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In Vitro Inhibition of Biofilm Formation and Phytochemical Analysis of Fractions of *Jatropha tanjorensis* against Clinical Bacterial Pathogens

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Abstract

The widespread use of antibiotics has driven the emergence of resistant bacteria, leading to longer treatments, higher costs, and increased mortality in humans. This study examined the antibacterial and antibiofilm activities of *Jatropha tanjorensis* leaf fractions and identified the major bioactive compounds responsible for these effects. The leaves were extracted using solvents of different polarities—n-hexane, dichloromethane (DCM), ethyl acetate, and water—and each fraction was tested against selected clinical bacterial isolates. Phytochemical screening of fractions was conducted following standard procedures. GC–MS analysis was carried out to confirm and quantify phytoconstituents in the fractions. Antibacterial activity of the fractions was evaluated using agar well diffusion methods. The antibiofilm effect of the fractions was determined by a slightly modified crystal violet microtiter plate assay. Extraction yields varied, with the n-hexane fraction giving the highest recovery (70.51%) and the aqueous fraction the lowest (63.71%). Phytochemical screening revealed the presence of flavonoids, saponins,

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tannins, steroids, and terpenoids, while GC–MS analysis confirmed compounds such as n-hexadecanoic acid, phytol, 9-octadecanamide, and benzenedicarboxylic acid in different concentrations across fractions. These compounds are known for their antimicrobial and antioxidant properties. All fractions showed varying degrees of antibacterial activity, but the n-hexane and ethyl acetate fractions were the most effective. The ethyl acetate fraction recorded the lowest minimum inhibitory and bactericidal concentrations (12.5 and 25 mg/mL, respectively) against *Escherichia coli* and *Proteus mirabilis*. Antibiofilm assays demonstrated concentration-dependent inhibition, with the n-hexane fraction exhibiting the strongest activity (0.014 OD against *E. coli*). In essence, the results indicate that *J. tanjorensis* is rich in biologically active compounds capable of inhibiting bacterial growth and biofilm formation. These findings provide scientific support for its traditional use in managing infections and highlight its potential as a natural source for developing new antibacterial and antibiofilm agents.

Keywords: *Jatropha tanjorensis*; Antibacterial Activity; Antibiofilm Activity; GC–MS Profiling; Phytochemical Constituents

INTRODUCTION

The increasing prevalence of antibiotic resistance among bacterial pathogens has become a major global health concern (Musini and Giri, 2019). A significant factor contributing to this problem is the formation of biofilms, which enable bacteria to withstand antibiotic treatments and evade host immune responses (Aiyegoro et al., 2019, Sofy et al., 2020). Pathogens such as *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* are well known for forming biofilms, resulting in persistent and recurrent infections that are difficult to eradicate (Jamal et al., 2018; Petruzzi et al., 2018; Aremu et al., 2019).

In response to this growing challenge, there has been a renewed interest in medicinal plants as alternative sources of antimicrobial agents (WHO, 2014; Frieri et al., 2017; Sadiq et al., 2017). These plants possess bioactive compounds—including tannins, alkaloids, flavonoids, and phenolics—that exhibit natural antibacterial properties with relatively low toxicity compared to conventional antibiotics (Al-Rifai et al., 2017; Piegerora et al., 2019; Rajkumari et al., 2019).

Among such plants, *Jatropha tanjorensis*, commonly referred to as *"Hospital-too-far"* or *"Catholic vegetable,"* has gained attention for its promising therapeutic potential (Babayemi et al., 2021; Obum-Nnadi et al., 2022). Widely distributed in West Africa, this herb is traditionally used for both nutritional and medicinal purposes. Empirical studies have demonstrated that extracts from its bark, leaves, and stems exhibit broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria (Kumar et al., 2016; Samatha et al., 2017; Abdallah & Ali, 2019; Udoh et al., 2024). These effects are attributed to its diverse phytochemical composition, which includes glycosides, flavonoids, saponins, alkaloids, steroids, terpenoids, and tannins (Datsugwai & Yusuf, 2017; Daniyan et al., 2018; Elinge et al., 2020).

Traditional medicinal plants, such as *Jatropha tanjorensis*, remain an important source of healthcare for many people, particularly in developing countries (Oyebode et al., 2016). Despite their widespread use, the biochemical mechanisms underlying the antimicrobial properties of these plants are not yet fully understood. This study seeks to identify and characterize the phytochemical constituents of *J. tanjorensis* at the molecular level and to evaluate its antibacterial and antibiofilm activities against selected bacterial strains. The research is especially relevant in light of the increasing global threat of antibiotic-resistant infections and the limited scientific data on the plant's potential to inhibit biofilm formation.

MATERIALS AND METHODS

Plant Species Collection and Identification

The plant (*Jatropha tanjorensis*) leaves were collected from the Botanical Garden of University of Cross River State, Calabar, Nigeria. The plant was then taken to the Herbarium unit, Department of Botany, Faculty of Biological Sciences, University of Calabar, Calabar, for proper identification, and authentication. The plant material was spread out to dry on wooden tables at room temperature in the laboratory, until it became brittle. It was then pulverized, using a mortar and pestle and stored in labelled polythene bag, until ready for use (Sofowora, 1984; Mukhtar and Tukur, 2000).



Jathropha tanjorensis (Hospital-too-far)

Preparation of Plant Extract

A total of 200 grams of air-dried *Jatropha tanjorensis* powder was soaked in 2000 ml of methanol (99.9% purity). The mixture was stirred and gently heated using a magnetic stirrer for 24 hours to enhance extraction. It was then transferred to a rotary shaker (LabTech Ltd., England) and agitated continuously for another 24 hours to ensure proper mixing. After this process, the suspension was filtered to separate the filtrate from the plant residue. The solvent concentration in the extract was gradually increased until complete solubility was no longer achieved (Shah et al., 2020).

The resulting filtrates were concentrated by evaporating the solvent at 64.7°C until dryness. The dry extract was then weighed using an electric balance (Gerhardt, England) and stored in a refrigerator at 4°C until needed for further analysis (Njeru *et al.*, 2021).

Fractionation of Plant Extract

The methanol leaf extract (100g) of the plant was dissolved in 1000 mL of deionized water and subjected to successive exhaustive partitioning with n-hexane, dichloromethane, and ethyl acetate using a separatory funnel (Pyrex, England). The resulting liquid fractions were individually weighed and stored at -4°C until further analysis. The percentage yield of the fractions of *J. tanjorensis* were calculated using the formula:

Percentage (%) recovery of fraction = W_a/W_b x 100/1

Where: W_a: Initial weight of fraction, W_b: Weight of plant material used.

Microbial-Free and Sterility Test of the fractions

The sterility of the fraction was evaluated for turbidity using the Millipore filtration method. In this procedure, 2 mL of the fraction was added to 10 mL of sterile Mueller-Hinton broth and incubated at 37°C for 24 hours. The absence of turbidity or a clear broth after incubation indicated that the fraction was free from microbial contamination (Sule & Agbabiaka, 2008; Bodunrinde et al., 2020).

To further confirm sterility and rule out microbial contamination, the fraction was re-dissolved in absolute ethanol. Appropriate concentrations for bioassay testing were prepared using sterile deionized distilled water and then sterilized by passing through a 0.5 µm Millipore membrane filter. Subsequently, 1 mL of each concentration was plated on nutrient agar and incubated at 30°C for 24 hours. The absence of microbial growth after incubation confirmed that the fraction was sterile (Ashish et al., 2016).

Qualitative Phytochemical Screening

A series of standard qualitative tests were conducted to identify the major classes of phytochemicals present in the various fractions of the plant in accordance with standard methods described by Sofowora (1993), Evans (2009) Boxi *et al.* (2010) and Fawehimi *et al.* (2013).

Saponins were detected using the *frothing test*, where persistent foam formation indicated their presence. Free anthraquinones were identified by treating samples with toluene and ammonia, resulting in pink to violet coloration. The presence of tannins was confirmed through the *ferric chloride test*, which produced a blue-black precipitate.

Flavonoids were assessed using three complementary methods: the *Shinoda reduction*, *aluminum chloride*, and *ammonia tests*. In all, the appearance of yellow, orange, or red coloration indicated positive results.

Cardiac glycosides were tested using *Salkowski's*, *Keller-Killiani*, and *Liebermann's* methods, all of which involved distinct colour changes or ring formation at the interface of reagent layers, confirming the presence of a steroidal or glycone nucleus.

Alkaloids were detected by the formation of orange to red precipitates upon addition of Dragendorff's reagent after acid extraction. Finally, triterpenes and steroids

were identified using the *Liebermann-Burchard test*, where colour transitions from violet to blue or the formation of a blue-green ring confirmed their presence.

Gas Chromatography-Mass Spectrometry Analysis

The GC-MS analysis of *J. tanjorensis* leaf fractions was carried out using a Shimadzu GCMS-QP2010 Plus system (Shimadzu, Japan), equipped with a fused silica capillary column coated with polymethylsiloxane (0.25 μm × 50 m). The analytical conditions were as follows: the oven temperature was programmed from 80°C to 200°C, initially held at 80°C for 1 minute, then increased at a rate of 5°C per minute and maintained at 200°C for 20 minutes. The flame ionization detector (FID) was set at 300°C, while the injection temperature was maintained at 220°C. Nitrogen was used as the carrier gas at a flow rate of 1 mL/min, with a split ratio of 1:75. For mass spectrometric detection, the GC-MS system (Shimadzu GCMS-QP2010 Plus, Japan) operated with an injector temperature of 220°C and a carrier gas pressure of 116.9 kPa. The column length was 30 m with an internal diameter of 0.25 mm, and the flow rate was maintained at 50 mL/min. The eluates were automatically introduced into the mass spectrometer, which operated at a detector voltage of 1.5 kV and a sampling rate of 0.2 seconds. The mass spectrometer was coupled to a computerized spectral library (Wiley MS libraries) for compound identification. A German Hermle Z233M-Z centrifuge was also employed during sample preparation. The chemical constituents of the fractions were identified by comparing the mass spectral peaks with those in the Wiley MS library database and further confirmed by comparing the obtained spectra with data from published literature.

