Phytochemical Analyses and Antibacterial Activities of *Jatropha tanjorensis* J. L. Ellis and Saroja Leaves Extract Against Selected Clinical Pathogens

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ABSTRACT

Aim: This study aimed to determine the phytochemical and antimicrobial properties of *Jatropha tanjorensis*leaf extracts on some selected clinical pathogens.
Methods: Fresh *Jatropha tanjorensis* leaves were collected, identified, dried at room temperature and finely ground. Two hundred grammes of the leaves were macerated in 1000ml of solvent to obtain the extracts. Phytochemical analyses of the extracts were done using appropriate standard methods. The leaf extracts and antibiotic sensitivity were evaluated against *Staphylococcus aureus, Bacillus subtilis, Enterococcus faecalis Escherichia coli, Bacillus subtilis* ATCC 6633*, Staphylococcus aureus* ATCC 25923*, Salmonella typhi* and *Pseudomonas aeruginosa* obtained from the stock culture of Microbiology Laboratory in LUTH and Bells University of Technologyusing the agar well diffusion method.
Results: The results of the qualitative phytochemical analysis revealed the presence of saponin, alkaloids, phenols, flavonoids, reducing sugars, terpernoids, steroids and tannins, whilephlobatannins was absent. Quantitative phytochemical analysis revealed that flavonoids from hot water extract had the highest quantity of 98.55mg/100g and reducing sugars from methanolic extract had the lowest quantity of 20.39mg/100g. The cold-water extract showed the highest inhibitory effect with the zone of inhibition of 19.83mm against *S. aureus,* while methanolic extract showed the lowest zone of inhibition of 13.17mm against *B. subtilis.* The lowest MIC value 10.24mg/ml was obtained against *B. subtilis* while the highest MIC value 20.48mg/ml was observed against other isolates. The GC-MS analysis revealed the presence of 57 bioactive compounds with 1,2,3-Benzenetriol having the highest percentage of 66.38%.
Conclusion: It can be inferred from this study, that*Jatropha tanjorensis*has the potential of a prospective antibacterial drug, although there is still need for extensive and synergistic study.

Keywords: Jatropha tanjorensis; antibacterial; bioactive compounds; extraction;clinical pathogens.

1. INTRODUCTION

"A major global public health problem is resistance to antimicrobial agents. Although a number of new antibiotics have been produced by pharmacological industries in the last three decades, microorganism resistance to these drugs has increased" [1]. "Incidents attributable to drug resistant microorganisms and the emergence of unknown disease-causing microbes, raised enormous public health concern despite the progress made in understanding microorganisms and their regulation in developed nations" [2]."In addition, some antibiotics have extreme unwanted side effects that restrict their use, so there is a pressing need to develop new antimicrobial agents with minimal unwanted side effects that are very successful. There is an endless search for new novel antimicrobials and a need to combat the incessant resistance of microorganisms to most antibiotics and plants as a potential source of novel prototypes of antibiotics. Bioactive compounds within plants are responsible for their medicinal benefit, hence the phytochemical screening of the *Jatropha tajonrensis* plant" [3].

"Medicinal plants are key sources of bio-active compounds for herbal medicine, antibiotics, antioxidants, and pharmaceutical drugs [4].Natural plants contain antibacterial and phytochemical properties similar to synthetic antibiotics and therefore have been used in traditional medicine to treat infections [5].The use of traditional medicines in treatment and management of various disease is now encouraged by World Health Organization (WHO) due to their ready availability, cost effectiveness and high potency against some diseases [1] and there has been a great deal of interest in

the investigation of various extracts obtained from traditional medicinal plants as prospective sources of new antimicrobial agents over the past two decades" [6].

"*J. tanjorensis* is a perennial herb that belongs to the family Euphorbiaceae;common name includes: "Jatropha", "Hospital too far", "Catholic vegetables" and "Iyana Ipaja" in Yoruba language [7]. The name "Jatropha" is derived from the Greek word "Jatros"(Doctor) and "trophe"(food) which connotes its medicinal uses" [8].
"*Jatropha tanjorensis* is a multipurpose plant, cultivated for medicinal applications and used as food. Virtually every part of the plant is beneficial and nutritional in various ways. Its many benefits depend on which part of the plant is being used. The leaves are commonly cooked and eaten like vegetables such as fluted pumpkin leaves (Ugu) and spinach"[9].

