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Nutritional Studies and Antimicrobial Activities of *Jatropha tanjorensis* Leaves Extracts against *Escherichia coli* Isolates

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Abstract:- This study was aimed at evaluating the extract of *Jatropha tanjorensis* leaves for bioactive components, proximate composition and antimicrobial activity. The collection of the plant leaves, processing of the leaves and extracts were all done using standard methodologies. Qualitative screening and gas chromatography (GC-MS) were used to screen for phytochemicals. Proximate composition, vitamins, minerals and anti-nutrients were all carried out using standard techniques. Standard microbiological techniques were used to isolate, characterize and identify *Escherichia coli* isolates from dumpsite soil samples. The standard disc method was used to assay the antibiotics sensitivity of the test antibiotics and that of the extracts. The proximate nutrients and their respective amounts in g/100g per dry matter were moisture (80.23±1.53), protein (5.01±0.17), ash (2.91±0.31), crude fiber (3.21±0.17) fat (1.94±0.11) and carbohydrate (86.93±1.47). Mineral analysis revealed the presence of sodium, potassium, calcium, magnesium, zinc, copper, and iron. Furthermore, vitamin analysis showed the presence of vitamins A, B, C and E. Anti-nutrient analysis revealed the presence of permissible levels of hydrocyanic acid, soluble oxalate, total oxalate, and phytate. Phytochemical analysis via GC-MS revealed a total of 16 compounds belonging to five different categories of phytochemical namely: alkanes and associated hydrocarbons, organic acid and fatty acid, flavonoids, alkaloids and amines. Organic and fatty acids had the highest concentration of 80.52% followed by flavonoids with a concentration of 18.44%. Alkaloids, amines and alkanes and associated hydrocarbons had concentrations of 0.18, 0.692 and 0.113, respectively. The test antibiotics showed zones that ranged from 12.00 to 23.00mm. The highest and least zones of inhibition were 28.00 and 10.00 mm for aqueous extract while it was 17.30 and 10.00 mm for ethanolic extract, respectively. *Jatropha tanjorensis* could be rich source of nutrients, vitamins, essential minerals as well as phytochemicals with effective antimicrobial potential against *Escherichia coli*.

Keywords:- *Nutrition, Phytochemicals, Jatropha tanjorensis Leaf Extract, Escherichia coli, Antimicrobial Susceptibility.*

I. INTRODUCTION

The discovery of the drug Penicillin by Alexander Flemmings from the Fungus: *Penicillium notatum* was timely since it significantly reduced the mortality and morbidity of infectious diseases globally.[01] Following its discovery, several other antibiotics have successfully been discovered and are currently in the global market where they have been mass produced. Besides the significant global reduction in morbidities and mortalities associated with antibiotics, they have been found to increase global life expectancy by atleast two decades.[01,02] Studies revealed that the advent of antibiotics paved way for advances in surgery, cancer treatment, and organ transplant, as well as influenced the expansion of livestock production amongst other uses. [02, 03]

Sadly, the very microorganisms and their associated infections that were effectively controlled and treated by these antibiotics are now becoming resistant to them. The challenge of antibiotics resistance has now assumed a global dimension as resistance to antibiotics has been reported in all continents of the world.[03] Even more worrisome, is the fact that resistance has completely outpaced the search and development of newer and more effective antibiotics.[04] Furthermore, microbes have the evolutionary capacity to acquire and spread resistance genes even via plasmids and transposons. [05, 06] Studies revealed that these genes abound in diverse environments including in the hospital samples, effluents and as well as other environments. [05, 07]

Despite advances in research development especially in the areas of medicine, lead compound discovery, molecular docking and combinatorial chemistry, only few antibiotics have made it to the market since the 1970s.[04] Given the gravity of this scourge and the global paucity of newer antibiotics to stem the tide of antibiotics resistance, alternative medicine has been one of the major interests of researchers currently. [08]

One of such alternatives is the use of ethno-medicinal plants. Since time immemorial, medicinal plants and its products have been utilized traditionally in the treatment of common ailments.[08,09] These plants and their products are currently gaining more attention because they have been reported to possess phytochemicals which exhibit noble antimicrobial properties against even the multidrug-resistant

microorganisms with little or no side effects to the host. [09, 10] One of such medicinal plants is *Jatropha tanjorensis* which belongs to the family *Euphorbiacea* and grows very well in the tropics.[11]

Jatropha tanjorensis commonly called "When hospital is too far" or "When the doctor is not near" in Cross River and Akwa Ibom States is used as a source of medicine and as a vegetable substitute for fluted pumpkin in these areas. In Nigeria, this plant has been utilized by the locals in the treatment of malaria and hypertension and has been reported to contain alkaloids, flavonoids, tannins, cardiac glycosides, saponins, and anthraquinones.[12] However, there is a dearth of information on the nutritional content and antimicrobial potential of this plant against microorganisms including the multidrug-resistant isolates. Therefore, this study was aimed at evaluating the nutritional and antimicrobial potentials of *Jatropha tanjorensis* extracts against *Escherichia coli* isolates.

II. MATERIALS AND METHODS

A. Materials:

➢ *Soil Samples and Plant Parts*

Soil samples from a dumpsite and plant parts of *Jatropha tanjorensis* were obtained and utilized for this work.