Collection of Sample at the Hospital Wards

A swab of sampling site was taken using a sterile cotton bud dipped into sterile distilled water. Samples were taken from the beds, tables, doors, and the floors of various wards, (Male, Female, and Children Wards) of General Hospital, Calabar, Cross River State. A total of 168 samples were collected from the three wards and coded appropriately, placed in a sterile swab bag, and transported immediately to Microbiology Laboratory, University of Cross River State. Collected samples were enriched in peptone broth and incubated at a temperature of 37°C for 48 h.

Isolation of test organisms

Staphylococcus aureus

Isolation of *S. aureus* was carried out by transferring a loopful of the enrichment culture onto Mannitol Salt Agar (MSA) plates, followed by incubation at 37°C for 24 hours. Colonies that appeared bright yellow on MSA were presumptively identified as *S. aureus*. These colonies were then sub-cultured onto Nutrient Agar and incubated again at 37°C for confirmatory analysis. Identification and characterization of the isolates were performed using standard biochemical tests, including Gram staining, methyl red-Voges Proskauer (MRVP), catalase, citrate utilization, and gelatin hydrolysis tests. All culture media used in this study were obtained from Oxoid, UK.

Salmonella typhi

Isolation of *S. typhi* was carried out following the techniques recommended by the International Organisation for Standardisation (ISO 6579, 2002) and the World Health Organization's Global Foodborne Infections Network Manual (2016), with minor modifications. A loopful of the pre-enriched culture was streaked onto Bismuth Sulphite Agar (BSA), *Salmonella-Shigella* Agar (SSA), and Xylose Lysine Deoxycholate Agar (XLD), then incubated at 37°C for 18–24 hours. Characteristic colonies – shiny black "rabbit-eye" colonies on BSA, red colonies with black centres on XLD, and colourless colonies with black centres on SSA – were presumptively identified as *Salmonella* species. These colonies were sub-cultured onto Nutrient Agar and incubated again at 37°C for 24 hours. Species confirmation was performed using standard biochemical tests, including Triple Sugar Iron (TSI) agar, Motility-Indole tests, and Citrate utilization tests. Serological identification using O and H antigens was also conducted. All culture media were sourced from Oxoid, UK.

Escherichia coli

The enriched sample was cultured onto MacConkey Agar, Eosin Methylene Blue Agar, and Nutrient Agar plates. These plates were streaked and incubated at 37°C for 24 hours. After incubation, the resulting bacterial colonies were subjected to Gram staining. Identification and characterization of the bacterial isolates were performed using standard microbiological techniques as described by Koneman et al. (2005).

Proteus mirabilis

The samples were first cultured on selective and differential media to obtain isolated colonies. Using a sterile inoculating loop, each sample was streaked on Blood Agar (BA) and MacConkey Agar (MAC) plates. The inoculated plates were incubated aerobically at 37°C for 18–24 hours. After incubation, growth characteristics were examined. On blood agar, *Proteus mirabilis* typically produces swarming colonies with a distinctive concentric ring pattern, while on MacConkey agar, colonies appear pale or colourless, indicating non-lactose fermentation. Suspected colonies were further examined through Gram staining and a few biochemical tests (urease, indole, oxidase, citrate and motility) were performed for confirmation.

Antibacterial Assay of *J. tanjorensis* Fractions against Bacterial Isolates

The antibacterial activity of the fractionated compounds against selected bacterial isolates was evaluated using the agar well diffusion method. Fractions that demonstrated inhibitory effects were further subjected to minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) testing. The MIC was determined following the broth microdilution method described by Novy *et al.* (2015) with slight modifications.

To assess the antibacterial effect, 100 µL of fresh bacterial culture (approximately 10^6 CFU/mL) was evenly spread on Mueller-Hinton agar (MHA) plates using a sterile glass spreader. The plates were allowed to dry at room temperature for 10 minutes. Using a sterile 6 mm cork borer, wells were created in the agar, and 100 µL of each fraction at varying concentrations (100, 50, 25, 12.5, and 6.25 mg/mL) was dispensed into the wells. All concentrations were prepared using 10% dimethyl sulfoxide (DMSO). The plates were incubated at 37°C for 24 hours. The antibacterial activity was determined by measuring the diameter of the inhibition zones around each well. DMSO served as the negative control, while chloramphenicol (100 mg/mL) was used as the positive control. Zones of inhibition were interpreted as resistant (≤14 mm) or sensitive (≥15 mm), according to Ndip *et al.* (2009) and the CLSI (2020) guidelines.

For MIC determination, 100 µL of Mueller-Hinton broth (Difco) containing varying concentrations of the fractions (ranging from 1.56 to 100 mg/mL) was added to wells of a 96-well microtiter plate. Then, 10 µL of standardized bacterial inoculum

(adjusted to 0.5 McFarland standard ≈ 1×10^6 CFU/mL) was introduced into each well. The plates were incubated at 37°C for 24 hours. The MIC was identified as the lowest concentration of the fraction showing no visible turbidity. Broth with bacterial inoculum served as the negative control, while broth containing chloramphenicol acted as the positive control (Ahmed *et al.,* 2020).

For MBC determination, 100 µL from each well without visible growth was sub-cultured onto fresh MHA plates and incubated again at 37°C for 24 hours. The MBC was defined as the lowest concentration of the fraction that completely inhibited bacterial growth.

Biofilm Formation Inhibition Activity of Plant Fractions

A slightly modified crystal violet microtiter plate assay was used to evaluate the effect of the fractions on biofilm formation, following the method of O'Toole and Kolter (1998). Two-fold serial dilutions of each fraction were prepared in sterile 96-well flat-bottom microplates, with each well containing 150 µL of Mueller-Hinton broth. The concentrations tested ranged from 1.56 mg/mL to 100 mg/mL. Then, 100 µL (0.1 mL) of a freshly prepared bacterial suspension, adjusted to 0.5 McFarland standard, was added to each well. A positive control (bacterial suspension in broth only) and a negative control (fraction in broth without bacteria) were included. After incubation at 37°C for 24 hours, the contents of the wells were gently discarded, and each well was washed with 200 µL of sterile distilled water to remove non-adherent (planktonic) cells. The remaining biofilm attached to the well surface was stained with 0.1% crystal violet and left at room temperature for 30 minutes. Excess stain was carefully rinsed off with distilled water, and the plates were fixed using 200 µL of 70% ethanol. The absorbance (OD_{600}) of the stained adherent biofilms was then measured using an ELISA microplate reader (Sunrise™ TECAN, Switzerland).

RESULTS AND DISCUSSION

The percentage recovery of leaf fractions of *J. tanjorensis*

Among the various fractions of *J. tanjorensis,* the n-hexane fraction showed the highest percentage yield at 70.51%, whereas the aqueous fraction recorded the lowest yield at 63.71%, as presented in Table 1.

Qualitative phytochemical screening of leaf fractions of *J. tanjorensis*

The phytochemical analysis of the different fractions indicated varying distributions of bioactive compounds. Saponins were detected in both the n-hexane and aqueous fractions, whereas tannins were found in the ethyl acetate and aqueous fractions. Flavonoids were present exclusively in the ethyl acetate fraction. In contrast, alkaloids were absent only in the ethyl acetate fraction. Triterpenes and steroids were identified across all fractions, while cardiac glycosides were missing from the n-hexane fraction. Additionally, free anthraquinones were detected solely in the ethyl acetate fraction. The detailed results are presented in Table 2.

GC-MS analysis of leaf fractions of *J. tanjorensis*

The spectral analysis of the n-hexane fraction of *J. tanjorensis* is presented in Figure 1 and Table 3. The GC–MS results identified and quantified 52 phytochemical constituents, with the most abundant compounds being n-Hexadecanoic acid (13.04%), Hexadecanoic acid (8.60%), Phytol (8.32%), 4-Acetoxy-6 (2.86%), and Methyl stearate (1.57%). The total ion chromatogram (TIC) and phytochemical profile of the dichloromethane fraction are shown in Figure 2 and Table 4, respectively. A total of 53 phytochemicals were identified and quantified, among which 9-Octadecanamide (15.08%), Trimethyl (7.36%), Phytol (3.57%), Cyclopentane-1-carboxylic acid (2.17%), and Butanedioic acid (1.78%) were prominent. Similarly, Figure 3 and Table 5 display the TIC and phytochemical constituents of the ethyl acetate fraction. GC–MS analysis revealed 50 compounds, including Benzenedicarboxylic acid (9.82%), 9-Octadecanamide (9.10%), 1-Nonadecane (5.87%), 1-Octadecanol (4.83%), and Benzofuran (4.15%). The aqueous fraction of *J. tanjorensis* represented in Figure 4 and Table 6, also yielded 50 phytochemical constituents. Major compounds identified include Benzenedicarboxylic acid (29.82%), 9-Octadecanamide (9.10%), 1-Octadecano (4.80%), Isopropyl palmitate (4.55%), and Benzofuran (4.15%).

Antibacterial assay of fractions of *J. tanjorensis*

Table 7 presents the antibacterial activity of the various fractions obtained from *J. tanjorensis*. The results indicate that the n-hexane fraction exhibited the strongest antibacterial effect, producing zones of inhibition ranging from 8.5 mm to 22 mm at different concentrations against the test isolates. The highest inhibitory activity was recorded against *E. coli* and *P. mirabilis*, both showing 22.5 mm zones of clearance. The dichloromethane (DCM) fraction also displayed notable antibacterial effects, with inhibition zones varying according to concentration. At 100 mg/mL, it produced 20.5 mm inhibition against *Escherichia coli*, 15.5 mm against *P. mirabilis*, 11.5 mm against *Staphylococcus aureus*, and 13.5 mm against *S. typhi*. However, the DCM fraction showed no activity against *S. aureus* at 25, 12.5, and 6.25 mg/mL concentrations, and no inhibition was recorded against *S. typhi* at 6.25 mg/mL. The ethyl acetate fraction demonstrated significant antibacterial activity, particularly against *E. coli*, with a 20 mm zone of inhibition at 100 mg/mL. The smallest inhibition zone (18.5 mm) at the same concentration was observed against *S. aureus*. However, at lower concentrations (6.25 mg/mL), the fraction showed no inhibitory effect against *S. typhi* and *S. aureus*. The aqueous fraction of *J. tanjorensis* produced inhibition zones of 18 mm against *E. coli*, 15 mm against *P. mirabilis*, 14.5 mm against *S. typhi* and 13.5 mm against *S. aureus*. No inhibitory activity was detected against *S. aureus*, *S. typhi*, and *P. mirabilis* at 25, 12.5, and 6.25 mg/mL concentrations. A general decline in antibacterial activity was observed with increasing dilution of the fractions.