"*Jatropha tanjorensis* leaves are consumed in Nigeria as soups and as a tonic with the claim that it increases blood volume. The leaves are also employed traditionally in the treatment of diabetes and cardiovascular diseases [10]. Extracts from the plant leaves have also been used in Nigeria to control sickle cell anaemia[11].Jatropha leaves are reported to be rich in beta blockers, anti-cancer agents, anti-anaemic, anti-microbial, anti-plasmodial and antioxidant effects caused by malaria parasite oxidative stress [12]. The leaf of *Jatropha tanjorensis* has an anti-anaemic effect (potential for blood replenishment). Some essential biogenic principles that are important for rapid haemopoiesis in the bone marrow were found to contain the leaf [13]. In Southwest Nigeria, infusion of *Jatropha tanjorensis* leaves is administered orally for diabetic symptoms. Three leaf extract fractions were assessed for their anti-diabetic potentials [14]. Hexane, chloroform and methanol leaf extracts exhibited varying degree of antimicrobial and anti-inflammatory activities" [15]. "Less study has been documented on *Jatropha tanjorensis* unlike other *Jatropha* species e.g*Jatropha curcas*. Hence this study aims to help clinicians in seeing the antimicrobial properties of the *Jatropha tajonrensis* leaves and the existence of highly potential, recognizable phytochemical and active compounds can be identified and thus, find their way into the manufacture of antimicrobial drugs".

2. METHODOLOGY

2.1 Collection of *Jatropha tanjorensis* leaves

Fresh *Jatropha tanjorensis* leaves were collected from a mini household farm in Federal College of Education, Osiele, Abeokuta, Ogun State, Nigeria and was identified by a Botanist, resident in the Department of Biological Sciences, Bells University of Technology, Ota, Ogun State. The leaves were dried under shade for about a month within a temperature range of 26-28°C and ground into fine powder with an electric blender. The pulverized plant material was kept in an air-tight plastic container for further use.The extraction method adopted was maceration using cold distilled water, warm distilled water and 100% methanol for 72hours.

2.2 Preparation of Plant Extract

A quantity of 200 g of the finely ground *J. tanjorensis* leaves were macerated in each solvent in a sterile clean plastic container for 72hours with frequent agitation, after which it was first filtered with muslin cloth, and then with Whatman No1 filter paper into a clean volumetric flask and the filtrates were concentrated using the rotary evaporator. The dried crude extracts were stored in air-tight containers.

2.2 Sterility Test of the Plant Extracts

One millilitre of each extract (methanolic, hot and cold water) was inoculated onto nutrient agar plates to determine its sterility and incubated at 37°C for 24 hours. The absence of microbial growth on the plates after incubation demonstrated the sterility of the extracts.

2.3 Test Organisms (Clinical Pathogens) Used

The test bacteria pathogens assayed in this study were Laboratory strains which include Clinical and Standard strains of *Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcusfaecalis* FELAO97, *Escherichia coli* NCTC 10418, *Bacillus subtilis* ATCC 6633,*Staphylococcus aureus* ATCC 25923, *Salmonella typhi* ATCC 13311 and *Pseudomonasaeruginosa* ATCC 27853 obtained from the stock culture of the Microbiology Laboratory in Lagos University Teaching Hospital(LUTH) and Bells University of Technology. They were primarily isolated on various diagnostic and selective media to suppress other contaminants. They were then subcultured unto a neutral medium; Mueller-Hinton Agar, to remove the effects of indicators and suppressive and selective chemical agents in primary isolation culture media. They also subcultured into sterile nutrient broth for optical density adjustment. Organisms were incubated for a period of 24 hours at 37 ^{o}C.

2.4 Standardization of Inoculum / Preparation of Bacterial Suspension

The bacterial biomass of the assay organisms was adjusted using a reference standard suspension. All the bacterial turbidities were adjusted using sterile distilled water. A loopful of the bacterial liquid cultures was added to the distilled water and the turbidity was matched with that of 0.5 McFarland turbidity standards which is equivalent to 10^8 CFU/ml. If the suspension was less turbid than that of the standard, then more isolates was added, but if more turbid, then more sterile distilled water were added so it could be as turbid as the standard. The adjusted suspension was used for the assay.

2.5 Preparation of Mcfarland Standard Solution

One millilitre of concentrated hydrogen tetraoxosulphate (VI)acid(H_2SO_4) was added to 99ml of distilled water to prepare a 1%v/v solution of the acid, the solution was mixed properly. Then 0.5 g of dihydrate barium chloride ($BaCl_2.2H_2O$) was added to50ml of sterile distilled water to prepare 1% w/v of barium chloride. Thereafter,0.6ml of the barium chloride solution was added to 99.4ml of the sulphuric acid solution, and then mixed properly. 9ml of the turbid solution was transferred into a test tube of the same type used in preparing the test inoculum.

2.6 Sample (Extract) Reconstitution

There were three working samples, 500 mg/ml, 250mg/ml, and 125mg/ml which were achieved by weighing 5g of extract and dissolving in sterile distilled water as solvent and then further diluting twice to have other working values.

