorinated derivatives, each with distinct applications. Alkenes, notably 13-docosenoic acid, account for a substantial 45.64% of the extract, indicating robust anti-inflammatory and antimicrobial properties, which aligns with studies of Gupta *et al.* (2021), who reported similar effects in long-chain alkenes from plant extracts. Fatty acids, such as n-Hexadecanoic acid (6.75%) and 9,12-octadecadienoic acid, show applications in cardiovascular health, as echoed in research finding by Zhang *et al.* (2019) on the cardiovascular benefits of similar fatty acids in natural products. Esters identified, including tridecanoic acid methyl ester, exhibit antimicrobial and flavor-industrial applications, reported by Singh *et al.* (2020) that highlight the antimicrobial effectiveness of esters in

plant-based extracts. Amides identified include 9-octadecenamide notable for its anti-inflammatory properties, aligning with Johnson *et al.* (2018) who reported similar effects in amide-rich botanical extracts. Alcohols and chlorinated compounds, such as 1-Dodecanol and 9,12-octadecadienoyl chloride, were detected, similar to the findings by Oliveira *et al.* (2017) on alcohols with emollient properties. Notably, chlorinated and sulfurous compounds, such as sulfurous acid esters, were identified and believed to contribute to antimicrobial activities and the antioxidant potential of the extract, aligning with findings by Kim *et al.* (2022) on sulfur-containing antioxidants in natural extracts. The diverse compound profile in this ethanol extract indicates its broad antibacterial spectrum, supporting the implication of the bioactive metabolites identified with the plant extract, suggesting potential anti-inflammatory and neuroprotective properties.

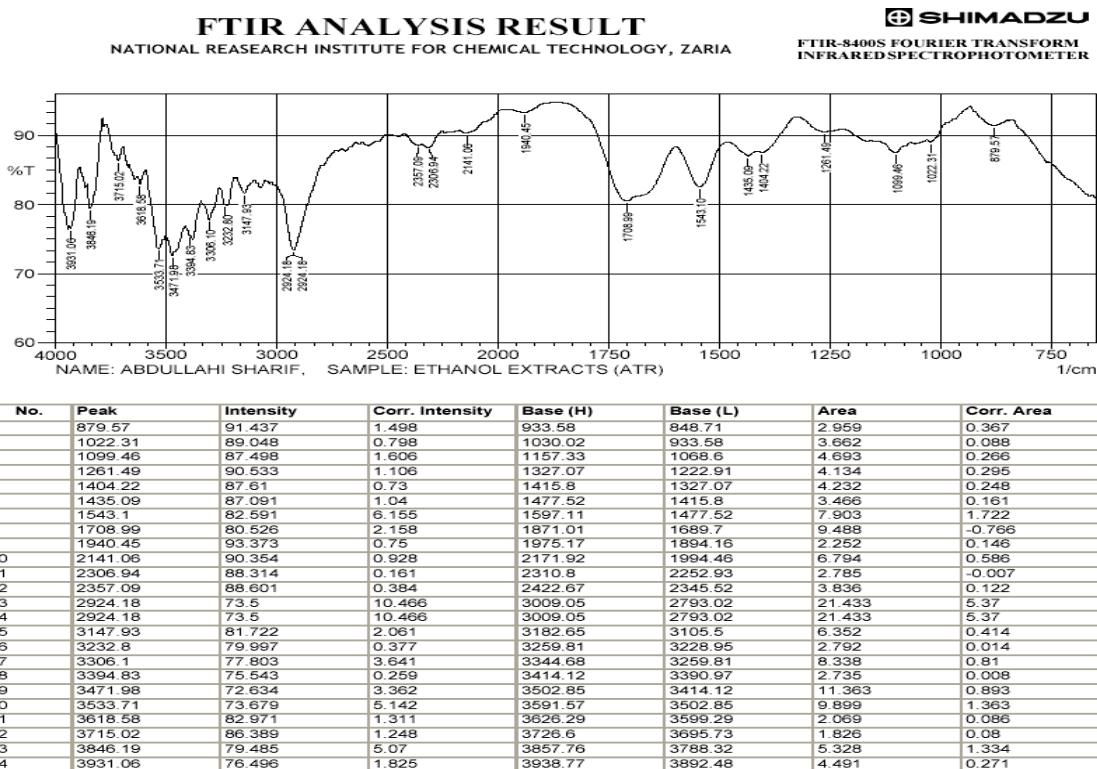


Figure 3: The FTIR spectral and Infrared Spectroscopy Absorption Table of the *J. curcas* ethanol Extract