MIC and MBC of fractions of *J. tanjorensis*

Table 8 presents the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of the different fractions of *J. tanjorensis* tested against bacterial isolates. The results show that *S. aureus, S. typhi* and *P. mirabilis* were inhibited and killed by the n-hexane fraction at relatively higher concentrations, with MICs and MBCs of 25 mg/mL, 25 mg/mL and 50 mg/mL, and MBCs of 100 mg/mL, 50 mg/mL and 50 mg/mL, respectively. The dichloromethane (DCM) fraction exhibited varying MICs across the test organisms: *P. mirabilis* showed inhibition at 25 mg/mL, *E. coli* at 12.5 mg/mL (the lowest MIC recorded), *S. aureus* at 50 mg/mL, and *S. typhi* at 100 mg/mL. However, no MBC values were observed for *S. aureus* and *S. typhi*. The ethyl acetate fraction demonstrated the highest antibacterial activity, recording the lowest MICs and MBC values against test organisms. Both *E. coli* and *P. mirabilis* exhibited identical MICs and MBCs of 12.5 mg/mL and 25 mg/mL, respectively. Notably, this was the only fraction that produced

an MBC (50 mg/mL) against *S. aureus*. The aqueous fraction of *J. tanjorensis* showed limited antibacterial activity. It demonstrated MICs of 100 mg/mL against *P. mirabilis*. *S. typhi* and *S. aureus*, while the only MBC recorded was 100 mg/mL against *E. coli* with an MIC of 25 mg/mL.

Antibiofilm activity of fractions of *J. tanjorensis*

Table 9 presents the optical densities (ODs) recorded, which indicate antibiofilm formation by different fractions of the plants against test isolates at various concentrations. The results clearly show that the antibiofilm activity of the different fractions was concentration-dependent. The best biofilm reduction is observed in higher concentrations of fractions (25mg/mL, 50mg/mL and 100mg/mL). However, n-hexane fraction produced the highest antibiofilm effect recording 0.014_{OD} against *E. coli*, which is the lowest optical density observed in the study.

Table 1: Percentage (%) recovery of leaf fractions of *Jatropha tanjorensis*

Fractions	Weight of leaves (Wa) (g)	Weight of fraction (Wb) (g)	% of fraction recovered
n-hexane	200	141.02	70.51
Dichloromethane	200	138.11	69.06
Ethyl acetate	200	129.51	64.76
Aqueous	200	127.42	63.71

% - Percentage; g – gram

Table 2: Result of preliminary screening of fractionated extract of *Jantropha tanjorensis*

S/N	Metabolite/Test	Partitioning solvents			
		Hexane	DCM	Ethyl acetate	Aqueous
1.	Saponins (Frothing test)	+	-	-	+++
2.	Tannins (5% $FeCl_3$ test)	-	-	++	+++
3.	Flavonoids (Mg metal test)	-	-	++	-
4	Alkaloids (10% NaOH test)	+	++	-	++
5.	Triterpenes/Steroids	++ (T)	++ (S)	++ (S)	+ (T)
6.	Cardiac glycoside (Salkowski test)	-	+	+	++
7.	Free anthraquinone	-	-	+	-

- = Not present; + = low presence; ++ = moderately present; +++ = high concentration of metabolite; DCM = dichloromethane; % = percentage; S = steroids; T = triterpenes.

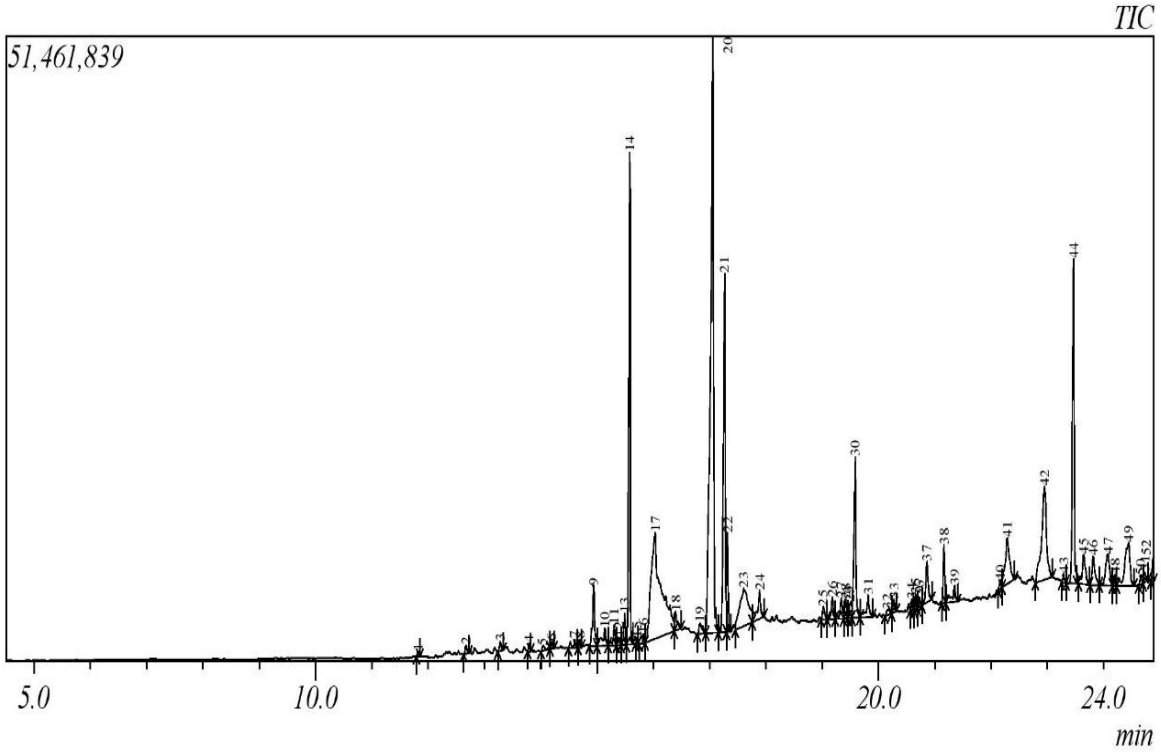


Figure 1: Total Ion Chromatogram (TIC) of n-hexane fraction of *Jatropha tanjorensis*

Table 3**:** Phyto-components generated in the n-hexane fraction of *J. tanjorensis* by GC-MS peak report TIC

Peak	Retention Time	Peak Area %	Molecular Formula	Molecular Weight	SI	Name
1	11.820	0. 08	$C_{11}H_{16}O_2$	180	90	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trim
2	12.676	0. 18	$C_{13}H_{20}O_2$	208	84	3-Hydroxy-.beta.-damascone
3	13.284	0. 21	$C_{11}H_{20}O_3$	200	68	Cyclopentane-1-carboxylic acid, 2-hydroxy-1,2,3-trime
4	13.796	0. 12	$C_{15}H_{30}O_2$	242	88	Tridecanoic acid, 12-methyl-, methyl ester
5	14.033	0. 16	$C_{14}H_{22}O_3$	238	68	Acetic acid, 2-(2,2,6-trimethyl-7-oxa-bicyclo[4.1.0]hep
6	14.198	0. 22	$C_{14}H_{28}O_2$	228	88	Tetradecanoic acid
7	14.610	0. 27	$C_{21}H_{44}$	296	89	Heneicosane
8	14.700	0. 13	$C_{16}H_{32}O_2$	256	84	Methyl 13-methyltetradecanoate
9	14.946	1. 56	$C_{22}H_{42}O_2$	338	89	Phytol, acetate

Peak	Retention Time	Peak Area %	Molecular Formula	Molecular Weight	SI	Name
10	15.144	0. 88	$C_{20}H_{40}O$	296	83	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
11	15.307	0. 55	$C_{20}H_{40}O$	296	89	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
12	15.392	0. 21	$C_{17}H_{24}O_3$	276	71	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-di
13	15.495	0. 62	$C_{17}H_{32}O_2$	268	88	7-Hexadecenoic acid, methyl ester, (Z)-
14	15.586	8. 60	$C_{17}H_{34}O_2$	270	97	Hexadecanoic acid, methyl ester
15	15.709	0. 07	$C_{16}H_{22}O_4$	278	89	Dibutyl phthalate
16	15.828	0. 14	$C_{20}H_{40}O$	296	94	Isophytol
17	16.036	13.04	$C_{16}H_{32}O_2$	256	93	n-Hexadecanoic acid
18	16.400	0. 50	$C_{18}H_{36}O_2$	284	90	Hexadecanoic acid, 15-methyl-, methyl ester
19	16.825	0. 38	$C_{13}H_{13}IO$	312	60	Pyrylium, 2,4-dimethyl-6-phenyl-, iodide
20	17.059	22.10	$C_{19}H_{32}O_2$	292	96	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-
21	17.272	8. 32	$C_{20}H_{40}O$	296	96	Phytol
22	17.321	1. 57	$C_{19}H_{38}O_2$	298	96	Methyl stearate
23	17.610	3. 93	$C_{18}H_{30}O_2$	278	91	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
24	17.892	1. 14	$C_{16}H_{33}NO$	255	93	Hexadecanamide
25	19.025	0. 36	$C_{24}H_{40}O_5$	408	67	Trihydroxycholanic acid, (3.alpha., 7.beta., 12.alpha.)-
26	19.184	0. 58	$C_{10}H_{18}O_4S$	234	79	Methanesulfonic acid, 9-oxabicyclo[3.3.1]non-3-yl
27	19.341	0. 64	$C_{21}H_{42}O_2$	326	91	Methyl 18-methylnonadecanoate
28	19.421	0. 47	$C_{26}H_{52}$	364	76	Cyclohexane, (1-butylhexadecyl)-
29	19.500	0. 49	$C_{19}H_{34}O_2$	294	76	Methyl 10,11-octadecadienoate
30	19.590	3. 91	$C_{18}H_{35}NO$	281	94	9-Octadecenamide, (Z)-
31	19.820	0. 78	$C_{18}H_{37}NO$	283	86	Octadecanamide
32	20.158	0. 20	$C_{27}H_{46}O_2$	402	58	Cholesterol epoxide
33	20.278	0. 35	$C_{15}H_{26}O$	222	75	1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahyd
34	20.592	0. 19	$C_{14}H_{22}O_2$	222	71	2,2,6-Trimethyl-1-(3-methylbuta-1,3-dienyl)-7-oxab