2.7 Antibacterial Susceptibility Test

The test bacteria were reconstituted to match the turbidity of 0.5 McFarland standards to be inoculated on Mueller-Hinton agar (Kumar *et al.,* 2012) [8]. The medium (Mueller-Hinton Agar) which was prepared for assaying was maintained at 45^OC so as to preserve molten nature until it was needed. Test organisms were mixed in vortex apparatus to homogenize the assay organisms. 1ml portion of standardized organisms was seeded into the 25ml agar and was mixed thoroughly using the roll-palm method before pouring in Petri dish. Agars were allowed to set and organised for cork boring.

After allowing all the seeded agars to solidify, a cork borer, size 10 mm diameter was used for boring the wells. It was flamed and allowed to cool before using it to bore a hole in each of the sectors of the Petri dishes. All the cut portions were carefully ejected and thrown into a dish of disinfectant. 150 µl portions of various working concentrations (500mg/ml, 250mg/ml and 125mg/ml) was dispensed into the wells and allowed to stand for 4 hours for diffusion to take place before incubation.In order to allow leaf extracts to diffuse through the agar media to form inhibition zones, plates were incubated uprightly for 24 hours at 37 ^OC [16]. Using a zone reader (ruler), the diameters of the inhibition zones for various leaf extract concentrations against different bacteria were measured in millimetres. All the antimicrobial tests were tripled, and the mean values were collected.

2.8 Antibiotic Standard (Ciprofloxacin) Preparation

Four working standards of pure Ciprofloxacin: 20µg/ml, 10µg/ml, 5µg/ml and 2.5µg/ml solutions of the standard solutions were achieved by weighing, dissolving and diluting the standard.

2.9 Determination of Minimum Inhibitory Concentration

This was carried out by adopting the prescribed method of the Clinical and Laboratory Standard institute[17]. Thirteen working concentrations of the samples were prescribed and were used in order to establish the minimum concentration that inhibited a particular assay organism that responded to the extracts. The concentrations were 0.005, 0.01, 0.02, 0.04, 0.08, 0.16, 0.32, 0.64, 1.28, 2.56, 5.12, 10.24, and 20.48mg/ml. Agar dilution technique of Hugo and Russell [18] was used because of the colourful nature of the extracts. To achieve these working concentrations, three different stock concentrations were prepared from which the various working concentrations were also prepared. These stocks concentrations were 10mg/ml, 20mg/ml, and 40mg/ml.

One millilitre portion of calibrated test organisms that responded to the extracts (*Staphylococcus aureus, Enterococcus faecalis, Bacillus subtilis*) was seeded into the 25ml molten agar and was mixed thoroughly using the roll-palm method before pouring in thirteen Petri dishes(containing the thirteen working concentrations). Each Petri dish was then divided into 3 compartments using a marker and each compartment had the test organisms each in a small ring. The plates were then incubated for 72hours at 24^oC.The lowest concentration of the extract that inhibited the visible growth of the test organisms is the minimum inhibitoryconcentration (MIC) [19].

2.10 Qualitative Phytochemical Screening of Leaf Extracts of *Jatropha tanjorensis*

Qualitative analysis was carried out to ascertain the presence of the different phytochemical compounds contained in the extract. The extracts were subjected to thin-layer and paper chromatographic procedures at the laboratory. This was done in order to separate the components into individual compounds for appropriate identification of all components of the

extracts. Preliminary phytochemical analysis of the extracts was carried out with reference to the standard methods of Sofowora[20),[21]. The extract solution was then used for phytochemical screening.

2.11 GC-MS Analysis (Gas Chromatography-Mass Spectrometry)

GC-MS extract analysis was performed with the GC-MS Clarus 500 Perkin Elmer method and gas chromatography interfaced with a mass spectrometer (GC-MS) using the following conditions: column Elite-1 fused capillary silica column (30 mm x 025 mm ID x 1 mdf, consisting of 100% Dimethyl poly silaxane), running in 70eV electron impact mode; helium (99.999%) was used in a carrier. The temperature of the oven was programmed from 110^{o}C (isothermal for 2min), with a rise of 10^{o}C / min to 200^{o}C from 5^{o}C / min to 280^{o}C, and isothermal at 280^{o}C for 9 minutes. At 70 eV, mass spectra were recorded: a scan interval of 0.5 seconds and 40 to 550 Da fragments. The average runtime for the GCMS was 36 minutes [22]. Through the National Institute of Standards and Technology (NIST) library, compounds were established.

