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Proximate Composition of *Jatropha curcas* Leaves, Phytochemical and Antibacterial Analysis of Its Ethyl Acetate Fraction

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Authors' contributions

This work was carried out in collaboration amongst all the three authors. Author QOA designed the study and wrote the protocol. Authors QOA, AYS and OJO managed the literature searches. Authors QOA, AYS and OJO wrote the first draft of the manuscript. All the three authors managed the analyses of the study, read and approved the final manuscript.

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ABSTRACT

Resurgence of public interest in the ethnomedical practices in both the developing and developed countries is increasing. Medicinal plants are parts of a plant or the whole plant that possess healing properties. *Jatropha curcas* (*Euphorbiaceae*) has been reported severally for its Folkloric uses as herbal remedy for the management of rheumatism, tumor etc. This study was carried out to screen the leaves of *Jatropha curcas* for its proximate nutrient constituents and also the phytochemicals and antibacterial analysis of the Ethyl acetate leaf extract. Proximate composition results showed high percentage moisture content of (89.70%) and percentage protein content of (4.35%) indicating that *Jatropha curcas* leaves is a good source of dietary protein. Qualitative and quantitative analysis of eight secondary metabolites (alkaloids, tannins, saponins, phenols, flavonoids, steroids, phlobataninns and cardiac glycosides) were undertaken. The result showed that all secondary metabolites analysed were present in the plant specie studied. Alkaloids and saponins had high percentage concentration (0.6280% and 0.4210%) respectively, which revealed the presence of

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bioactive compounds. The antibacterial activity of the ethyl acetate leaf extract against *Staphylococcus sp, Bacillus cereus, Clostridium sp, Escherichia coli, Proteus sp* and *Pseudomonas sp* showed average zone of inhibition of (17, 31, 33, 52, 37, and 33) respectively. This result confirms the potency of this plant in treating human infections. Overall this study indicates that previously reported antibacterial, anti-inflammatory properties of this herb may be due to their inherent bioactive constituents and its proximate components, thus supporting the claims of the traditional healers.

Keywords: Jatropha curcas; proximate; phytochemical; ethylacetate; antibacterial.

1. INTRODUCTION

1.1 Ethnomedicine

The practice of ethnomedicine is a complex multi-disciplinary system constituting the use of plant, spirituality and the natural environment and has been the source of healing for people for millennia [1]. Today about 80% of the world's population rely predominantly on plants and plant extracts for healthcare [2]. Ethnomedicine is the study or a comparism of the traditional medicines practiced by various ethnic groups and especially by indigenous people [3]. [4] also referred to ethnomedicine as the study of traditional medical practice which is concerned with the cultural interpretation of health, diseases and illness and also addresses the healthcare seeking and healing practices. Traditional medicines are potential source of new drug, a source of cheap starting product for the synthesis of known drugs. They are mostly compounded from natural product. Traditional medicines are cheaper than orthodox medicine which serves as therapeutic agent as well as important raw material for manufacture of traditional modern medicines. Studying medicinal plants helps to understand plants toxicity and protect human and animal from natural poisons [5].

1.2 Justification

Synthetic drugs have been proven to have adverse effects on human health, become increasingly expensive and also relatively out of reach. Therefore natural products are gaining attention as an alternative for health care [6]. Traditional medicine is cheaper than synthetic medicines and is a potential source of new drugs. Most of plant species are used as food supplement along with their oral decoctions. *Jatropha curcas* L is a specie of flowering plant in the spurge family *Euphorbiaceae*. It is a multipurpose shrub that grows throughout the arid, semi-arid tropical and subtropical regions of the world [7]. It is called Lapalapa by the Yorubas, Cinidazugu by the Hausas, Olulu idu/Uru by the Igbos, Omangba by the Iyedes in Benue State and Itiakpa by the Urhobos in Delta State. It is now widely cultivated in both tropical and sub tropical regions around the world [8]. *Jatropha curcas* leaves have been widely used (sometimes) indiscriminately as remedies for various diseases and ailment, therefore this study is poised to evaluate the proximate composition of *Jatropha curcas* leaves, phytochemicals present in its ethyl acetate fraction and the antibacterial activities of its ethyl acetate fraction. All is aimed at confirming its use in traditional remedies.