Peak	Retention Time	Peak Area %	Molecular Formula	Molecular Weight	SI	Name
35	20.664	0. 27	$C_{16}H_{22}OSi$	258	52	Diisopropyl(1-naphthyloxy)silane
36	20.726	0. 28	$C_3H_{12}Si_3$	132	66	1,3,5-Trisilacyclohexane
37	20.864	1. 18	$C_9H_{11}FN_2O_5$	246	71	Floxuridine
38	21.166	1. 03	$C_{24}H_{38}O_4$	390	90	Bis(2-ethylhexyl) phthalate
39	21.355	0. 75	$C_{10}H_{24}Si_4$	356	52	1,3,5,7-Tetrasilatricyclo[3.3.1.1<3,7>]decane,1,3,5,
40	22.167	0. 26	$C_9H_{14}O_2S$	186	66	3-(3-Methylbutyl)thiophene-1,1-dioxide
41	22.291	2. 47	$C_{19}H_{30}O_2$	290	68	2,5-Octadecadiynoic acid, methyl ester
42	22.951	5. 60	$C_{22}H_{42}O_2$	338	92	Phytol, acetate
43	23.293	0. 15	$C_{20}H_{34}O$	290	83	trans-Geranylgeraniol
44	23.471	7. 14	$C_{30}H_{50}$	410	98	Squalene
45	23.647	1. 17	$C_{28}H_{44}N_2O_7$	520	54	1-Pyrrolidinebutanoic acid, 2-[(1,1-dimethylethoxy)
46	23.819	1. 02	$C_{28}H_{44}N_2O_7$	520	53	1-Pyrrolidinebutanoic acid, 2-[(1,1-dimethylethoxy)
47	24.078	1. 55	$C_{20}H_{20}O_8$	388	58	4H-1-Benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-5
48	24.204	0. 34	$C_{32}H_{60}O_4$	508	37	Sebacic acid, cis-hex-3-enyl hexadecyl ester
49	24.446	2. 868	$C_{26}H_{20}O_8$	460	56	4-Acetoxy-6',7-dimethyl-5',8'-dimethoxy-1,2'-binaph
50	24.658	0. 26	$C_{35}H_{46}O_2$	498	54	Anthraergostatetraenol benzoate
51	24.732	0. 26	$C_{31}H_{54}O_3$	474	55	3-[3-(1,5-Dimethylhexyl)-7-(2-hydroxy-1-methyleth
52	24.892	0. 45	$C_{30}H_{50}$	410	60	Squalene

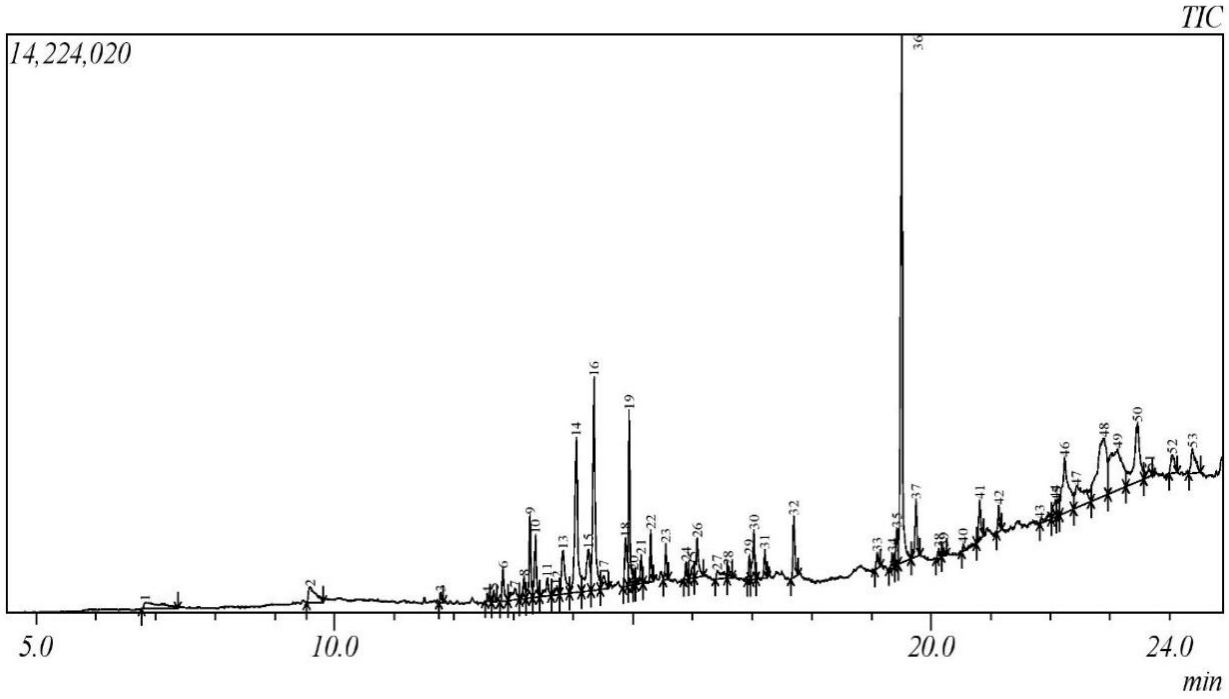


Figure 2: Total Ion Chromatogram (TIC) of dichloromethane fraction of *Jatropha tanjorensis*

Table 4**:** Phyto-components generated in the dichloromethane fraction of *J. tanjorensis* by GC-MS peak report TIC

Peak	Retention Time	Peak Area %	Molecular Formula	Molecular Weight	SI	Name
1	6. 833	1. 78	$C_7H_{10}O_4$	158	92	Butanedioic acid, methylene-, dimethyl ester
2	9. 596	1. 69	$C_9H_{10}O_2$	150	90	2-Methoxy-4-vinylphenol
3	11.780	0. 22	$C_8H_{14}O_2$	142	72	Cyclohexanone, 2-(1-hydroxyethyl)-
4	12.572	0. 15	$C_{13}H_{20}O_2$	208	69	3-(1-Methylhept-1-enyl)-5-methyl-2,5-dihydrofuran-2-one
5	12.674	0. 22	$C_{13}H_{20}O_2$	208	81	3-Hydroxy-.beta.-damascone
6	12.825	0. 94	$C_{13}H_{20}O_2$	208	80	3-Hydroxy-7,8-dihydro-.beta.-ionol
7	13.027	0. 82	$C_{13}H_{20}O_2$	208	77	2-Cyclohexen-1-one, 4-(3-hydroxy-1-butenyl)-3,5,5-trime
8	13.178	0. 61	$C_{13}H_{22}O$	194	78	2-Butanone, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-
9	13.280	2. 17	$C_{11}H_{20}O_3$	200	74	Cyclopentane-1-carboxylic acid, 2-hydroxy-1,2,3-trimethy
10	13.372	1. 88	$C_{10}H_{18}O_2$	170	75	4-Octen-3-one, 6-ethyl-7-hydroxy-
11	13.571	0. 81	$C_{13}H_{22}O_2$	210	83	2-Cyclohexen-1-one, 4-(3-hydroxybutyl)-3,5,5-trimethyl-
12	13.733	0. 36	$C_{13}H_{18}O_4$	238	63	Benzo-1,4,7,10-tetraoxacyclotridecane