4.0 RESULTS

4.1 Antibacterial Screening of Methanolic and Aqueous Extract of *Jatropha tanjorensis*

The results of the antibacterial screening of methanolic and aqueous (cold and hot) extracts of *Jatropha tanjorensis*leaves on *Staphylococcus aureus* ATCC *25923, Enterococcus faecalis* FELAO97, *Salmonella typhi* ATCC13311*, Staphylococcus aureus* (clinical isolate), *Escherichia coliNCTC 10418*, *Pseudomonas aeruginosa ATCC 27853, Bacillus subtilis* (clinical strains)*, Bacillus subtilis ATCC 6633* showed that there was minimal activity of the plant on the test organisms as represented in table 1 to 3. From this study, it was observed that the methanolic extract had effect on *Bacillus subtilis* (clinical isolate) and no effect on all other test organisms (table 2). The hot water extract of the plant showed no activity on all test organisms as depicted in table 4, while the cold-water extract had better activity on some of the test organism as depicted in Table 3.

Table 1: The antibacterial activities of methanolic extract of *Jatropha tanjorensis*leaves against bacterial test organisms

ASSAY ORGANISM	500mg/ml	250mg/ml	125mg/ml	S0LVENT
	Zone of Inhibition (mm)			
Staphylococcus aureus ATCC 25923	N.I	N.I	N.I	N.I
Bacillius subtilis clinical strain	13.17±0.28	0.00	0.00	0.00
Escherichia coli NCTC 10418	N.I	N.I	N.I	N.I
Pseudomonas aeruginosa ATCC 27853	N.I	N.I	N.I	N.I
Salmonella typhi ATCC 13311	N.I	N.I	N.I	N.I
Staphylococcus aureus Clinical strain	N.I	N.I	N.I	N.I
Enterococcus faecalis FELAO97	N.I	N.I	N.I	N.I
Bacillus subtilis ATCC 6633	N.I	N.I	N.I	N.I

Key: N.I= No Inhibition

Table 2: The antibacterial activities of cold-water extract of *Jatropha tanjorensis*leaves against bacterial test organisms

ASSAY ORGANISM	500mg/ml	250mg/ml	125mg/ml	S0LVENT
	Zone of inhibition (mm)			
Staphylococcus aureus ATCC 25923	N.I	N.I	N.I	N.I
Bacillus subtilis ATCC 6633	N.I	N.I	N.I	N.I
Escherichia coli NCTC 10418	N.I	N.I	N.I	N.I
Pseudomonas aeruginosa ATCC 27853	N.I	N.I	N.I	N.I
Salmonella typhi ATCC 13311	N.I	N.I	N.I	N.I
Staphylococcus aureus Clinical strain	19.83±0.76	N.I	N.I	N.I
Enterococcus faecalis FELAO97	14.17±0.29	N.I	N.I	N.I
Bacillius subtilis clinical strain	17.67±0.56	N.I	N.I	N.I

Key: N.I= No Inhibition

Table 3: The antibacterial activities of hot water extract of *Jatropha tanjorensis*leaves against bacterial test organisms

ASSAY ORGANISM	500mg/ml	250mg/ml	125mg/ml	S0LVENT
	Zone of inhibition(mm)			
Staphylococcus aureus ATCC 25923	N.I	N.I	N.I	N.I
Bacillus subtilis ATCC 6633	N.I	N.I	N.I	N.I
Escherichia coli NCTC 10418	N.I	N.I	N.I	N.I
Pseudomonas aeruginosa ATCC 27853	N.I	N.I	N.I	N.I
Salmonella typhi ATCC 13311	N.I	N.I	N.I	N.I
Staphylococcus aureus	N.I	N.I	N.I	N.I

Clinical strain				
Enterococcus faecalis FELA 097	N.I	N.I	N.I	N.I
Bacillius subtilis clinical strain	N.I	N.I	N.I	N.I

Key: N.I= No Inhibition

4.2 Qualitative Phytochemical Screening of Aqueous and Methanolic Leaf Extracts of *Jatropha tanjorensis*

The qualitative phytochemical screening of aqueous(hot and cold) and methanolic leaf extracts of *Jatropha tanjorensis* revealed the presence of phenols, saponins, terpenoids, steroids, flavonoids, tannins,phlobatanin, reducing sugars and alkaloids as depicted in Table 4. It was observed that both aqueous and methanolic leaf extract did not contain phlobatanin (Table 4). Steroids and trepernoids were not detected in the aqueous leaf extract of *J. tanjorensis* while the methanolic leaf extract of *J. tanjorensis* revealed the absence of saponins and Phlobatanin as shown in table 4.