➢ Antibiotics discs employed were commercially sourced for and included; gentamycin (CN) (10µg), ofloxacin (OFX) (10µg), ceporex (CEP) (10µg), amplicin (PN) (10µg),ciprofloxacin (CPX) (10µg), reflacine (PEF) (10µg), amoxicillin+clavulanic acid (AU) (30µg), streptomycin (S) (30µg), sulfamethoxazole+ trimethoprim (SXT) (30µg), nalidixic acid (NA) (30µg), (Optun laboratories, Nigeria).

➢ Media and Reagents employed included Eosin Methylene blue medium, Muller Hinton Agar, Nutrient agar, Citrate agar, Urease agar, Grams reagents, indole, H_2O_2, oxidase reagent, methyl red, alcohol, among others.

➢ *Other Materials*

Other materials employed for this study included incubator, GC-MS, rotary evaporator, petri dishes, among others.

B. Methodology:

➢ *Collection of Plant Parts*

The leaves of the plant employed for this study were freshly collected from Obong University Community with the help of locals. The plant parts were identified scientifically at the Botany Department, University of Calabar, Cross River State as *Jatropha tanjorensis.* The freshly collected leaves were immediately taken to the Microbiology laboratory for further analysis.

➢ *Processing of Leaves Sample*

The collected leaves were processed following procedures previously described.[13] Briefly, the freshly collected leaves were first washed and allowed to air dried. There were then chopped into tiny pieces using a clean stainless kitchen knife and dried using an electric oven maintained at 60^{o}C for atleast 1 hour. Afterwards, the dried leaves were then grinded into powder using a clean Mortar and pestle and the powdered leaves were then stored in a clean, dry sample bottle and kept in a dry place till required.

➢ *Preparation of Extracts*

Both aqueous and ethanolic extracts were prepared as previously reported.[09] Briefly, 20g each of the powdered leaves was weighed out and dissolved separately into beaker containing 200ml each of distilled water and freshly prepared 75% ethanol. The mixtures were then stirred gently to ensure proper mixing. The flasks were sealed and incubated for 24 hours. Following incubation, the content of the flasks were then filtered into separate conical flasks using Whitman No.1 filter papers neatly folded into glass funnels. The filtrates from both flasks were allowed time to filter properly. At the end of the filtration, the residues were discarded while some portions of both filtrates were heated using an electric water bath maintained at 100^{0}C till slurries of both filtrates were obtained. The slurries were then stored away at room temperature till further use.

➢ *Qualitative Screening for Phytochemical*

Qualitatively, the remaining filtrates (extracts) were screened for crude phytochemicals using slightly modified methods previously described.[10, 14, 15, 16, 17] The phytochemicals screened for were alkaloids, tannins, saponins, flavonoids, glycosides and polyphenol.

- *Screening for Alkaloids*

Briefly, 5ml of 1 % HCl was added to exactly 2ml of each extract and gently stirred after which they were placed on steam bath. Then, a few drops of Mayer's reagent and 1ml of Dragendorff's reagent were added to the mixtures. The presence of a precipitate with either of these reagents was taken as a positive result.

- *Screening for Tannins*

To the extracts, exactly 2mls of 5% ferric chloride was added to 1ml of each of the extracts. The formation of a dark blue or greenish black coloration on addition of ferric chloride indicated the presence of tannins.

- *Screening for Saponins*

To each of the extracts, 2ml of distilled water was added and the resulting aqueous mixtures shaken vigorously for about 5 – 10minutes. The formation of a stable and persistent froth of about 1cm in length was taken as positive result.

- *Screening for Flavonoids*

To exactly 2ml of both extracts, a few pieces of aluminum metal and concentrated HCl were added. Following addition, the formation of an orange or red or crimson or magnetic coloration was regarded as a positive result for flavonoids.

- *Screening for Glycosides*

Exactly 2ml of chloroform with 10% ammonia solution was added to 2ml of each of the extracts. Formation of a pink color was regarded as positive test for glycosides.

- *Screening for Polyphenol*

Briefly, 2ml of both extracts were treated with 5ml of distilled water and heated for 30min in a water bath. After heating, 1ml of 1% potassium ferrocyanide solution was added to it. The formation of green-blue coloration indicated the presence of polyphenol.

➢ *GC-MS Analysis*

This was done at Mifor Consult Laboratory located at Marian Calabar, Cross River State. Briefly, exactly 10g of the powdered leaves was weighed and introduced into an extraction thimble of a soxhlet extractor. Then, 100ml of methanol was measured and poured into the round bottom flask attached to the soxhlet extractor. This was refluxed for at least three times. The extract was concentrated to about 2mL using a rotary evaporator after which it was then transferred into a Teflon screw-cap vial and appropriately labeled. Subsequently, the extract was cleaned up with 3g of anhydrous sodium sulfate in a well packed column, conditioned with methanol to form slurry after which it was injected into GC-MS for qualitative and quantitative analyses.