2. MATERIALS AND METHODS

2.1 Plant Collection

The fresh leaves of *Jatropha curcas* were collected within the premises of the Federal College of Animal Health and Production Technology. They were rinsed with tap water followed by rinsing in distilled water, air dried at room temperature for three weeks and pulverized into fine powder.

2.2 Extraction

Using cold extraction, exactly 200 ml of ethyl acetate was added to 100 g of the pulverized leaves in a covered bottle. The supernatants were sieved into another covered bottle after 24 hours intervals of soaking and this process was repeated for 10 days. This was the point when the added solvent showed no more sign of extraction. Thereafter the recovered supernatant containing the extract was concentrated using a rotary evaporator.

2.3 Proximate Analysis

Portions of *J. curcas* leaves were analysed for their proximate compositions using the [9] methods.

2.3.1 Moisture determination

It was determined by drying empty porcelain crucible in the oven at 105 ± 50℃ for 30 minutes

to get rid of the moisture present on the dish. The porcelain crucible was transferred into a desiccator and allowed to cool at room temperature for about 20 minutes. The weight of the empty porcelain crucible was taken and recorded as W_0. The sample was blended into powder using Cyclotec sample mill to increase the surface area. 1.0 g of the sample were weighed into the porcelain crucible (recorded as W_1) and dried in the oven at 105 ± 50℃ till constant weight. The porcelain crucible containing the sample was allowed to cool for about 10 minutes in the oven. The porcelain crucible was transferred into the desiccator and allowed to cool at room temperature for about 30 minutes. The final weight of the porcelain crucible and content was recorded as W_2. Therefore, the % moisture content was calculated using the formula:

$$\%\ \text{Moisture} = \frac{(W_0 + W_1) - (W_0 + W_2)}{W_1} \times 100$$

2.3.2 Crude fat determination

The crude fat was determined by placing thimble fitted with the adapter on a balance and tare. 1.0 g of well-prepared sample were weighed into the thimble and tare then moved to the thimble stand using the thimble handler. The soxhlet solvent extraction unit was switched on. The temperature according to the solvent used to achieve a reflux of solvent that is 3–5 drops per second was set. The proper program and check time settings for boiling/rinsing/evaporation/pre-drying on the control unit were selected. The cold water tap for the reflux condenser was opened with cooling water at approximately 15℃ adjusted to 2 L/min to prevent solvent evaporation from the condenser. A thin layer of defatted cotton was put on the top of the sample. The thimble was moved to the thimble support using thimble handler. The thimble was inserted into the extraction unit and attached to the magnet. The aluminum extraction cup (pre-dried at 103 ± 2℃) was weighed, and then loaded with petroleum ether which was inserted using cup holder. The soxhlet auto fat extraction system now performed the extraction automatically. The cup was removed, dried at 103 ± 2℃ for 30 min and weighed. The % fat was calculated thus:

$$\%\ \text{Fat} = \frac{W_3 - W_2}{W_1} \times 100$$

W_1 = Weight of sample (g)
W_2 = Empty extraction cup weight (g)
W_3 = Extraction cup + residue weight (g)

2.3.3 Crude fiber determination

The crude fiber which is the residue left after alkaline and acid digestion of organic matter was determined by pre-drying crucible at 130 ± 2˚C for 30 minutes. The pre-dried crucible was placed on a balance and tare. 1.0g of well-prepared sample was weighed into the crucible containing celite.

2.3.3.1 Hot extraction

Prepared 1.25% H_2SO_4 was heated on a hot plate.

The crucible was inserted using the holder and locked into position in front of the radiator in the fibertec hot extraction unit ensuring that the safety latch engages. The reflector was placed in front of the crucible and all valves were put to closed position. Cold water tap (1 – 2 L/min) was opened for reflux system. 150 ml of preheated 1.25% H_2SO_4 was added into each column (reagent 1), 2 – 4 drops of n – Octanol was also added to prevent foaming and turn on 'Heater' control fully clockwise. When the reagents started to boil, it was adjusted to moderate boiling using the heater control. The boiling time from the time when the solution has reached the boiling point (30 min) were measured and at the elapse of 30 min (end of extraction), the heater was turned off. The valves in 'Vacuum' were positioned and the cold water tap was opened to full flow rate for the water suction pump and the filtration started. The sample was washed three times with hot deionized water using reversed pressure. 150 ml of preheated 1.25% NaOH solution was added into each column (reagent 2). The operation above was repeated. The crucible was released with the safety hook and using the crucible holder, the crucible was transferred to the fibertec cold extraction unit.