Table 4: The Qualitative phytochemical screening of the crude extracts of leaf *J.tanjorensis*

Jatropha tanjorensis	Phenol	Alkaloid	Flavonoid	Tannin	Saponin	Reducing Sugar	Terpernoid	Steroid	Phlobatanin
Methanolic extract	+	+	+	+	-	+	+	+	-
Hot water extract	+	+	+	+	+	+	-	-	-
Cold water extract	+	+	+	+	+	+	-	-	-

Key: (+) =Present, (-) =Not detected

4.3 Quantitative Phytochemical Screening of Methanolic and Aqueous Leaf Extracts of*Jatropha tanjorensis*

Table 5: The quantitative phytochemical screening of the methanolic and aqueous leaf extract of *J. tanjorensis*

Jatropha tanjorensis	Phenol	Flavonoid	Steroid	Alkaloid	Reducing sugar	Tannin	Terpernoid	Saponin
	Concentrations (mg/100g)							
Methanolic extract	29.90	85.01	91.45	50.88	20.22	21.75	50.43	-
	29.20	85.56	91.86	50.62	20.55	21.24	44.47	-
MEAN VALUE	29.55±0.50	85.29±0.39	91.66±0.29	50.75±0.18	20.39±0.23	21.49±0.36	47.45±4.21	-
Hot water extract	67.45	97.82	-	61.06	25.77	49.06	-	31.01
	70.73	99.27	-	58.85	26.05	51.45	-	32.06
MEAN VALUE	69.09±2.32	98.55±1.02	-	59.95±1.56	25.91±0.20	50.26±1.69	-	31.54±0.49
Cold water extract	66.59	78.11	-	48.50	48.26	48.44	-	43.63
	67.68	80.29	-	48.76	49.21	49.23	-	42.97
MEAN VALUE	67.14±0.91	79.20±1.54	-	48.63±0.18	48.74±0.67	48.84±0.56	-	43.30±0.46

The Quantitative phytochemical screening of the crude extracts of *J. tanjorensis*leaf revealed that Steroid(91.66±0.29mg/100g) was the most abundant, followed by alkaloids (50.75±0.18mg/100g), phenol (29.55±0.50 mg/100g), terpernoids (47.45±4.21mg/100g), and tannins (21.49±0.36 mg/100g), reducing sugars(20.39±0.23mg/100g), flavonoids (85.29±0.39mg/100g) respectively in the methanolic leaf extract. The quantitative phytochemical analysis of hot

aqueous leaf extract of *J. tanjorensis* showed that flavonoid (98.55±1.02mg/100g) was the most abundant, followed by phenol (69.09±2.32mg/100g), alkaloids (59.95±1.56mg/100g), tanins (50.26±1.69mg/100g), saponin (31.54±0.49 mg/100g), reducing sugar (25.91±0.20mg/100g) respectively.

4.4 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The GC-MS analysis of chemical composition of *Jatropha tanjorensis* leaf extract indicated a total of 57 compounds were identified in the leaf extract of *Jatropha tanjorensis* accounting for 100% of the total components in the extract and the main constituents identified were 1,2,3-Benzenetriol (66.38%), 1,3,5-Benzenetriol (8.82%), Catechol (6.84%), n-Hexadecanoic acid (2.08%), and 9-Octadecadienoic acid E (2.00%) as represented in table 6.

Table 6: The Gas Chromatography-Mass Spectrometry (GC-MS) analysis showing the chemical composition of *J. tanjorensis*

S/N	Compound	Retention Time(Min)	Percentage (%)
1	Cyclotrisiloxane, hexamethyl-	3.370	0.26
2	Oxime-, methoxy-phen+yl-_	4.450	0.32
3	Cyclotetrasiloxane, octamethyl-	5.460	0.10
4	Phenol	5.611	0.17
5	Ether, methyl 1-octadecenyl	6.182	0.05
6	Phenol, 2-methoxy-	6.777	0.18
7	Ethyl 4-hydroxymandelate, 2TMS derivative	6.985	0.10
8	Cyclopentasiloxane, decamethyl-	7.447	0.05
9	Cyclododecane	7.915	0.28
10	N-Methylsalicylamide	8.082	0.12
11	1H-benzimidazole, 2-[2-(2-thienyl)ethenyl]-	8.839	1.15
12	1,2-Benzenediol, 3-methoxy-	8.926	0.33
13	Catechol	9.151	6.84
14	1,2-Benzenediol, 4-methyl-	9.457	0.54
15	Resorcinol, 2TMS derivative	9.855	0.08
16	5-Tridecene, (E)-	10.225	0.29
17	2,4-Di-tert-butylphenol	11.571	0.35
18	1,2,3-Benzenetriol	12.108	66.38
19	1-Octadecene	12.281	0.96
20	Pyrazole-5-carboxylic acid, 3-methyl-	12.512	0.75
21	5-Octadecene, (E)-	14.135	0.61
22	1,3,5-Benzenetriol	14.776	8.82
23	9-Heptadecanone	14.868	0.08
24	Hexadecanoic acid, methyl ester	15.290	0.28
25	n-Hexadecanoic acid	15.660	2.08
26	Trifluoroacetic acid, pentadecyl ester	15.821	0.66
27	Benzoic acid, 3,4,5-trihydroxy-, methyl ester	15.931	1.02
28	Benzoic acid, 4-fluoro-3-methoxy-, methyl Ester	16.243	0.46
29	1,1'-Biphenyl, 2,3'-dimethyl-	16.405	0.25
30	Uracil, 5-allyl-6-methyl-2-thio-	16.549	0.08
31	9-Octadecenoic acid, methyl ester,(E)-	16.688	0.48
32	Methyl stearate	16.872	0.18
33	9-Octadecenoic acid, (E)-	17.040	2.00
34	Octadecanoic acid	17.190	0.49
35	Cyclopentadecane	17.363	0.46
36	2-Chloro-5-methoxybenzimidazole	17.635	0.20
37	Benz[d]isoxazole-5-ol-4-one,4,5,6,7-tetrahydro-3-methyl-	17.843	0.27
38	1,1'-biphenyl, 2,6-dimethyl-	18.120	0.11