➢ *Proximate Composition and Mineral Analysis*

The leaves were also evaluated for proximate nutrients and mineral elements. These were all done following the methods of the Official Analytical Chemists (A.O.A.C). The analysis included moisture content, ash content, crude protein, lipid, crude fiber, carbohydrate, vitamins, calorific value and mineral elements (Ca, Fe, Mg, Zn, Cu, Na, K, and P).

- *Vitamin Analysis*

Vitamins A, B, C and E were also evaluated for in the leaves of our study plant. These were done using methods previously described.[18, 19, 20, 21]

- *Anti -Nutrients*

The anti-nutrients examined were hydrocyanic acid, soluble oxalate, phytate and total oxalate. These were estimated using procedures previously described.[22, 23, 24]

➢ *Collection and Processing of Soil Samples*

This was done following methods previously described.[24] Briefly, soil samples from three different locations in Uyo Village dumpsite were collected into sterile nylon bags and transported immediately to the laboratory for further analysis. Approximately, 1g of each soil sample was introduced into test tubes containing 10mL of distilled water. Then, ten-fold serial dilutions were carried out on the soil samples.

➢ *Isolation and Characterization of E. Coli Isolates*

This was performed following methods previously described.[25] Briefly, the 10^{-3} dilutions were then plated out on freshly prepared Eosin Methylene Blue (EMB) Agar and incubated at room temperature overnight. Following incubation, the plates were examined for growth. Following purification of test isolates, they were characterized following methods described previously.[26]

➢ *Antimicrobial Susceptibility Testing*

- *Antibiotics Susceptibility against Test Isolates*

This was performed using the Agar Disc diffusion method previously described.[27, 28,29] Briefly, exactly, 3-5 colonies of each test organism was selected using a sterile inoculating loop and suspended in saline after which the inoculum was adjusted to a turbidity equivalent to a 0.5 McFarland standard (corresponds to approximately 1.5 x 10^8 CFU/ml). The suspension was then vortexed to make sure it was well-mixed. Then a fresh, sterile cotton-tipped swab was dipped into the suspension, the excess liquid from the swab removed by pressing it against the side of the tube. Subsequently, the swab was inoculated unto a plate containing freshly prepared Muller Hinton Agar (MHA) starting at the top, the surface was inoculated with the swab covering the entire plate by spreading back and forth from edge to edge, rotating the plate approximately 60° and repeating the swabbing procedure thrice ensuring that the entire surface was properly covered. Then the disc containing the antimicrobial agents was applied using a sterile pair of forceps within 15 minutes of inoculating the MHA plate and then pressed down firmly to ensure firm, leveled contact with the agar. The plate was inverted and incubated at 35^0C for 16-18 hours. After incubation, the clear zone around each disc was measured and interpreted using procedures already described (CLSI, 2014). Isolates showing resistance to atleast two antibiotics were regarded as multidrug-resistant *E. coli* isolates. This procedure was carried out on all bacterial isolates.

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- *Antimicrobial Susceptibility Test of Extracts against Test Isolates*

This was carried out using agar disk diffusion methods previously described.[27,28] Briefly, the test isolates were first inoculated on nutrient broth and allowed to stand overnight. After overnight incubation, 0.1ml (a loopful) of each test isolate was inoculated unto plates containing freshly prepared MHA. Then, a manual borer was used to obtain discs of about 6mm from Whitman filter paper No1. The discs were sterilized in an oven for 30minutes at 60ºC. Following sterilization, these discs were soaked in different concentrations (0.1, 0.3, 0.5mg/ml) of each extract for 15minutes and were placed gently on the Mueller Hinton Agar (MHA) plates pre-inoculated with the test organisms. This was done in duplicates for each isolate and concentration. The plates were then incubated at 37ºC overnight. Following incubation, zone diameter were obtained and compared with appropriate standards.

➢ *Statistical Analysis*

Replicate readings obtained from this study were analyzed using Analysis of Variance (ANOVA) at 95% confidence level using Microsoft Excel version 2007. Results analyzed using mean ± Standard deviation.

III. RESULTS

➢ *Phytochemical Analysis*

The leaves when subjected to phytochemical screening using crude means as well as via GC-MS revealed that *Jatropha tanjorensis* leaves contain various phytochemicals. The results of the crude qualitative screening revealed the presence of alkaloids, tannins, flavonoids, saponins, glycosides, glycosides and polyphenol. Among the phytochemicals, alkaloids, flavonoids and polyphenol were present in excess while the remaining phytochemicals were just present but not in excess as presented in Table 1. Similarly, the results of the GC-MS as presented in Tables 2 and 3 as well as in Fig. 1 further confirm the presence of different bioactive compounds in the leaf extracts. The 16 compounds observed via GC-MS belong to five different categories of phytochemicals namely alkanes and associated hydrocarbons, organic acid and fatty acid, flavonoids, alkaloids and amines. Amongst these categories of phytochemicals, the most diverse was the organic and fatty acid family which had a total of 13 compounds namely thiazole, 2-amino-4-(p-methoxyphenyl), 1,16-Cyclocorynan-17-oic acid, 19,20-didehydro, methyl ester, 2-(Acridin-9-ylamino)-3-(3H-imidazol-4-yl)-propionic acid, 2-Hydroxy-2-methylbutyric acid, Butanoic acid, 4-[(2,4-dichlorophenyl)oxy]-, butyl ester, 2-propenoic acid, 2-methyl-, 3-[(phenylsulfonyl)amino]phenyl ester, Heptyl isobutyl carbonate, Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, n-Hexadecanoic acid, n-Hexadecanoic acid, Octadecanoic acid, Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl), and 3-pyridinecarboxylic acid, 2,7,10-tris(acetyloxy)-1,1a,2,3,4,6,7,10,11,11a-decahydro-1,1,3,6,9-pentamethyl-4-ox. In the organic and fatty acid family of phytochemicals, thiazole, 2-amino-4-(p-methoxyphenyl) was the most abundant with a concentration of 41.04%. Flavonoids and amines had 3 compounds each while alkaloids, and the alkanes and associated hydrocarbons had 2 compounds each. As shown in Fig. 2, the combined concentration of the various phytochemicals revealed that the organic and fatty acids had the highest abundance of 80.52% followed by flavonoids (18.44%). Alkaloids, amines, alkanes and associated hydrocarbons had concentrations of 0.18, 0.692 and 0.113, respectively.