Peak	Retention Time	Peak Area %	Molecular Formula	Molecular Weight	SI	Name
13	13.832	2. 23	$C_{13}H_{20}O_3$	224	71	Ppropiolic acid, 3-(1-hydroxy-2-isopropyl-5-methylcycloh
14	14.059	6. 56	$C_{12}H_{20}O_2$	196	72	9,10-Dimethyltricyclo[4.2.1.1(2,5)]decane-9,10-diol
15	14.257	2. 56	$C_{16}H_{24}O_2$	248	69	Cyclobuta[1,2:3,4]dicyclooctene-1,7(2H,6bH)-dione, dod
16	14.355	7. 36	$C_{12}H_{20}O$	180	82	5,5,8a-Trimethyl-3,5,6,7,8,8a-hexahydro-2H-chromene
17	14.516	0. 74	$C_{14}H_{24}O$	208	68	3-Buten-2-ol, 3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-
18	14.881	1. 56	$C_7H_{10}N_2O_3$	170	73	Propionic acid, 3-(3-methyl-5-oxo-4,5-dihydro-1H-pyrazo
19	14.943	3. 57	$C_{22}H_{42}O_2$	338	91	Phytol, acetate
20	15.017	0. 34	$C_{15}H_{32}O$	228	76	1-Dodecanol, 3,7,11-trimethyl-
21	15.141	0. 73	$C_{20}H_{40}O$	296	91	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
22	15.302	1. 17	$C_{20}H_{40}O$	296	92	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
23	15.557	0. 68	$C_{17}H_{34}O_2$	270	93	Hexadecanoic acid, methyl ester
24	15.895	0. 43	$C_{16}H_{32}O_2$	256	88	n-Hexadecanoic acid
25	16.000	0. 52	$C_{14}H_8O_2$	208	51	1,4-Anthracenedione
26	16.081	1. 47	$C_{14}H_{22}$	190	68	Benzene, 1-ethyl-3,5-diisopropyl-
27	16.417	0. 71	$C_{13}H_{15}NO_3$	233	78	4-Oxazolecarboxylic acid, 4,5-dihydro-2-phenyl-, 1-methy
28	16.605	0. 32	$C_{19}H_{30}$	258	59	Octadecahydro-cyclopenta[e]pyrene
29	16.957	0. 49	$C_{19}H_{34}O_2$	294	85	9,12-Octadecadienoic acid, methyl ester, (E,E)-
30	17.033	1. 34	$C_{19}H_{36}O_2$	296	93	10-Octadecenoic acid, methyl ester
31	17.216	0. 80	$C_{20}H_{40}O$	296	90	Phytol
32	17.701	1. 63	$C_{14}H_{29}NO$	227	93	Tetradecanamide
33	19.102	0. 49	$C_{16}H_{30}O$	238	81	cis-9-Hexadecenal
34	19.352	0. 40	$C_{26}H_{52}$	364	69	Cyclohexane, (1-butylhexadecyl)-
35	19.427	0. 94	$C_{14}H_{24}O$	208	76	13-Tetradece-11-yn-1-ol
36	19.514	15.08	$C_{18}H_{35}NO$	281	94	9-Octadecenamide, (Z)-
37	19.750	1. 53	$C_{18}H_{37}NO$	283	94	Octadecanamide
38	20.131	0. 32	$C_{30}H_{52}O_3Si$	488	61	9,10-Secocholesta-5,7,10(19)-triene-1,3-diol, 25-[(t
39	20.217	0. 17	$C_{15}H_{26}O$	222	66	1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahy
40	20.542	0. 30	$C_{15}H_{24}O_2$	236	61	Bicyclo[4.4.0]dec-2-ene-4-ol, 2-methyl-9-(prop-1-e
41	20.817	1. 23	$C_{16}H_{38}O_2Si_2$	318	68	Decane, 1,9-bis[(trimethylsilyl)oxy]-
42	21.136	0. 63	$C_{24}H_{38}O_4$	390	86	Diisooctyl phthalate
43	21.833	0. 46	$C_{30}H_{54}O_2$	446	34	Cholestane, 3-(2-methoxyethoxy)-, (3.beta.,5.alpha.
44	22.083	0. 79	$C_{29}H_{48}O$	412	54	Stigmasta-4,22-dien-3.beta.-ol
45	22.117	0. 49	$C_{32}H_{52}O_6$	532	43	Ergostan-6-one, 3,25-bis(acetyloxy)-5-hydroxy-, (3
46	22.241	4. 71	$C_{27}H_{52}O_2Si_2$	464	66	Silane, [[(3.alpha.,5.beta.,20S)-pregnane-3,20-diyl]

Peak	Retention Time	Peak Area %	Molecular Formula	Molecular Weight	SI	Name
47	22.433	3. 11	$C_{15}H_{18}N_4O_4$	318	55	Glycyl-L-tryptophylglycine
48	22.899	7. 73	$C_{22}H_{42}O_2$	38	88	Phytol, acetate
49	23.129	6. 96	C21H36O2	320	71	i-Propyl 9,12,15-octadecatrienoate
50	23.464	4. 95	$C_{21}H_{34}O_3$	334	55	Pregnan-3,11-diol-20-one
51	23.667	0. 43	$C_{14}H_{26}O_4Se_2$	418	39	Heptanoic acid, 7,7'-diselenodi-
52	24.042	0. 98	$C_{27}H_{45}F$	388	54	3-Fluorocholest-5-ene
53	24.382	1. 45	$C_{26}H_{20}O_8$	460	54	4-Acetoxy-6',7-dimethyl-5',8'-dimethoxy-1,2'-binap

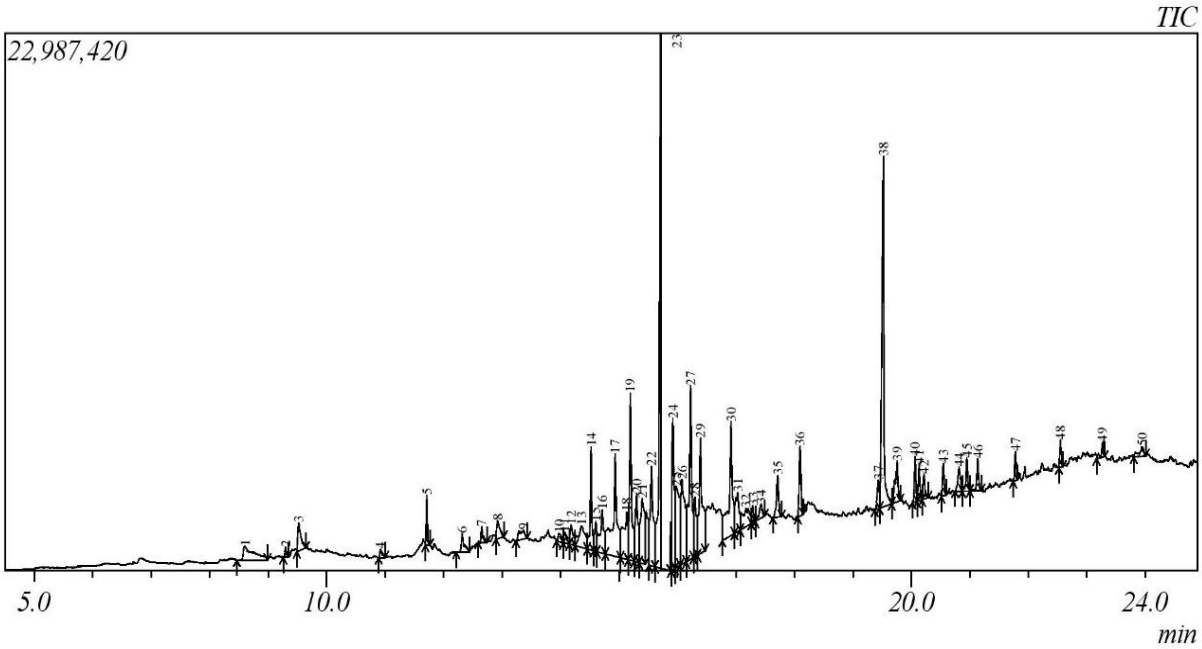


Figure 3: Total Ion Chromatogram (TIC) of ethyl acetate fraction of *Jatropha tanjorensis*

Table 5**:** Phyto-components generated in the ethyl acetate fraction of *J. tanjorensis* by GC-MS peak report TIC

Peak	Retention Time	Peak Area %	Molecular Formula	Molecular Weight	SI	Name
1	8. 605	1. 86	C_8H_8O	120	86	Benzofuran, 2,3-dihydro-
2	9. 303	0. 20	$C_{13}H_{20}O$	192	82	2(1H)-Naphthalenone, 3,4,4a,5,6,7-hexahydro-1,1,4a
3	9. 524	1. 07	$C_9H_{10}O_2$	150	94	2-Methoxy-4-vinylphenol
4	10.924	0. 23	$C_{10}H_{10}O_4$	194	90	Dimethyl phthalate
5	11.717	0. 68	$C_{14}H_{22}O$	206	95	Phenol, 2,4-bis(1,1-dimethylethyl)-
6	12.323	0. 66	$C_{12}H_{24}O_2$	200	94	Dodecanoic acid
7	12.655	0. 36	$C_{16}H_{32}$	224	93	Cetene
8	12.927	0. 69	$C_{12}H_{10}O_2$	186	83	1,4-Naphthalenedione, 2-ethyl-
9	13.367	0. 64	$C_8H_{14}O_3$	158	72	1-(1-Hydroxy-1-methyl-ethyl)-cyclobutanecarboxylic

Peak	Retention Time	Peak Area %	Molecular Formula	Molecular Weight	SI	Name
10	13.975	0. 37	$C_{15}H_{20}O$	216	67	Octanal, 2-(phenylmethylene)-
11	14.075	0. 45	$C_{13}H_{12}N_2O$	212	59	Benzoic acid, 2-phenylhydrazide
12	14.184	0. 74	$C_{14}H_{28}O_2$	228	91	Tetradecanoic acid
13	14.360	1. 74	$C_{12}H_{21}N$	179	78	2,3-Bis(1-methylallyl)pyrrolidine
14	14.526	2. 09	$C_{19}H_{38}$	266	97	1-Nonadecene
15	14.606	0. 75	$C_{16}H_{34}$	226	86	Hexadecane
16	14.715	2. 00	$C_{17}H_{34}O_2$	270	88	Isopropyl myristate
17	14.939	5. 36	$C_{22}H_{42}O_2$	338	89	Phytol, acetate
18	15.137	2. 68	$C_{20}H_{40}O$	296	88	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
19	15.199	4. 06	$C_{16}H_{34}O$	242	97	1-Hexadecanol
20	15.301	2. 32	$C_{20}H_{40}O$	296	89	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
21	15.400	3. 38	$C_{17}H_{24}O_3$	276	63	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-d
22	15.558	3. 59	$C_{17}H_{34}O_2$	270	94	Hexadecanoic acid, methyl ester
23	15.717	9. 82	$C_{20}H_{30}O_4$	334	94	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl este
24	15.923	3. 96	$C_{16}H_{32}O_2$	256	86	n-Hexadecanoic acid
25	15.983	4. 15	$C_{15}H_{10}N_4O_2S$	310	69	1H-Benzofuro[3,2-e]indole, 1-[2-(aminocarbonothio
26	16.079	4. 11	$C_{13}H_{18}O$	190	69	Phenol, 2-(1,1-dimethyl-2-propenyl)-3,6-dimethyl-
27	16.228	5. 87	$C_{19}H_{38}$	266	97	1-Nonadecene
28	16.300	1. 92	$C_{21}H_{44}$	296	93	Heneicosane
29	16.396	4. 55	$C_{19}H_{38}O_2$	298	93	Isopropyl palmitate
30	16.918	4. 83	$C_{18}H_{38}O$	270	96	1-Octadecanol
31	17.033	1. 72	$C_{19}H_{36}O_2$	296	90	11-Octadecenoic acid, methyl ester
32	17.175	1. 59	$C_{13}H_{28}$	184	68	Tridecane
33	17.292	0. 36	$C_{19}H_{38}O_2$	298	88	Methyl stearate
34	17.432	0. 56	$C_{18}H_{34}O_2$	282	87	cis-Vaccenic acid
35	17.715	1. 08	$C_{16}H_{33}NO$	255	93	Hexadecanamide
36	18.098	1. 50	$C_{19}H_{38}$	266	76	1-Nonadecene
37	19.436	0. 72	$C_{10}H_{16}O$	152	75	cis-Verbenol
38	19.523	9. 10	$C_{18}H_{35}NO$	281	94	9-Octadecenamide, (Z)-
39	19.757	1. 38	$C_{18}H_{37}NO$	283	93	Octadecanamide
40	20.067	0. 81	$C_{28}H_{58}O$	410	96	Octacosanol
41	20.135	0. 70	$C_{18}H_{26}O$	258	71	(3E,5E,7E)-6-Methyl-8-(2,6,6-trimethyl-1-cyclohex