39	2,2'-Dimethylbiphenyl	18.334	0.06
40	9-Octadecenamide, (Z)-	18.651	0.27
41	Nonacos-1-ene	18.778	0.16
42	Benzene, 1-methyl-2-(phenylmethyl)	19.685	0.13
43	Bis(2-ethylhexyl) phthalate	19.847	0.15
44	1-Docosene	20.095	0.07
45	2-Dimethylamino-6,7-dihydro-4H-oxazolo[3,2-a]-1,3,5-triazin-4-one	20.742	0.05
46	Thiazolo[3,2-a]pyridinium, 2,3-dihydro-8-hydroxy-5-methyl-, hydroxide, inner salt	21.729	0.06
47	2-Octenal, 2-butyl-	21.851	0.11
48	Eicosane	22.093	0.09
49	1-Ethyl-2-hydroxymethylimidazole	22.873	0.21
50	cis-9-Tetradecenoic acid, propyl ester	23.081	0.14
51	Hexahydropyridine, 1-methyl-4-[4,5-dihydroxyphenyl]-	23.179	0.10
52	Vitamin E	23.364	0.18
53	2,2-Dimethylpropanoic acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester	24.074	0.06
54	Trimethyl-(2-(phenylthio)methoxyethyl)silane	24.305	0.05
55	Cholesta-22,24-dien-5-ol, 4,4-dimethyl-	24.634	0.11
56	.beta.-Sitosterol	24.767	0.14
57	[1,2,4]Triazolo[1,5-a]pyrimidine-6-carboxylic acid, 4,7- dihydro-7-imino-, ethyl ester	25.495	0.06
	Total		**100.03**