Phytochemicals	Aqueous extract	Ethanolic extract
Alkaloids	++	++
Tannins	+	+
Flavonoids	++	++
Saponins	+	+
Glycosides	+	+
Polyphenol	++	++

Table 1:- Qualitative screening for phytochemicals in *Jatropha tanjorensis*
Key:- + = present and ++ = present in excess.

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S/N	Phytochemical groups	Compounds	Concentration (%)
1	Alkanes and associated hydrocarbons	Cyclohexane, 1,1'-[4-(3-cyclohexylpropyl)-1,7-heptanediyl]bis-	0.053
2		2-Isopentyl-N-(1-naphthyl)-1-cyclopropanecarboxamide	0.060
3	Organic acid and Fatty acid	Thiazole, 2-amino-4-(p-methoxyphenyl)-	41.040
4		1,16-Cyclocorynan-17-oic acid, 19,20-didehydro-, methyl ester,	7.281
5		2-(Acridin-9-ylamino)-3-(3H-imidazol-4-yl)-propionic acid	0.061
6		2-Hydroxy-2-methylbutyric acid	0.361
7		Butanoic acid, 4-[(2,4-dichlorophenyl)oxy]-, butyl ester	0.113
8		2-propenoic acid, 2-methyl-, 3-[(phenylsulfonyl)amino]phenyl ester	0.057
9		Heptyl isobutyl carbonate	0.057
10		Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	0.056
11		n-Hexadecanoic acid	0.059
12		n-Hexadecanoic acid	9.476
13		Octadecanoic acid	10.474
14		Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-	8.138
15		3-Pyridinecarboxylic acid, 2,7,10-tris(acetyloxy)-1,1a,2,3,4,6,7,10,11,11a-decahydro-1,1,3,6,9-pentamethyl-4-ox	3.347
15	Flavonoids	2-Pentanone, 4-hydroxy-4-methyl-	18.380
17		4"-Dehydroxy-2",3',3",4',5,6",7-hepta-O-methylisoorientin	0.062
18		1-Dimethyl(chloromethyl)silyloxydecane	0.055
19	Alkaloid	2,16-Dihydro-iboxyphylline	0.072
20		3,5-Dimethyl-1-dodecylpyrazole	0.105
21	Amine	5-benzoxazolamine, 2-[4-[2-[4-(2-benzoxazolyl)phenyl]ethyl]phenyl]-	0.070
22		Heptadecanenitrile, 6-aza-2-(4-hydroxy-3-methoxyphenyl)-2-propyl	0.561
23		Cyclohexanecarbonitrile, 4-[4'-(nonyloxy)[1,1'-biphenyl]-4-yl]-1-octyl-	0.061