2.3.3.2 Cold extraction

The crucible in the fibertec cold extraction unit was positioned and valves closed. 25 ml acetone was added to each crucible. Placing the valve in vacuum position, solvent was extracted and filtered and was repeated three times. Crucible was removed and transferred to a crucible stand, it was left at room temperature until the acetone was evaporated, in order to increase the burning of the fiber during the drying process. The crucible was dried for 2hours at 130 ± 20℃. The

crucible was cooled at room temperature in a desiccator and weighed. The sample in the crucible was ashed for 3 hours at 525 ± 150℃ and the crucible was heated and cooled with caution. The crucible was slowly cooled at room temperature in a desiccator and weighed. Hence % fiber was calculated using;

$$\%\ \text{Crude Fiber} = \frac{W_2 - W_3}{W_1} \times 100$$

W_1 = Sample weight (g)
W_2 = Crucible + residue weight after drying (g)
W_3 = Crucible + residue weight after ashing (g)

2.3.4 Crude protein determination

Crude protein was determined by weighing 1 gram of well-prepared sample to an accuracy of 0.1 mg into a 250 ml digestion tube. 2 kjeltabs of Cu 3.5 were added, 12 ml of concentrated H_2SO4 was added and shaked gently to wet the sample with the acid. The exhaust system was attached to the digestion tube in the rack and the water aspirator was set to full effect. The sample was digested for 1 h at 420℃. The rack of tube was removed and placed in a stand and allowed to cool for 10 – 20 minutes. Tube was inserted into the distillation unit and the safety door closed. 80 ml deionized water was added into the tube, 25 – 30 ml receiver solution was added into the conical flask and was placed into the distillation unit in the platform that the distillate outlet was submerged in the receiver solution. 50 ml of 40% NaOH was dispensed into the tube. It was distilled for about 4 minutes and the distillate was titrated with standardized HCl, (usually 0.1 or 0.2N) until the blue grey end point was achieved. The volume of acid consumed in the titration was noted and blank was run through every batch. Then the % protein was calculated using;

$$\%\ \text{Protein} = \frac{(\text{T}-\text{B}) \times \text{N} \times 14.007 \times 100}{W_1} \times \text{F}$$

Volume sample (ml)
W1 = Sample weight (mg)
T = Titration volume of sample (ml)
B = Titration volume of blank (ml)
N = Normality of acid to 4 decimal places
F = Conversion factor for nitrogen to protein = 6.25 for food & feeds gN/l = Gram Nitrogen per Liter.

2.3.5 ASH content determination

The ash content was determined by drying empty crucible in the oven at 130 ± 15℃ for 30 minutes to get rid of moisture present on the crucible. The crucible was transferred into a desiccator and allowed to cool at room temperature for about 20 minutes. The weight of the empty crucible was taken and recorded as W_0. The sample was blend into powder using cyclotec sample mill to increase the surface area. 1.0g of sample was weighed using an analytical balance into the crucible (recorded as W_1) and ash it in the furnace at 500±15℃ for 5 – 6 hours. The crucible containing the sample was allowed to cool for about 30 minutes in the furnace. The crucible was transferred in the desiccator using a crucible tongue and allowed to cool at room temperature for about 45 minutes and the final weight of the crucible and content were recorded as W_2. The ash content was then determined with the formula:

$$\%\ \text{Ash Content} = \frac{(W_2 - W_0)}{W_1} \times 100$$

2.3.6 Nitrogen free extract determination

NFE (carbohydrate) was determined by calculating the difference after analysis of all the other content in the proximate analysis.

NFE = (100 - % moisture + % ash + % crude protein + % crude fat + Crude fiber).

2.4 Phytochemical

The extract was subjected to qualitative and quantitative phytochemical tests for tannins, alkaloids, saponins, flavonoids, steroids, phenols, phlobatannin and cardiac glycosides. Qualitative test was carried out on the extract in the Federal College of Animal Health and Production Technology using standard procedures as described by [10,11,12,13].