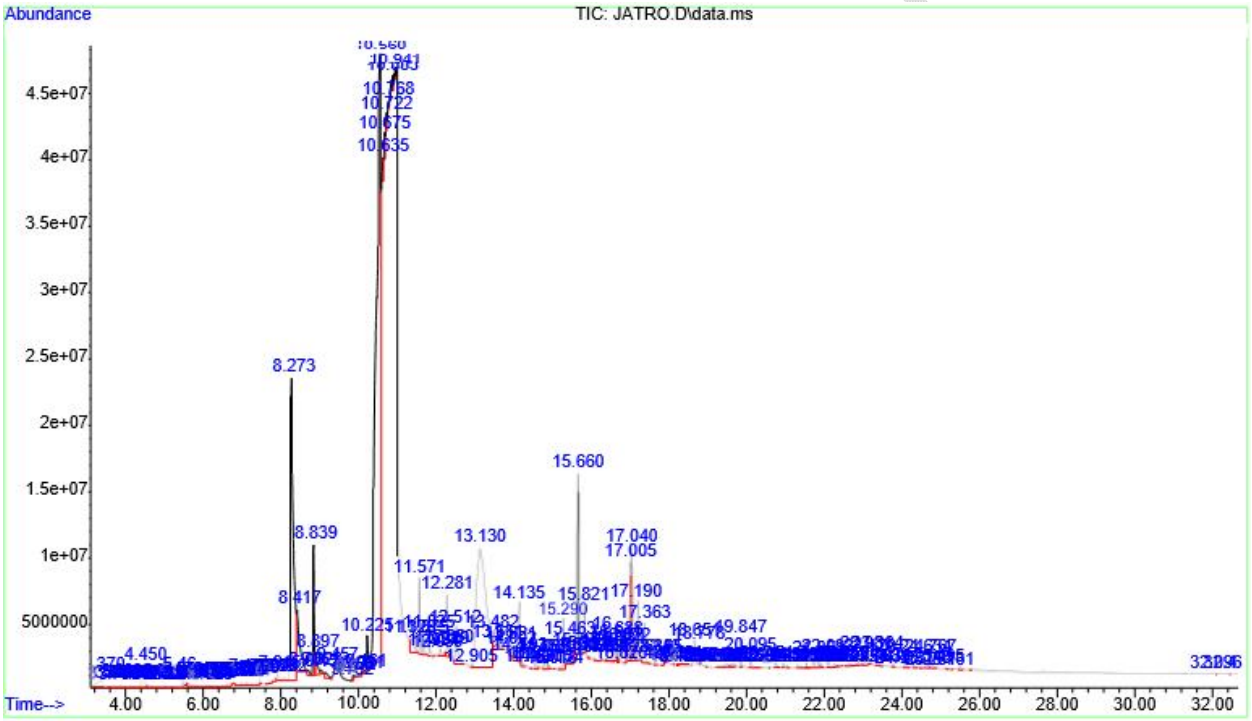

Figure 1: The chromatogram of the GC-MS analyses of the methanolic extract of *Jatropha tanjorensis* leaves.

Table 7: The antibiotic susceptibility of ciprofloxacin standard on test bacteria

ASSAY ORGANISM	20µg/ml	10µg/ml	5 µg/ml	2.5 µg/ml
	Zone of Inhibition(mm)			
Staphylococcus aureus ATCC 25923	28.83±0.29	22.33±0.58	0.00	0.00
Bacillus subtilis ATCC 6633	26.33±0.29	23.17±0.29	19.33±0.29	17.33±0.29
Escherichia coli NCTC 10418	>45	>45	>45	>45
Pseudomonas aeruginosa ATCC 27853	22.17±0.29	18.83±0.29	13.50±0.50	0.00
Salmonella typhi ATCC 13311	30.33±0.58	26.83±0.29	21.83±0.29	17.83±0.29
Staphylococcus aureus Clinical strain	29.33±0.58	26.33±0.58	18.67±0.58	0.00
Enterococcus faecalis FELA 097	39.33±0.58	35.67±0.58	30.67±0.58	26.67±0.58
Bacillius subtilis clinical strain	30.50±0.50	27.33±0.58	22.83±0.29	18.83±0.29

Table 8: The minimum inhibitory concentration values (mg/ml) of cold *Jatropha tanjorensis* extract on responsive organisms

ASSAY ORGANISM	**0.005MG/ ML**	**0.01MG/ML**	**0.02MG/ML**	**0.04MG/ML**	**0.08MG/ML**	**0.16MG/ML**	**0.32MG/ML**	**0.64MG/ML**	**1.28MG/ML**	**2.56MG/ML**	**5.12MG/ML**	**10.24MG/ML**	**20.48MG/ML**	**MINIMUM INHIBITORY CONCENTRATION VALUE (MG/ML)**
CONCENTRATION CODE ON SAMPLE BOTTLE	A	B	C	D	E	F	G	H	I	J	K	L	M	
Staphylococcus aureus clinical strain	+	+	+	+	+	+	+	+	+	+	+	+	-	20.48
Bacillus subtilis clinical strain	+	+	+	+	+	+	+	+	+	+	+	+	-	20.48
Enterococcus faecalis FELA 097	+	+	+	+	+	+	+	+	+	+	+	+	-	20.48

Table 9: The minimum inhibitory minimum inhibitory concentration values (mg/ml) of methanolic *Jatropha tanjorensis* extract on responsive organisms

ASSAY ORGANISM	0.005MG/ ML	0.01MG/ML	0.02MG/ML	0.04MG/ML	0.08MG/ML	0.16MG/ML	0.32MG/ML	0.64MG/ML	1.28MG/ML	2.56MG/ML	5.12MG/ML	10.24MG/ML	20.48MG/ML	MINIMUM INHIBITORY CONCENTRATION VALUE (MG/ML)
CONCENTRATION CODE ON SAMPLE BOTTLE	A	B	C	D	E	F	G	H	I	J	K	L	M	
Bacillus subtilis clinical strain	+	+	+	+	+	+	+	+	+	+	+	-	-	10.24

4. **DISCUSSION**

Recently, increasing concern about health problems has led to the production of natural antibacterial for disease control. One of the most widely used natural antibacterial agents is medicinal plants, which have historically been used by many cultures for thousands of years to control common health complications. The discovery of antimicrobial drugs based on natural plant products reached critical importance as newly discovered drugs are likely to be successful against microbes resistant to multiple drugs.

In order to investigate the antibacterial activities of *Jatropha tanjorensis* leaves, this research was carried out.

The antibacterial activity of cold-water extract against *Staphylococcus aureus* was the highest, with mean zone of inhibition of 19.83±0.76 with an MIC of 20.48mg/ml, while the antibacterial activity of methanolic extract against *Bacillus subtilis* was low with the mean zone of inhibition of 13.17±0.28 with a MIC of 10.24mg/ml.