Table 2:- GC-MS analysis of *Jatropha tanjorensis* leaves

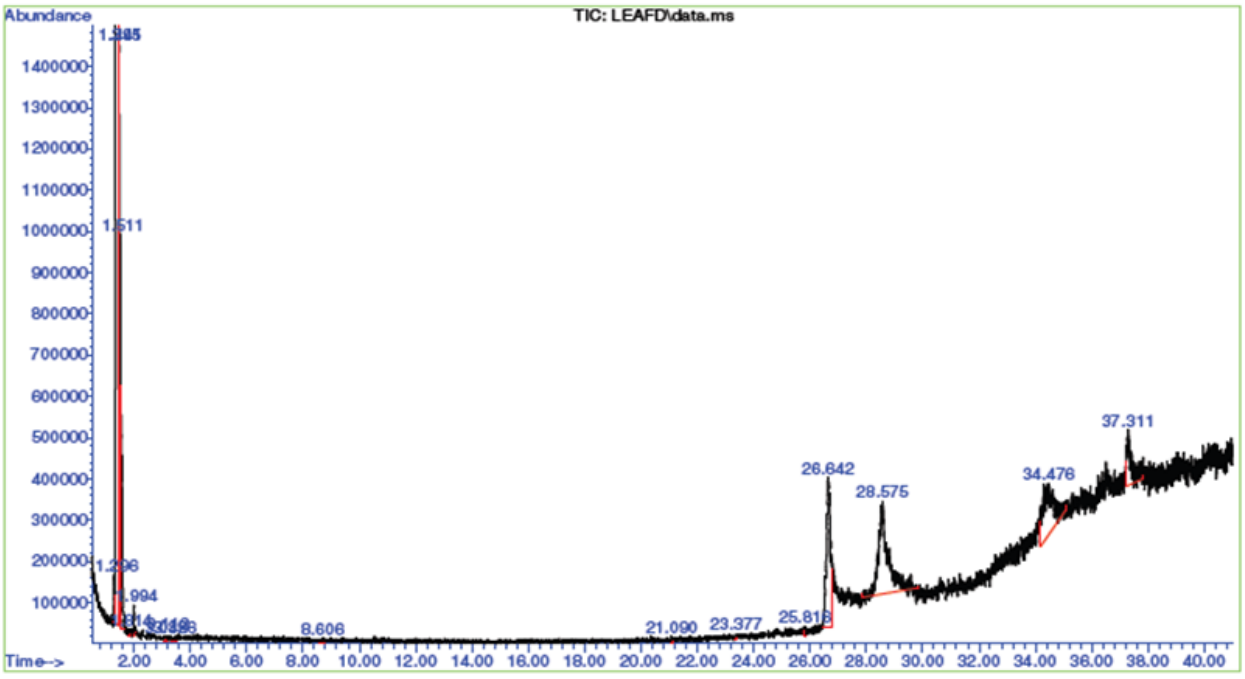

Fig 1:- Chromatogram of the various phytochemicals in the leave extract using GCMS

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peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	1.296	134	139	143	rBV	114323	211622	1.37%	0.549%
2	1.395	143	156	162	rVV3	2984248	15474367	100.00%	40.145%
3	1.441	162	164	174	rVV	4228204	6930176	44.78%	17.979%
4	1.511	174	176	199	rVB2	956263	2745417	17.74%	7.122%
5	1.814	222	228	231	rBV3	14142	23025	0.15%	0.060%
6	1.994	254	259	269	rVV2	73178	135936	0.88%	0.353%
7	3.042	436	439	446	rVB5	9693	23495	0.15%	0.061%
8	3.112	446	451	455	rBV4	15793	26296	0.17%	0.068%
9	3.356	490	493	500	rBV5	9709	22623	0.15%	0.059%
10	8.606	1390	1395	1402	rVB2	9084	27269	0.18%	0.071%
11	21.090	3534	3540	3543	rBV4	14775	22979	0.15%	0.060%
12	23.377	3924	3933	3939	rBV8	14923	42534	0.27%	0.110%
13	25.816	4348	4352	4355	rVB7	23443	39752	0.26%	0.103%
14	26.642	4461	4494	4517	rBV7	364656	3573075	23.09%	9.270%
15	28.575	4748	4826	5047	rM 7	235156	5599990	36.19%	14.528%
16	34.476	5757	5840	5983	rM 7	115521	3647460	23.57%	9.463%
						Sum of corrected areas:	38546016		