2.5 Antibacterial

2.5.1 Tested bacteria

The bacteria used as the test organisms were Gram positive (*Staphylococcus sp, Bacillus cereus* and *Clostridium sp,*). Gram negative (Escherichia *coli, Proteus sp and Psedomonas sp*).

Media used were Nutrient agar and MacConkey agar.

2.5.2 Preparation of culture media

2.5.2.1 Nutrient agar

The medium was prepared by weighing 14 g of agar powder into 500 ml of distilled water in a conical flask and shaken very well to dissolve. It was autoclaved at 121℃ for 15 minutes and allowed to cool at 47℃ before pouring into agar plates aseptically in the required amount. The medium was then allowed to solidify.

2.5.2.2 MAC conkey agar

This medium was prepared by weighing 26 g of agar powder into a conical flask with 500 ml of distilled water. The flask was corked, shaken to soak and autoclaved at 121℃ for 15 minutes. After autoclaving, it was allowed to cool at 47℃ before pouring into the plates aseptically in the required amount and allowed to solidify.

2.5.3 Inoculation and incubation

2.5.3.1 Streaking method

An inoculating loop or wire loop which had been sterilized by direct flame was used to scrape the bacteria isolate surface. The wire loop was used to streak the surface of each labeled petri dishes containing the prepared agar. The streaked media was incubated for 24 hours at 27℃ and was checked for surface colony growth. Streaking method was done to subculture the organisms.

2.5.3.2 Pour plate method

2 ml of distilled water was measured into a MacCartney bottle and the already streaked plates were brought out, inoculums were picked from the plates and this was dropped into the MacCartney bottle. The mixture was then poured into different labeled sterile petri dishes containing prepared agar. Sensitivity disc containing the conventional antibiotics to be used was introduced into the plates by placing it on the media that contains the inoculums. Agar well diffusion method was used in the assessment of the antibacterial activity of the extract as described by [14]. This method was achieved by using a sterile cork borer to make well at the centre of the prepared media (nutrient and MacConkey agar). 0.2 ml of the extract was dropped into the well and allowed to diffuse for 5 minutes and incubated for 24 hours at 37℃.

3. RESULTS AND DISCUSSION

The proximate composition of the fresh leaves of *Jatropha curcas* is as presented in Table 1. The fresh leaves of the plant had a high moisture value (89.70%); this shows it's important, since high moisture levels accelerate all types of food deterioration like chemical, enzymatic and microbial actions. The significant value (4.35%) of crude protein shows that the leaves are good source of protein. *Jatropha curcas* can provide with appreciable amount of protein which provides enormous benefits such as maintenance of fluid balance, optimal functioning of hormones, enzymes and also contributes to immune function. The crude fat content has the least value (1.16%) when compare with other content value in the table. Crude protein, Crude fat, and total Ash content of (4.35%, 1.16%, and 1.38%) obtained respectively from the leaves in this study was higher than (1.90, 0.69 and 0.55) values reported in the leaves of *Jatropha curcas* by [15]. Moreso, Fat and oils are the most abundant lipids found in nature. They are a heterogeneous group of organic compounds, which are important constituents of plants and animal tissue. The crude fiber of the leaves was founds to be (1.93%). Crude fiber in diet consists mostly of plant polysaccharides that cannot be digested by human dietary enzymes such as cellulose, hemicellulase and some materials that encrust the cell wall [16]. Fibre content is a significant component of the diet. It increases stool bulk and decreases the time that waste materials spend in the gastrointestinal tract. Ash content which is an index of mineral contents in the leaves was (1.38%). The function of carbohydrate in the body is to supply energy. The leaves had carbohydrate content of (1.48%). This indicates that the leaves are good source of energy.

Table 1. Proximate analysis of fresh leaves of *Jatropha curcas*

Parameter	Values (%)
Moisture	89.70
Crude protein	4.35
Crude fat	1.16
Crude fiber	1.93
Total ash	1.38
NFE	1.48

Key: NFE – Nitrogen free extract

Phytochemical constituent (Table 2) indicates some chemical substances that produce a

definite physiological action on the human body. They are secondary plant metabolites responsible for many observed bioactivity of plants. Tannins present in the leave with a value of 0.0034%. They have been reported to form irreversible complexes with proline rich protein, [17] thereby inhibiting cell protein synthesis [18]. Intestinal disorders such as diarrhea and dysentery have been treated with herbs that have tannins as their main components [19].