Peak	Retention Time	Peak Area %	Molecular Formula	Molecular Weight	SI	Name
42	20.218	0. 53	$C_{15}H_{26}O$	222	80	1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahyd
43	20.548	0. 67	$C_{15}H_{24}O$	220	74	1H-3a,7-Methanoazulen-5-ol, octahydro-3,8,8-trime
44	20.817	0. 77	$C_7H_{12}O_5$	176	65	Dimethyl 2-hydroxy-2-methylbutane-1,4-dioate
45	20.951	0. 77	$C_{18}H_{36}O_2$	284	83	Decanoic acid, 2-ethylhexyl ester
46	21.136	0. 77	$C_{24}H_{38}O_4$	390	94	Bis(2-ethylhexyl) phthalate
47	21.782	0. 53	$C_{28}H_{58}O$	410	94	Octacosanol
48	22.551	0. 47	$C_{18}H_{36}O_2$	284	82	Decanoic acid, 2-ethylhexyl ester
49	23.273	0. 38	$C_{28}H_{58}O$	410	88	Octacosanol
50	23.946	0. 48	$C_{30}H_{60}O_2$	452	73	Hexadecanoic acid, tetradecyl ester

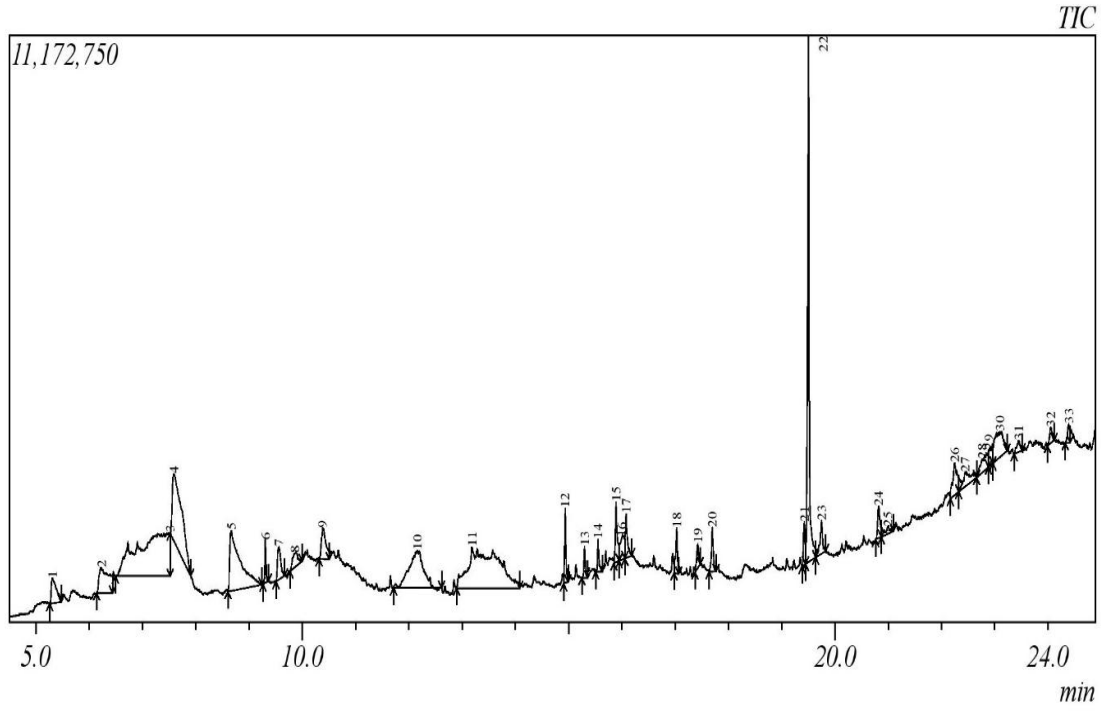


Figure 4: Total Ion Chromatogram (TIC) of aqueous fraction of *Jatropha tanjorensis*

Table 6**:** Phyto-components generated in the aqueous fraction `of *J. tanjorensis* by GC-MS peak report TIC

Peak	**Retention Time**	**Peak Area %**	**Molecular Formula**	**Molecular Weight**	**SI**	**Name**
1	5. 313	1. 64	$C_6H_6O_2$	110	92	2-Furancarboxaldehyde, 5-methyl-
2	6. 232	3. 13	C_6H_8OS	128	66	2-Methyl-3-(methylthio) furan
3	7. 516	17.21	$C_{27}H_{52}O_4Si_2$	496	63	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-
4	7. 595	8. 39	$C_6H_8O_4$	144	82	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
5	8. 670	7. 99	$C6H_6O_3$	126	76	5-Hydroxymethylfurfural
6	9. 310	0. 60	$C_{13}H_{20}O$	192	84	2(1H)-Naphthalenone, 3,4,4a,5,6,7-hexahydro-1,1,4a-tri
7	9. 564	1. 36	$C_9H_{10}O_2$	150	89	2-Methoxy-4-vinylphenol
8	9. 867	1. 01	$C_7H_{14}O_5$	178	86	.alpha.-L-Galactopyranoside, methyl 6-deoxy-
9	10.388	1. 77	$C_7H_{14}O_5$	178	91	2-Methyl-l-methylmannopyranoside
10	12.167	6. 70	$C_6H_{12}O_6$	180	91	D-Allose
11	13.187	15.28	$C_{38}H_{60}O_{18}$	804	66	Stevioside
12	14.940	1. 28	$C_{20}H_{40}O$	296	89	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
13	15.301	0. 62	$C_{20}H_{40}O$	296	88	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
14	15.557	0. 66	$C_{17}H_{34}O_2$	270	89	Hexadecanoic acid, methyl ester
15	15.893	1. 51	$C_{16}H_{32}O_2$	256	95	n-Hexadecanoic acid
16	16.000	1. 29	$C_{15}H_{10}N_4O_2S$	310	57	1H-Benzofuro[3,2-e]indole, 1-[2-(aminocarbonothioyl)h
17	16.081	1. 13	$C_{13}H_{18}O$	190	65	2,3,3,4,7-Pentamethyl-2,3-dihydro-benzofuran
18	17.032	1. 02	$C_{19}H_{36}O_2$	296	92	10-Octadecenoic acid, methyl ester
19	17.426	0. 60	$C_{18}H_{34}O_2$	282	84	cis-Vaccenic acid

20	17.699	1. 08	$C_{16}H_{33}NO$	255	92	Hexadecanamide
21	19.425	0. 89	$C_{21}H_{38}O_4$	354	74	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl
22	19.508	12.11	$C_{18}H_{35}NO$	281	94	9-Octadecenamide, (Z)-
23	19.750	1. 13	$C_{18}H_{37}NO$	283	90	Octadecanamide
24	20.821	0. 98	$C_{25}H_{42}$	342	64	1H-Indene, 1-hexadecyl-2,3-dihydro-
25	20.992	0. 55	$C_{20}H_{26}O_2$	298	36	Norethindrone
26	22.247	1. 78	$C_{18}H_{23}N$	253	59	3-(2,6-Dimethylocta-2,7-dienyl)-1H-indole
27	22.442	1. 58	$C_8H_{15}NO_6$	221	49	.alpha.-D-Glucopyranosiduronamide, methyl 3-O-methyl
28	22.767	1. 38	$C_{28}H_{52}$	388	46	4,7-Methano-1H-indene, octahydro-5-(2-octyldecyl)-
29	22.892	0. 81	$C_{11}H_{16}Cl_2O_2$	250	47	Dichloroacetic acid, 2-methyloct-5-yn-4-yl ester
30	23.103	2. 95	$C_{21}H_{36}O_2$	320	70	i-Propyl 9,12,15-octadecatrienoate
31	23.454	0. 50	$C_{25}H_{36}O_5$	416	51	Pregn-5-en-20-one, 3,21-bis(acetyloxy)-, (3.beta.)-
32	24.053	0. 54	$C_{27}H_{48}O$	388	54	Cholestanol
33	24.392	0. 52	$C_{21}H_{36}Cl_2O_2Si$	418	45	Silane, dimethyl(2,5-dichlorophenyloxy) tridecyloxy-

Table 7: Antibacterial activity of the fractions of methanolic leaf extract of *Jatropha tanjorensis*