Table 3:- Peaks properties of the various phytochemicals

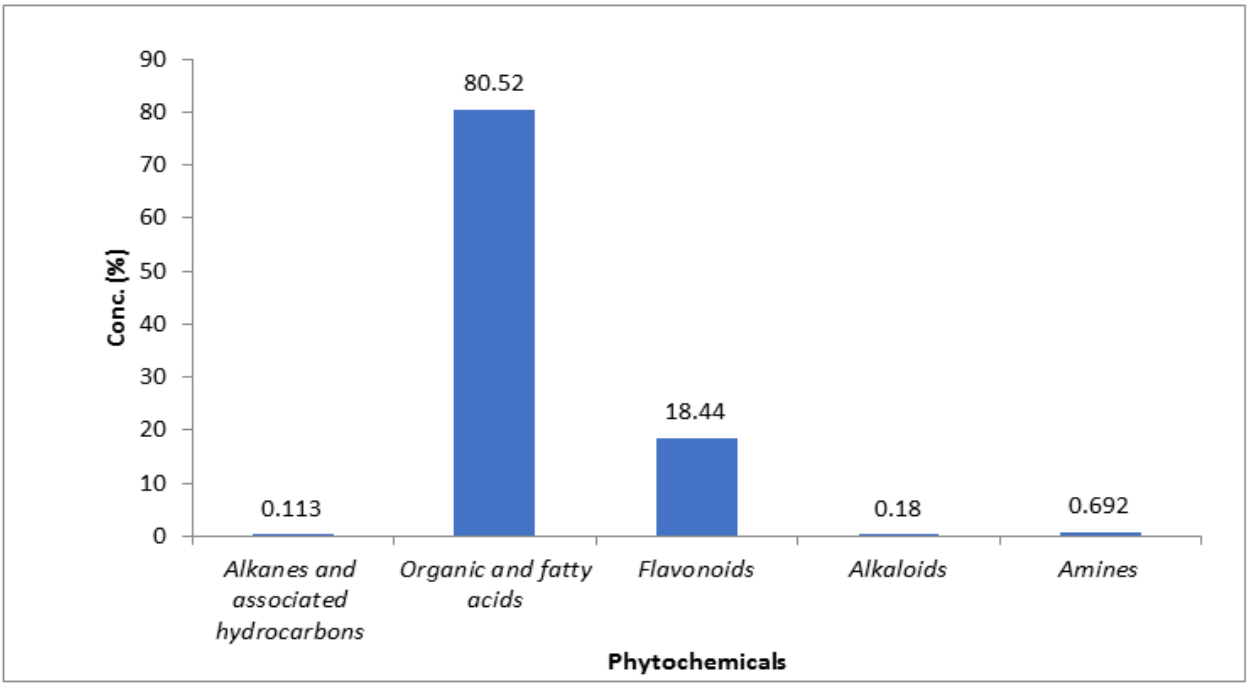

Fig 2:- Combined concentrations of the various phytochemical categories

➢ *Nutrients and Anti-Nutrient Analyses*

The results of the nutrient analyses are presented in Tables 4-7 as mean ± SD (standard deviation). The results of the proximate composition presented in Table 4 indicate that the leaves of the study were rich in nutrients. The proximate nutrients and their respective amounts in g/100g per dry matter were moisture (80.23±1.53), protein (5.01±0.17), ash (2.91±0.31), crude fiber (3.21±0.17) fat (1.94±0.11) and carbohydrate (86.93±1.47). It can be seen that the carbohydrate was the most abundant nutrient in the study plant part. This was followed by moisture, protein, ash, crude fiber and fat. The least abundant nutrient was fat. The leaves employed in this study were also analyzed for the presence of mineral elements and the results indicate the presence of sodium, potassium, calcium, magnesium, zinc, copper, and iron. The most abundant mineral element was magnesium which had an abundance of 19.23±1.71 mg/100g per dry matter. The second most abundant mineral element was calcium with an abundance of 18.64±1.33 mg/100g per dry matter. These were followed by potassium and sodium that had abundance of 16.22±1.57 and

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14.14±2.14 mg/100g per dry matter. The least abundant element was copper with a value of 1.21±0.10 ±0.10.

Table 5 shows the presence of vitamins. From the results, the leaves had vitamins A, B, C and E in varying amounts. The most abundant vitamin was B with a mean value of 102.81 mg/100mL followed by vitamin A with a mean value of 81.09 mg/100mL. Vitamins C and E had mean values of 6.13 and 3.05 mg/100mL, respectively. In addition to the nutrient analysis presented in Tables 4-7, the leaves of the study plant were also evaluated for anti-nutrients. The anti-nutrients evaluated were hydrocyanic acid, soluble oxalate, total oxalate, and phytate. The results indicate that total oxalate was the most abundant anti-nutrients with a mean value of 9.27 followed by soluble oxalate with a mean value of 8.23. The least abundant was hydrocyanic acid which had a mean value of 4.07.

Proximate nutrients	Amount (g/100g per dry matter)
Moisture	80.23±1.53[a]
Protein	5.01±0.17
Ash	2.91±0.31
Crude fiber	3.21±0.17
Fat	1.94±0.11
Carbohydrate	86.93±1.47

Table 4:- Proximate composition of the *Jatropha tanjorensis*
Key:- [a]Superscript represents significant Mean±SD ($p < 0.05$) following analysis of variance

Elements	Amount (mg/100g per dry matter)
Sodium	14.14±2.14[a]
Potassium	16.22±1.57
Calcium	18.64±1.33
Magnesium	19.23±1.71
Zinc	3.64±0.17
Copper	1.21±0.10
Iron	3.80±0.20

Table 5:- Mineral levels in the leaves
Key:- [a]Superscript represents significant Mean±SD ($p < 0.05$) following analysis of variance.

Vitamins	Amount (mg/100mL)
A	81.09±2.14[a]
B	102.81±2.89
C	6.13±1.41
E	3.05±0.17

Table 6:- Estimation of vitamin contents
Key: - [a]Superscript represents significant Mean±SD ($p < 0.05$) following analysis of variance.

Anti-nutrients	Amount (mg/100mL)
Hydrocyanic acid	4.07±0.14[a]
Soluble oxalate	8.23±0.17
Total oxalate	9.27±2.11
Phytate	7.18±0.10

Table 7:- Anti-nutrients levels in the leaves
Key:- [a]Superscript represents significant Mean±SD ($p < 0.05$) following analysis of variance.

- *Microbiological Analysis*

A total of ten (10) test isolates were recovered from soil samples and characterized as presented in Table 8. In addition to showing a green metallic sheen on EMB plates, the isolates were subjected to a battery of various biochemical tests. The isolates were positive for catalase, indole and H_2S/Gas, in addition to appearing as rods and showing motility. However, they were negative for oxidase, MR-VP, citrate and urease tests.

- *Antimicrobial Analysis of Antibiotics and Extracts against Test Isolates*

Table 9 shows the antibiotics sensitivity profile against test isolates. The isolates showed varying zones of inhibition against the various antibiotics. For Ciprofloxacin, the zones ranged from 12.00-21.50 mm, 8.50-20.50mm for OFX and 15.50-21.10mm for NA. CN and PEF recorded zones of inhibition ranging from 14.00-23.00mm and 12.50-20.00, respectively. 9.50-21.20mm and 10.50-19.50mm were the zones of inhibition recorded for AU and CPX, respectively. For SXT, S and PN, the zones were 13.50-23.00, 13.20-22.00mm and 12.00-21.50 mm, respectively.