Another secondary metabolite observed in the leave extract of *J. curcas* was alkaloid at a value of 0.6280%. Alkaloids have analgesic effects [20]. Alkaloids have been acclaimed for their antimicrobial activities, especially against gram negative bacteria [21]. Other secondary metabolites present in *J. curcas* whose antimicrobial activities have been documented are flavonoids and saponins with values of 0.0052% and 0.4210% respectively. Saponins are the plants immune system acting as in antibiotic to protect the plant against microbes and fungi and also have haemolytic activity against red blood cell (RBC). Flavonoids are group of polyphenolic compounds with known properties of free radical scavenging, antibacterial and anti inflammatory. Steroids have been reported to have antibacterial properties and they are very important compounds due to their relationship with compounds such as sex hormones [22] and its percentage after analysis gave 0.0068 in this study.

Various effects of different antibiotics on each bacterial isolates were observed during this experiment. After incubation, it was observed that there were zones of inhibition in both the conventional antibiotics plate and that of ethyl acetate extract plate. Antibiotic Gentamycin and Ofloxacin inhibited the growth of both gram positive and gram negative but not all the conventional antibiotics used show significant zones of inhibitions like ethyl acetate extract. The ethyl acetate extract was found to be effective against gram positive and gram negative than known conventional antibiotics. From the result of this analysis, the effect of ethyl acetate extract (33mm) against *clostridium sp* was more than antibiotic (Gentamycin 15mm and Ofloxacin 20mm). This shows that it can be a better cure for bacterial disease such as tetanus [23]. The zone of inhibition value of *Jatropha curcas* leave extract on *Bacillus cereus* and *Pseudomonas sp.* are higher compared to a similar work done [24].

Table 2. Qualitative and quantitative phytochemical analysis of the ethyl acetate fraction of *Jatropha curcas* leaves

Phytochemical	Qualitative	Quantitative (%)
Alkaloids	+	0.6280
Saponins	+	0.4210
Tannins	+	0.0034
Flavonoids	+	0.0052
Steroids	+	0.0068
Philobatannins	+	0.0016
Phenols	+	0.0970
Cardiac glycosides	+	0.2210

Table 3. Average values of antibacterial activities of the ethyl acetate fraction of *Jatropha curcas* leaves

	Zone of inhibition (mm)	
Isolate	**Antibiotics**	**Ethyl acetate extract**
(Gram +ve)		
Staphylococcus sp	32(Gen), 29(Ofl)	17
Bacillus cereus	22(Gen), 20(ofl)	31
Clostridium sp	15(Gen), 20(ofl)	33
(Gram -ve)		
Escherichia coli	28(Gen), 25(Ofl)	52
Proteus sp	20(Ch)	37
Pseudomonas sp	40(Cpx), 35(Pef, Ofl)	33

Key: Gen – Gentamycin, Ofl – Ofloxacin, Ch – Chloramphenicol, Cpx – Ciprofloxacin, Pef – Pefloxacin

4. CONCLUSIONS

This study has revealed the presence of some biological active compounds and nutrients in the leaves of *Jatropha curcas.* It has further confirmed that the plant extracts could be used for the treatment of various diseases and infections based on the results of its phytochemical and antibacterial analysis. The result corroborates the folkloric use of this plant in treating microbial infections and diseases. It also shows that *Jatropha curcas* could be exploited for new potent antibiotics. Based on this studies work it can be concluded that the leaves of *Jatropha curcas* can be a very good source for herbal drugs.

5. RECOMMENDATIONS

Indigenous knowledge systems are culturally valued and scientifically important. Strengthening the wise use and conservation of indigenous knowledge of useful plants may benefit and improve the living standard of poor people. It is therefore recommended that this work can be explored further for the extraction of antimicrobial agents by more sophisticated procedures for extraction. In addition, herbal therapy trials should be carried out so as to verify the medicinal potential of *Jatropha curcas leaves*.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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