		Concentrations (mg/mL)/ zone of inhibition (mm)					Controls	
Plant fractions	Isolate	100	50	25	12.5	6.25	CPC	DMSO
n-hexane	*P. mirabilis*	22.5±0.5	18.5±0.7	14.5±0.0	11±0.5	9±0.0	27±0.0	0±0.0
	S. typhi	21±0.0	17.0±0.	14±0.5	12±0.5	8.5±0.5	29±0.0	0±0.0
	E. coli	22.5±0.5	18±0.0	16±0.0	13±0.0	11±0.0	24±0.5	0±0.0
	S. aureus	20±0.0	18.5±0.0	15±0.0	13.5±0.0	11±0.0	22±0.0	0±0.0
Dichloromethane	*P. mirabilis*	15.5±0.5	14±0.7	12.0±0.0	10±0.5	8±0.0	26±0.0	0±0.0
	E. coli	20±0.0	18±0.0	16.5±0.5	13.5±0.5	11.5±0.5	28±0.5	0±0.0
	S. typhi	13.5±0.5	11.5±0.5	9.5±0.5	8±0.0	0±0.0	21±0.0	0±0.0
	S. aureus	11.5±0.5	9±0.0	0±0.0	0±0.0	0±0.0	20±0.5	0±0.0
Ethyl acetate	*P. mirabilis*	19±0.5	17.5±0.7	15±0.0	13±0.5	10±0.0	25±0.0	0±0.0
	E. coli	20±0.0	17.0±0.0	15.5±0.5	13.5±0.5	11.5±0.5	29±0.0	0±0.0
	S. typhi	19.5±0.5	16±0.0	14±0.0	12±0.0	10±0.0	24±0.5	0±0.0
	S. aureus	18.5±0.5	15±0.0	12±0.0	10±0.0	0±0.0	22±0.0	0±0.0
Aqueous	*E. coli*	18±0.5	15±0.7	13.5±0.0	11±0.5	0±0.0	27±0.0	0±0.0
	S. typhi	14±0.0	12.0±0.0	9.5±0.5	0±0.0	0±0.0	30±0.5	0±0.0
	P. mirabilis	15.5±0.5	13.5±0.5	10.5±0.5	0±0.0	0±0.0	23±0.0	0±0.0
	S. aureus	13.5±0.5	11±0.0	0±0.0	0±0.0	0±0.0	23±0.0	0±0.0

Values are mean of three replicates DMSO – dimethyl sulphur oxide; CPC – chloramphenicol; ± - mean standard deviation; mg/mL – milligram per millitre; mm - millimetre

Table 8: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of fractions of *J. tanjorensis* on clinical bacterial isolates

Plant fraction	Isolate	MIC (mg/mL)	MBC (mg/mL)
n-hexane	*E. coli*	12.5	25.0
	S. typhi	25.0	50.0
	P. mirabilis	25.0	50.0
	S. aureus	50.0	100.0
Dichloromethane	*P. mirabilis*	25.0	100.0
	E. coli	12.5	25.0
	S. aureus	100.0	0. 0
	S. typhi	50.0	0. 0
Ethyl acetate	*E. coli*	12.5	25.0
	S. typhi	12.5	50.0
	P. mirabilis	12.5	25.0
	S. aureus	12.5	50.0
Aqueous	*E. coli*	25.0	100.0
	S. typhi	100.0	0. 0
	S. aureus	100.0	0. 0
	P. mirabilis	100.0	0. 0

mg/mL – milligram per millitre

Table 9: Antibiofilm activity of the fractions of *Jatropha tanjorensis* against biofilm formation by clinical bacterial isolates

		Optical Density (OD_{600}nm) / Concentration (mg/mL)					Controls	
Plant fractions	**Isolate**	**100**	**50**	**25**	**12.5**	**6.25**	+	-
n-hexane	*E. coli*	0.014	0.044	0.297	0.417	0.532	1.624	0.000
	P. mirabilis	0.033	0.038	0.286	0.452	0.556	1.598	0.000
	S. typhi	0.039	0.047	0.388	0.463	0.558	1.624	0.000
	S. aureus	0.040	0.079	0.477	0.596	0.663	1.623	0.000
Dichloromethane	*E. coli*	0.016	0.038	0.396	0.417	0.532	1.624	0.000
	P. mirabilis	0.032	0.039	0.396	0.423	0.536	1.633	0.000
	S. typhi	0.035	0.038	0.397	0.422	0.565	1.625	0.000
	S. aureus	0.037	0.049	0.397	0.497	0.665	1.598	0.000
Ethyl acetate	*E. coli*	0.017	0.038	0.396	0.417	0.532	1.627	0.000
	P. mirabilis	0.033	0.039	0.396	0.413	0.576	1.625	0.000
	S. typhi	0.035	0.038	0.397	0.423	0.555	1.621	0.000
	S. aureus	0.036	0.048	0.396	0.428	0.655	1.622	0.000
Aqueous	*E. coli*	0.011	0.035	0.096	0.317	0.532	1.623	0.000
	P. mirabilis	0.448	0.477	0.496	0.513	0.576	1.644	0.000
	S. typhi	0.446	0.476	0.497	0.525	0.588	1.611	0.000
	S. aureus	0.549	0.569	0.591	0.620	0.669	1.633	0.000

+ = Positive control (bacterial suspension in broth); - = negative control (fraction in broth); nm = nanometre

Discussion

The findings of this study revealed that *Jatropha tanjorensis* possesses a rich composition of bioactive phytochemicals with significant antibacterial and antibiofilm properties. The fractionation of the leaf extracts yielded varying quantities, with the n-hexane fraction recording the highest recovery rate (70.51%), while the aqueous fraction showed the lowest yield (63.71%). This variation may be attributed to the polarity differences of solvents used in the extraction process, which influence the solubility of phytochemicals (Evans, 2009). Similar trends have been reported in studies where non-polar solvents such as n-hexane extracted more lipophilic compounds compared to polar solvents like water (Sofowora, 1993).

Phytochemical screening indicated the presence of saponins, tannins, flavonoids, alkaloids, triterpenes, steroids, cardiac glycosides, and anthraquinones in varying combinations across the fractions. These compounds are known to contribute to the antimicrobial properties of plants. For instance, saponins and flavonoids disrupt microbial cell membranes, while tannins and alkaloids inhibit enzymatic activities essential for bacterial survival (Fawehinmi et al., 2013; Musini & Giri, 2019). The broad phytochemical diversity observed suggests that *J. tanjorensis* contains multiple metabolites that may act synergistically against microbial infections.

The GC–MS analysis further identified several key compounds such as n-Hexadecanoic acid, Phytol, Benzenedicarboxylic acid, and 9-Octadecanamide, which have previously been reported for their antibacterial, antioxidant, and anti-inflammatory activities (Boxi et al., 2010; Shah et al., 2020). The predominance of fatty acids and terpenoids in the n-hexane and dichloromethane fractions aligns with findings from Elinge et al. (2020), who reported similar compounds in *J. tanjorensis* extracts with broad antimicrobial activity. The identification of these compounds supports the traditional use of this plant for treating infections and wounds (Babayemi et al., 2021; Obum-Nnadi et al., 2022).

The antibacterial assay demonstrated that all fractions exhibited inhibitory effects against the tested pathogens—*E. coli*, *P. mirabilis*, *S. typhi*, and *S. aureus*—though the degree of inhibition varied by solvent fraction and concentration. The n-hexane and ethyl acetate fractions showed the strongest antibacterial effects, producing zones of inhibition up to 22.5 mm. This high activity may be attributed to the abundance of non-polar and semi-polar bioactive constituents such as fatty acids and phenolic derivatives that can readily penetrate bacterial membranes (Aiyegoro et al., 2019; Abdallah & Ali, 2019). A similar trend of concentration-dependent inhibition has been observed in other medicinal plants, indicating that higher doses enhance bactericidal efficiency (Ndip et al., 2009; Daniyan et al., 2018).

The MIC and MBC results further confirmed the potency of the n-hexane and ethyl acetate fractions, which displayed lower inhibitory and bactericidal concentrations compared to the aqueous fraction. The ethyl acetate fraction, in particular, exhibited the lowest MIC (12.5 mg/mL) and MBC (25 mg/mL) values against *E. coli* and *P. mirabilis*, suggesting a strong bactericidal effect. These findings corroborate those of Udoh et al. (2024), who reported potent antibacterial effects of *J. tanjorensis* against similar Gram-negative bacteria. The limited activity of the aqueous fraction may be due to the poor extraction efficiency of non-polar compounds, which are often responsible for antibacterial actions (Muktar & Tukur, 2000).

Biofilm inhibition assays showed that all fractions reduced biofilm formation in a concentration-dependent manner. Notably, the n-hexane fraction recorded the most pronounced antibiofilm effect, with the lowest optical density (0.014 OD) observed against *E. coli*. This finding suggests that the non-polar phytochemicals in this fraction disrupt

quorum sensing and inhibit bacterial adherence—key steps in biofilm formation (O'Toole & Kolter, 1998; Piegerova et al., 2019). The ability of *J. tanjorensis* fractions to inhibit biofilm formation aligns with previous reports highlighting the role of plant-derived compounds in combating chronic infections caused by biofilm-forming bacteria (Sofy et al., 2020; Anderson & O'Toole, 2018).

In general, the combined findings demonstrate that *J. tanjorensis* is a promising source of natural antibacterial and antibiofilm agents. Its activity against both Gram-positive and Gram-negative bacteria underscores its therapeutic potential in managing infections, particularly those associated with multidrug-resistant pathogens (Frieri et al., 2017; WHO, 2014).

Conclusion

This study confirms that *Jatropha tanjorensis* possesses significant antibacterial and antibiofilm activities, largely attributed to its rich content of phytochemical constituents, including fatty acids, terpenoids, and phenolic compounds. The n-hexane and ethyl acetate fractions exhibited the most potent antibacterial and antibiofilm effects, suggesting that bioactive metabolites within these fractions could serve as potential leads for developing new antimicrobial agents. The concentration-dependent inhibitory effects observed in both planktonic and biofilm assays emphasize the importance of dosage in maximizing therapeutic outcomes. Given the growing global concern over antibiotic resistance, *J. tanjorensis* presents a valuable natural alternative that warrants further purification, characterization, and *in vivo* evaluation to explore its clinical relevance.

Competing Interests

The authors declare that they have no competing interests.

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References

Abdallah, M. S., & Ali, M. (2019). Antibacterial activity of leaf and stem bark extracts of *Adansonia digitata* against *Escherichia coli* and *Salmonella typhi* grown in Potiskum, Yobe State, Nigeria. *International Journal of Research in Business Studies and Management, 2*(1), 1–7.

Ahmed, R. S., Akram, A. A., El-Shahat, E., Heba, A. A., & Ahmed, E. A. (2020). Evaluation of antimicrobial and antibiofilm activity of new antimicrobials as an urgent need to counteract stubborn multidrug-resistant bacteria. *Journal of Pure and Applied Microbiology, 14*(1), 595–608.