Similarly, Tables 10 and 11 show the antimicrobial activities of the aqueous and ethanolic extracts of our study plant, against the test isolates. Generally, as the concentration of the extract increased, there was a gradual increase in the zone inhibition. Isolate 1 showed no susceptibility to the aqueous extracts at concentration of 0.1 mg/ml as shown in Table 10. Isolate 7 also showed resistant at 0.1 and 0.2 mg/ml. The highest zone of inhibition obtained was 28.00mm against isolate 10 while the least was 10.00mm. Furthermore, only isolate 10 showed complete resistances to all the concentrations of the ethanolic extract used in our study as shown in Table 11. The highest susceptibility observed was 17.30 mm while the least was 10.00 against isolate 2 and 1, respectively. Apart from isolate 10, all other isolates gave zones that increased with increasing concentrations of the ethanolic extract.

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Isolates	Grams Reaction	Shape	Motility	Catalase	Oxidase	Methyl Red	Voges Proskauer	Indole	Citrate	Urease	H_2S/Gas
Isolate 1	-	Rods	Yes	NA	-	-	-	+	-	-	-/+
Isolate 2	-	Rods	Yes	NA	-	-	-	+	-	-	-/+
Isolate 3	-	Rods	Yes	NA	-	-	-	+	-	-	-/+
Isolate 4	-	Rods	Yes	NA	-	-	-	+	-	-	-/+
Isolate 5	-	Rods	Yes	NA	-	-	-	+	-	-	-/+
Isolate 6	-	Rods	Yes	NA	-	-	-	+	-	-	-/+
Isolate 7	-	Rods	Yes	NA	-	-	-	+	-	-	-/+
Isolate 8	-	Rods	Yes	NA	-	-	-	+	-	-	-/+
Isolate 9	-	Rods	Yes	NA	-	-	-	+	-	-	-/+
Isolate 10	-	Rods	Yes	NA	-	-	-	+	-	-	-/+

Table 8:- Biochemical characterization of the microbial isolates
Keys: + = Positive, - = Negative, NA=Not applicable

E. coli isolates	CEP	OFX	NA	PEF	CN	AU	CPX	SXT	S	PN
Isolate 1	18.00	17.20	20.30	12.50	14.00	15.20	17.50	14.30	15.00	16.00
Isolate 2	-	-	-	-	-	-	-	-	-	12.00
Isolate 3	16.00	18.30	15.50	13.00	17.30	18.30	15.50	14.30	13.20	16.00
Isolate 4	12.00	16.50	17.30	14.00	15.50	16.20	17.30	16.30	22.00	17.20
Isolate 5	19.00	20.50	18.10	18.00	23.00	21.20	19.50	23.00	22.00	21.50
Isolate 6	20.00	8.50	19.30	18.50	18.30	9.50	10.50	13.50	17.30	10.80
Isolate 7	21.50	15.30	20.50	20.00	22.00	21.50	19.50	18.00	15.00	12.00
Isolate 8	-	-	-	-	-	-	-	-	-	-
Isolate 9	15.30	13.20	21.10	20.00	17.50	18.00	19.00	14.50	16.00	17.30
Isolate 10	14.20	16.40	15.20	19.00	22.00	19.30	18.30	21.00	19.00	18.50

Table 9:- Antibiotics sensitivity of the various *E. coli* isolates
Key: - = No zone of inhibition, Key: - = No zone of inhibition, CPX, SXT, S, PN, CEP, OFX, NA, PEF, CN, and AU respectively.

Isolates	Concentration (mg/ml)		
	0.1	0.2	0.3
1	-	15.00	16.00
2	13.10	14.00	15.20
3	12.20	14.00	15.50
4	14.30	15.00	16.70
5	11.00	12.99	14.78
6	11.19	12.00	13.89
7	-	-	10.00
8	12.11	13.28	15.56
9	12.50	13.10	14.00
10	-	10.00	28.00

Table 10:-Antimicrobial sensitivity (mm) of aqueous extracts of *Jatropha tanjorensis* against *E. coli*
Key: - = No zone of inhibition.

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Isolates	Concentration (mg/ml)		
	0.1	0.2	0.3
1	10.00	11.00	12.00
2	11.50	12.00	17.30
3	12.50	13.00	14.60
4	13.50	16.40	20.00
5	12.50	15.00	17.00
6	11.10	12.00	13.90
7	12.00	14.11	15.00
8	12.00	12.50	13.00
9	13.00	13.90	14.00
10	-	-	-

Table 11:- Antimicrobial sensitivity (mm) of ethanolic extracts of *Jatropha tanjorensis* against test isolates
Key: - = No zone of inhibition.