Remarkably, the results of antibacterial activity of the leaf extract of *Jatropha tanjorensis*on the test organisms revealed that the cold-water leaf extract had the best effect, and this contrasts with previously reported studiesby Oyewole and Akingbala (2011 [23]. This could probably be due to biodiversity, or environmental conditions as organisms have different soluble portions of the phytochemicals. Van (2008) [24], [25] have reported that geographical and seasonal variations affect the phytochemical constituents of plant and this in turn affects the result of antimicrobial activity.
Staphylococcus aureus, *Enterococcus faecalis* FELAO97 and *Bacillus subtilis* responded to the cold-water leaf extract of *Jatropha tanjorensis*. Only *Bacillus subtilis* showed inhibitory effect on the methanolic leaf extract. Cold water extracts of *Jatropha tanjorensis*was more effective on the bacterial isolates. The result, however, is in disparity with an earlier study by [26] which reported that methanolic plant extracts are more active against bacteria. The aqueous extract of *Jatropha tanjorensis* leaves inhibited the growth of *Staphylococcusaureus* and this conforms to the study carried out by[27].

In this study, the leaves of *Jatropha tanjorensis*plant were effective against only Gram-positive bacteria and this contrasts with an earlier study by[26]which reported that the plant extracts are more active against Gram-negative bacteria.

Qualitative phytochemical analyses of crude methanolic, cold and hot water extracts of the plants revealed the presence of eight bio active compounds, and this agrees with the work of [28]. Seven compounds namely: flavonoids, terpenoids, tannins, alkaloid, reducing sugar, steroids and phenol were detected in the methanolic extract. Earlier studies by Oyewole and Akingbala (2011) [23] have reported the presence of flavonoids, terpenoids, and cardiac glycosides. Five compounds namely, saponins, flavonoids, tannins, alkaloid, reducing sugar and phenol and steroids were detected in both hot and cold-water extracts. Alkaloids, tannin, flavonoid, phenol and reducing sugars were found in all extract types. Saponins were found exclusively in both cold and hot water extracts. Terpenoids and steroids were found exclusively in the methanolic extract.This difference in phytochemical constituents of extracts from the same plant could be due to the solvent of extraction [29].

The presence of these secondary metabolites in the extracts of this plant part (leaf) could probably be responsible for its ethnomedical importance. Plants rich in alkaloids have been reported to possess antimalarial, analgesic and/or stimulant and antidiabetic effects, those with saponins, are useful in the treatment of hypercholesterolemia [30]. While plants rich in tannins, are useful in combating diarrhoea, headache, poor appetite, haemorrhoids, bactericides [31]. Phenol, when present in a plant, helps the plant to act as an oral analgesic/anaesthetic and to temporarily treat pharyngitis and prevention of UV-induced skin damage [32]. Flavonoids components may confer on the plant an anti-allergic, anti-inflammatory, anti-oxidant,anti-microbial, anti-bacterial, anti-fungal, anti-viral, anti-cancer, anti-diarrhoeal activity and anti-anaemic potential [33].

The Gas Chromatography and Mass Spectrometry (GC-MS) analysis using the methanolic extract of the leaves for the identification of the chemical components of *Jatropha tanjorensis* leaf extract showed a total of 57 compounds which accounts for the 100% of the total components in the extract and the main constituents identified were 1,2,3-Benzenetriol (66.38%), 1,3,5-Benzenetriol (8.82%), Catechol (6.84%), n-Hexadecanoic acid (2.08%), and 9-Octadecadienoic acid E (2.00%).

The result of the GC-MS analysis showed that the leaves of *Jatropha tanjorensis* grown in Nigeria have various medicinally active compounds which justify the claims of its use traditionally for the treatment of several bacterial ailments. From the results of this study, it can be inferred that *Jatropha tanjorensis* may be an excellent source for the development of novel antibiotics. However, further studies will need to be carried out extensively to exploit the potential of this plant and also for synergistic studies.

5.2 CONCLUSION

The results of this study on *Jatropha tanjorensis* have led to the following conclusions that:
The extracts of *Jatropha tanjorensis*leaves possessed antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Enteroccocus faecalis. Jatropha tanjorensis* has low antimicrobial activity on the test pathogens as compared to the Ciprofloxacin standard.
The Minimum Inhibitory Concentration (MIC) of *Jatropha tanjorensis*reveals that a higher dose of the plant extract is required to bring about a significant activity in the treatment of infection/ailment.
Eight active ingredients were identified in the plant leaves which include flavonoids, terpenoids, tannins, alkaloid, reducing sugar, saponins, steroids and phenol.
It is concluded that *Jatropha tanjorensis* should be extensively studied in future research designed to produce lead compounds and biologically active molecule to be incorporated in the arsenal of new antimicrobial drugs.

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