Aiyegoro, O. A., Afolayan, A. J., & Okoh, A. I. (2009). In vitro antibacterial activities of crude extracts of the leaves of *Helichrysum longifolium* in combination with selected antibiotics. *African Journal of Pharmacy and Pharmacology, 3*(6), 293–300.

Al-Rifai, A. A., Aqel, A., Al-Warhi, T., Wabaidur, S. M., Al-Othman, Z. A., & Badjah-Hadj-Ahmed, Y. (2017). Antibacterial and antioxidant activity of ethanolic plant extracts of some *Convolvulus* species and their DART-ToF-MS profiling. *Evidence-Based Complementary and Alternative Medicine, 2017*, 5694305. https://doi.org/10.1155/2017/5694305

Anderson, G. G., & O'Toole, G. A. (2008). Innate and induced resistance mechanisms of bacterial biofilms. In T. Romeo (Ed.), *Bacterial biofilms* (Current Topics in Microbiology and Immunology, Vol. 322, pp. 85–105). Springer. https://doi.org/10.1007/978-3-540-75418-3_5

Ashish, K. N., Kunjabihari, S., & Sabuja, S. (2016). Comparative studies on the phytochemistry, antimicrobial and antioxidant activities of *Jatropha* species (*J. curcas* L. and *J. gossypifolia* L.) of Odisha. *International Journal of Pharmacognosy and Phytochemical Research, 8*(10), 1614–1624.

Babayemi, O. O., Oke, E. A., & Bayode, M. T. (2021). Antibacterial activity of *Jatropha tanjorensis* leaf extracts against bacteria associated with wound infections from the clinical setting. *Nusantara Bioscience, 13*, 239–246. https://doi.org/10.13057/nusbiosci/n130215

Bodunrinde, R. E., Oladunmoye, M. K., Bayode, M. T., Adetunji, C. O., & Gabriel, P. O. (2020). Synergistic antibacterial activity of *Hibiscus sabdariffa*, honey and ciprofloxacin on selected uropathogens. *Archives of Science and Technology, 1*, 75–87.

Boxi, M., Rajesh, Y., Kumar, V. R., Praveen, B., & Mangamma, K. (2010). Extraction, phytochemical screening and in vitro evaluation of antioxidant properties of *Commicarpus chinensis* (aqueous leaf extract). *International Journal of Pharma and Bio Science, 1*(4), 537–547.

Braun, V., & Clarke, V. (2006). Using thematic analysis in psychology. *Qualitative Research in Psychology, 3*(2), 77–101. https://doi.org/10.1191/1478088706qp063oa

CLSI. (2020). *Performance standards for antimicrobial susceptibility testing* (Approved standard M100-17, 27[1]). National Committee for Clinical Laboratory Standards.

Daniyan, S. Y., Ukubuiwe, C. C., Ukubuiwe, A. C., Oluwafemi, O. J., & Chukwudi, P. O. (2018). Antibacterial activities of leaf extracts of *Jatropha tanjorensis* Ellis and Saroja (Euphorbiaceae). *Medicinal Plant Research, 8*(4), 21–26.

Datsugwai, M. S. S., & Yusuf, A. S. (2017). Phytochemical analysis and antimicrobial activity of baobab (*Adansonia digitata*) leaves and stem bark extracts on *Staphylococcus aureus* and *Escherichia coli*. *Journal of Bioscience and Biotechnology, 6*(1), 9–16.

Elinge, C. M., Yanah, Y. M., Habiba, A., Obaro, I. O., Ogunleye, A. O., Yusuf, H., & Elinge, R. I. (2020). Phytochemical screening and antimicrobial activity of ethanolic leaves and stem bark extract of *Jatropha tanjorensis*. *Direct Research Journal of Health and Pharmacology, 8*(1), 7–13.

Evans, W. C. (2009). *Trease & Evans' pharmacognosy* (16th ed.). Saunders.

Fawehinmi, A. B., Lawal, H., Etatuvie, S. O., & Oyedeji, F. O. (2013). Preliminary phytochemical screening and antimicrobial evaluation of four medicinal plants traditionally used in Nigeria for skin infection. *African Journal of Pure and Applied Chemistry, 7*(2), 44–49.

Frieri, M., Kumar, K., & Boutin, A. (2017). Antibiotic resistance. *Journal of Infection and Public Health, 10*(4), 369–378. https://doi.org/10.1016/j.jiph.2016.08.007

Jamal, M., Ahmad, W., Andleeb, S., Jalil, F., Imran, M., Nawaz, M. A., Hussain, T., Ali, M., Rafiq, M., & Kamil, M. A. (2018). Bacteria biofilm and associated infections. *Journal of the Chinese Medical Association, 81*(1), 7–11. https://doi.org/10.1016/j.jcma.2017.07.012

Kumar, C. M. K., Yugandhar, P., & Savithramma, N. (2016). Biological synthesis of silver nanoparticles from *Adansonia digitata* L. fruit pulp extract, characterization, and its antimicrobial properties. *Journal of Intercultural Ethnopharmacology, 5*(1), 79–85.

Muktar, M. D., & Tukur, A. (2000). In vitro screening for antimicrobial activity of *Pistia stratiotes* L. extract. *Journal of Experimental Biology, 1*(1), 59–69.

Musini, A., & Giri, A. (2019). Investigation of mode of action of antibacterial activity of *Salacia oblonga* extract against drug-resistant pathogens. *Brazilian Archives of Biology and Technology, 62*, e19180051.

Ndip, R. N., Ajonglefac, A. N., Wirna, T., Luma, H. N., Wirmum, C., & Efange, S. M. (2009). In vitro antimicrobial activity of *Ageratum conyzoides* (Linn) on clinical isolates of *Helicobacter pylori*. *African Journal of Pharmacy and Pharmacology, 3*(11), 585–592.

Novy, P., Davidova, H., Serrano-Rojero, C. S., Rondevaldova, J., Pulkrabek, J., & Kokoska, L. (2015). Composition and antimicrobial activity of *Euphrasia rostkoviana* Heyne essential oil. *Evidence-Based Complementary and Alternative Medicine, 2015*, 734101. https://doi.org/10.1155/2015/734101

Obum-Nnadi, C. N., Fredrick, C. C., Ezenwa, C. M., Amaechi, D., Ozoemena, N., & Ohabughiro, N. (2022). Assessment of antibacterial properties of *Jatropha tanjorensis*. *Asian Journal of Applied Chemistry Research, 12*(4), 13–19.

O'Toole, G. A., & Kolter, R. (1998). Initiation of biofilm formation in *Pseudomonas fluorescens* WCS365 proceeds via multiple, convergent signaling pathways: A genetic analysis. *Molecular Microbiology, 28*(3), 449–461. https://doi.org/10.1046/j.1365-2958.1998.00797.x

Oyebode, O., Kandala, N., Chilton, P. J., & Lilford, R. J. (2016). Use of traditional medicine in middle-income countries: A WHO-SAGE study. *Health Policy and Planning, 31*(8), 984–991. https://doi.org/10.1093/heapol/czw022

Petruzzi, B., Ra, D., Leraith, T., Np, E., Fw, P., & Tj, L. (2018). Biofilm formation and avian immune response following experimental acute and chronic avian cholera due to *Pasteurella multocida*. *Veterinary Microbiology, 222*, 114–123.

Piegerova, A., Koscovia, J., Schusterova, P., Nemcova, R., & Kryvtsova, M. (2019). In vitro inhibition of biofilm formation by *Staphylococcus aureus* under the action of selected plant extracts. *Folia Veterinaria, 63*(1), 48–53.

Rajkumari, J., Bhattacharjee, A., & Bora, S. S. (2019). Biofilm inhibition and antibiofilm activity of phytochemicals against clinical isolates. *Journal of Applied Microbiology, 126*(3), 866–879. https://doi.org/10.1111/jam.14176

Sadiq, M. B., Taming, J., Cho, T. Z. A., & Anal, A. K. (2017). Antibacterial activities and possible mode of action of *Acacia nilotica* (L.) Del. against multidrug-resistant *Escherichia coli* and *Salmonella*. *Molecules, 22*(1), 47. https://doi.org/10.3390/molecules22010047

Samatha, T., Shama, N., Chandrakala, G., Thirupathi, K., & Rama, S. N. (2017). Antibacterial activity of *Adansonia digitata* L.: A globally endangered tree. *International Journal of Pharmacognosy and Phytochemical Research, 9*(11), 1410–1413.

Shah, S., Dhanani, T., Sharma, S., Singh, R., Kumar, S., Kumar, B., Srivastava, S., Ghosh, S., Kumar, R., & Juliet, S. (2020). Development and validation of a reversed-phase high-performance liquid chromatography–photodiode array detection method for simultaneous identification and quantification of coumarin, precocene-I, β-caryophyllene oxide, α-humulene, and β-caryophyllene in *Ageratum conyzoides* extracts and essential oils. *Journal of AOAC International, 103*(3), 857–864. https://doi.org/10.1093/jaoacint/qsz038

Sofowora, E. A. (1984). *Medicinal plants and traditional medicine in Africa* (4th ed.). John Wiley & Sons.

Sofy, A. R., Aboseldah, A. A., El Morsi, E. S., Azmy, H. A., & Ahmed, A. A. (2020). Evaluation of antibacterial and antibiofilm activity of new antimicrobials as an urgent need to counteract stubborn multidrug-resistant bacteria. *Journal of Pure and Applied Microbiology, 14*(1), 595–608. https://doi.org/10.22207/JPAM.14.1.62

Sule, I. O., & Agbabiaka, T. O. (2008). Antibacterial effect of some plant extracts on selected Enterobacteriaceae. *Ethnobotanical Leaflets, 12*, 1035–1042.

Udoh, I. P., Eze, C. U., & Berebon, D. P. (2024). Antibacterial activity of aqueous and methanol extracts of *Jatropha tanjorensis* and *Adansonia digitata* against selected clinical bacteria pathogens. *Scientia Africana, 23*(2), 403–414.

World Health Organization. (2014). *Antibiotic resistance: Global threat to medicine* (Technical Report Series, 98[67], 876–884).