IV. DISCUSSION

Jatropha tanjorensis is popular plant that is well used as a leafy vegetable and as a medicinal plant. Analysis of the plant parts for nutrients indicate that the leaves have a very high moisture content of 80.23%. This slightly higher than the 78.77% previously reported.[30] The moisture contents of various edible leafy vegetables such as cassava, cabbage, cowpea and sweet potato have been observed to range from 79.00-85.00 with cabbage having the least and cowpea with the highest amounts of moisture.[30] Compared to other common tropical edible leafy vegetables including *Lasianthera africana* and *Dennettia tripetala*, which gave moisture contents of 78.95 and 76.62 %, respectively, the moisture content of our sample plant was only slightly higher.[09] Similarly, the carbohydrate content was observed to be 86.93 g/100g per dry matter. This is somewhat higher than the carbohydrate contents of *Lasianthera africana* (73.04g/100g) and *Dennettia tripetala* (49.70g/100g).[09]

The values for the protein, ash, crude fiber and fat were observed to be 5.01, 2.91, 3.21 and 1.94, respectively. The 5.01% protein content observed in this study although lower than the values of moisture and that of carbohydrate recorded, it is slightly higher than the 2.04% previously reported from Benin City, Nigeria. Generally, this low protein value may be indicative of the fact that leafy vegetables are usually low in protein.[30] The protein content of this leaf extract has also been observed similar to other leafy vegetables such as cassava, cowpea and sweet potato which have reported protein contents of 6.00, 4.70 and 4.60, respectively.[30] The protein content observed in this study is consistent with earlier findings where protein content was reported to be 4.75 %. [11]

The low amount of fiber in this study correlates with those of earlier studies and this nutrient is usually an indication of low mineral content.[11, 30] The presence of little fat observed in this study agrees with previous findings.[11] This makes the leafy vegetable a good option for those that are dieting and/or diabetic. Studies revealed that the presence of low fiber in leafy vegetables is always an indication of low mineral content.[09,30] In line with this assertion, it is therefore not surprising that our leafy vegetable was low in minerals analyzed in this study. The highest was magnesium with a value of 19.23 and this was followed by calcium with a value of 18.64 mg/100g per dry matter. Compared to earlier findings, all the observed minerals were slightly higher.[31] However; minerals observed in this study were somewhat lower than previously reported.[11]

The presence of essential vitamins such as A, B, C and E in this study is a further indication of the nutritional capacity of the plant. The level of vitamins in this study was agreeable to those of mushroom in our earlier study.[24] Since *Jatropha tanjorensis* is widely eaten in the Southern part of the country, the anti-nutrient levels present in the leaves were consistent with anti-nutrients in other widely eaten leafy vegetables such as *Lasianthera Africana*, *Dennettia tripetala* , cassava, water leaf, bitter leaf, Ugu leaf and green vegetable. [09, 32]

Jatropha tanjorensis has been adjudged to have atleast eight benefits ranging from increasing men vitality (increasing blood circulation and size of the penis during sex), placing slices of the leaves on the forehead of children cures fever, and treatment of vaginal discharge.[33] Other beneficial uses include curing skin problems and rheumatism, relieve for inflammation, smoothing defecation and curing of the scratch and skin blisters.[31, 33]

The medicinal properties of all ethno-medicinal plants have been associated with the presence of phytochemicals in various parts of the plants.[04, 31] Phytochemicals including alkaloids, tannins, flavonoids, saponins, glycosides and polyphenols observed in this study are similar to those earlier reported. [31, 34] A total of 23 individual phytochemicals classified into five categories namely alkanes and associated hydrocarbons, organic acid and fatty acid, flavonoids, alkaloids and amines were quantified. The organic and fatty acid class were the most abundant with a total of 13 compounds and a combined total of 80.52%. This was followed by flavonoids, amines and alkaloids with combined concentrations of 18.44, 0.692 and 0.18%, respectively. This observation is contrary to an earlier report.[31] Flavonoids is a diverse group of compounds have

been shown to possess anti-oxidant and anti-inflammatory properties in humans.

The antimicrobial susceptibility profile of the plant extracts revealed consistent and increasing inhibitions against test isolates as the concentrations increased from 0.10-0.30mg/ml and none of the isolates showed absolute resistance to the extracts. However, isolate 10 showed mild resistances to all concentration of the ethanolic extract. The highest and least inhibitions recorded were 28.00 and 10.00mm against isolates 10 and 7 at a concentration of 0.30 mg/ml for aqueous extract and 10.00-20.00mm for ethanolic extract. Similarly, test isolates showed complete resistance to the antibiotics used and these were isolates 2 and 8. These two isolates were however, sensitive to plant extracts and gave zones of 13.10-15.20 and 12.11-15.56mm, respectively. The highest zone of inhibition for the antibiotics was 23.00mm against isolate 5 with gentamycin and sulfamethoxazole+ trimethoprim. The aqueous extract was earlier reported to inhibit the growth of both Gram negative and positive bacteria (*Staphylococcus aureus* and *E. coli*) with inhibition zones of 16.00 and 11.30 mm, respectively.[31]

V. CONCLUSION

This study shows that *Jatropha tanjorensis* is indeed a rich source of nutrients with features comparable to other known edible leafy vegetables. Further evaluations revealed that they are a rich source of vitamins and essential minerals as well as low in anti-nutrients. This plant has been observed to possess phytochemicals and possess effective antimicrobial potential comparable to those of routinely used antibiotics against *Escherichia coli* in our study.

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