Table 5: The Functional Group characteristic of FTIR spectral of ethanol extracts of *J. cactus* indicating Peak, compound class, appearance and frequency range

Ethanol Extract Peak (Absorption (cm ⁻¹))	Compound Class	Group	Appearance	Frequency Range
879.57	halo compound	C-Cl stretching	strong -	850-550
1022.31	vinyl ether	C-O stretching	strong -	1075-1020
1099.46	Secondary alcohol	C-O stretching	strong -	1124-1087
1261.49	alkyl aryl ether	C-O stretching	strong -	1275-1200
1404.22	Sulfonyl chloride	S=O stretching	strong -	1075-1020 1410-1380
1435.09	carboxylic acid	O-H bending	medium -	1204-1177
1543.10	Nitro compound	N-O stretching	strong -	1440-1395 1550-1500
1708.99	Conjugated aldehyde	C=O stretching	strong -	1372-1290
1940.45	alkene	C=C=C stretching	medium -	2000-1900
2141.06	carbodiimide	N=C=N stretching	strong -	2145-2120
2306.94	carbon dioxide	O=C=O stretching	strong -	About 2349
2357.09	Amino-related component	NH component		about 2350
2924.18	alkane	C-H stretching	medium -	3000-2840
3147.93	alcohol	O-H stretching	weak broad	3200-2700
3232.8	carboxylic acid	O-H stretching	strong broad	3300-2500
3306.1	Secondary amine	N-H stretching	medium -	3350-3310
3394.83	Aliphatic primary amine	N-H stretching	medium -	3400-3300 3330-3250
3471.98	Primary amine -	N-H stretching	medium -	3500- 3400
3533.71	alcohol	O-H stretching	strong broad	3550-3200
3618.58	alcohol free	O-H stretching	medium sharp	3700-3584
3715.02	alcohol free	O-H stretching		About 3700
3846.19	alcohol free	O-H stretching		About 3700
3931.06	alcohol free	O-H stretching		About 3700

The Table 5 details the infrared (FTIR) spectroscopy data for various functional groups as presented in Figure 3 as FTIR spectrum of the ethanol extract of *J. curcas*, offering insights into the chemical structure of compounds present in the extract. This information complements the Gas Chromatography-Mass Spectrometry (GCMS) data in Table 4, aiding in the comprehensive interpretation of the compounds identified in the ethanol extract of *J. curcas*. The FTIR spectroscopy is a very strong technique that reveals details about the molecular vibrations of chemical bonds, aiding in identification of functional groups in a compound. The absorption peaks at the region 686.68 cm^{-1} (- C-H stretching), indicates the presence of aromatic rings, suggesting the possible existence of aromatic compounds which Correlates with GCMS showing compounds related to the presence of benzene rings or similar structures identified in the GCMS Table 4. The adsorption peak 756.12 cm^{-1} (- C-H bending): Indicates the presence of ethers, which Correlates with GCMS identified compounds Such as Octadecadienoyl ethers identified in the GCMS Table 4, the absorption peak 887.28 cm^{-1} (- C=C bending), indicates the presence of alkene groups and Correlates with GCMS indicated Alkene structures, such as 9, 12-Octadecadienoyl chloride (Z) as in the Table 4. In the same narration the absorption peak 964.44 cm^{-1} indicated (Alkene disubstituted (Trans) - C=C bending) showing the presence of trans-disubstituted alkenes in similar correlation with the alkene structures identified in the GCMS spectra. The spectral 1026.16 cm^{-1} (- C-O stretching) peak indicates the presence of vinyl ether groups Correlated with GCMS compounds containing oxygenated groups in the spectral above. Together, the IR analyses offer a very good understanding of the metabolites composition such as 9,12-Octadecadienoyl chloride (Z) and others that appear to contain multiple functional groups, including alkene, ether, and aromatic moieties, all of which are consistent with the diverse structures present in the GCMS data. The correlation between the two strengthens the confidence in the identification and characterization of the compound identified.

CONCLUSION

In conclusion the study on *J. curcas* ethanol leaf extract reveals its potential as a bioactive compound source with various metabolites identified. The extract was identified with 19 compounds with antibacterial, anti-inflammatory, and possible antioxidant, properties, making it suitable antimicrobial drug development. However, further studies are needed to validate the safety and efficacy of these compounds other microbial agents.

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