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Proximate Composition of Leaves and Root extract of *Jatropha tanjorensis* (*Ellis* & Saroja)

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ABSTRACT

Jatropha tanjorensis is herbaceous taxon belong to the Euphorbiaceae family commonly called hospital too far. The leaves are consumed as vegetables, fodder and medicine The study determined proximate composition of leaves and root extract of Jatropha tanjorensis. The result shows that Carbohydrate, Crude Protein and moisture had high concentrations of 80.04±0.55, 8.82 ± 0.09, and 5.29 ± 0.14 respectively. Crude Fat, Crude Fiber and Ash contents were low in concentrations of 2.67±0.12, 1.33±0.07 and 1.15±0.16 respectively. Phyto-chemical constituent present in the extract of J. tanjorensis leaves and roots metabolites which includes tannin, saponins among others. The results obtained from this study contribute to the scientific validation for the use of this medicinal plant in traditional medicine which could be standardized culturally to as a broad spectrum for curing diseases.

Introduction

The history of studying and working with medicinal plants is quite chemists and botanist are interested in studying medicinal plant which has not been research before to identify which component in the plant are active and to see how those compound works (wasan, 2014) use of plants sources of medicine has been inherited and is an important component of the health care System. Herbal medicine derived from plant extracts are being utilized The increasingly to treat wide variety of clinical disease studies have shown that commonly consumed medicines plants are good sources of polyphenols, saponin, flavonoids and phenypreponoids. This compounds display a vast variety of pharmacological activities such as anti-inflammatory, anti-cancer, anti-bacteria, anti-oxidant, anti-fungal, anti-viral activities. The purpose of identifying of phyto-chemical in plant was to attain the therapeutically deferred active portion and eliminate unwanted materials. A knowledge of chemical constituent of plant is desirable not only for the discovered of the therapeutic agents, but also for disclosing new sources of economics phyto-compounds for the synthesis of complex chemical substances and for discovering the actual significant of folkloric.

The study identified the chemical components and bioactive properties Phyto-chemical constituent present in the extract of *J. tanjorensis* leaves and roots. Jatropha tanjorensis is herbaceous taxons belong to the euphorbiaceae family commonly called hospital too far. The leaves are consumed as vegetables, fodder and medicine (Olayiwola, 2004). But the economic values of the root are yet to be known. The leaves of *J. tanjorensis* based of claims by traditional healers have the efficacy in the treatment of anaemia and malaria fever (Omoregie and Sisodia, 2011

Materials and methods

Study Area

The analysis was carried out federal university of technology Minna Niger state. Collection and identification of plant material Jatropha tanjorensis root and leaf material were collective on February 2019 from kontagora , Niger state. And

conveyed for identification and authentication at biology department, federal college of education kontagora, the leaves and root were dried in a room temperature for 24hours to remove moisture content and the ground using mechanical grinder put in air tight container and store in desiccators for the further analysis.

Preparation of plant extract
Fresh *J. tanjorensis* leaves and roots were dried under shade within a temperature range of 26-28°C and ground into fine powder with an electric blender

Authentication of the Plant
The Plants was Authenticated and Identified at Department of Plant Science Bayero University. Kano with Herbarium Accession Number Bukhan 666

Determination of Moisture Content
2.0 grams of the sample was accurately weighed into a previously cleaned, dried and weighed crucible. The crucible with its content was put into a Gallenkamp drying oven at 105^{o}C for 3 hours. The sample was then cooled in desiccators and weighed. The process was repeated until a constant weight was obtained. The loss in weight expressed as a percentage of the initial weight of sample gave the percent moisture (AOAC, 2000).

$$\%\text{Moisture} = \frac{(\text{weight of wet sample} - \text{weight of dry sample})}{\text{sample weight}} \quad \ldots\ldots\ldots\ldots (1)$$

Determination of Ash Content
1 gram of the samples was weighed into a clean dried and cooled crucible. It was incinerated in a furnace at 550 to 600°C for 3 hours. It was then removed and allowed to cool in desiccators and weighed again. The percentage ash content was calculated as:

$$\text{Ash content} = \frac{\text{wt of ash (g)}}{\text{wt of original sample (g)}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Crude Protein Content Determination
2.0 g of the sample was weighed into a digestion flask containing 0.5g of selenium catalyst. 25 cm^3 of concentrated H_2SO_4 was added and the contents thoroughly mixed. The flask was then heated on a digestion burner for 8 hours until the solution was green and clear. The solution was transferred into a 100cm^3 volumetric flask and made up to the mark with distilled water. 25cm^3 of 2% boric acid was pipette into a 250cm^3 conical flask and 2 drops of mixed indicator (20cm^3 of bromocresol green and 4cm^3 of methyl red) solution were added 10cm^Z of the digested sample solution was then introduced into a Kjeldahl flask, the condenser tip if the distillation apparatus containing 15cm^3 if 40% NaOH was dipped into the boric acid contained in the conical flask. The ammonia in the sample solution was then distilled into the boric acid until it became bluish green. The distillate was titrated with 0.1M HCl solution colourless end point. The percent total nitrogen and crude protein were calculated (AOAC, 2000).

Crude protein determination:

$$\%\text{Total nitrogen} = \frac{(100 \times (VA - VB) \times M \times 0.01401) \times 100}{10W} \quad \ldots\ldots\ldots\ldots (3)$$

Where:
VA = volume (cm^3) of HCl used in the sample titration
VB = volume (cm^3) of HCl used in the blank titration
M = Molarity of HCl
W = weight of sample (g)
% Crude protein = % nitrogen x 6.25

Crude Fibre Content Determination
2.0g of the defatted sample (from crude fat determination) was transferred into a 250cm^3 Erlenmeyer flask and 2.5cm^3 of 1.25% H_2SO_4 was added. The content of the flask was boiled under reflux and digested for 30 minutes. At the end of the 30minutes, the content was filtered and subsequently washed with boiling water until the washings were no longer acidic using blue litmus paper. The sample was washed back into the flask with 200cm^3 boiling 1.25% NaOH solution and boiled for 30 minutes. It was then filtered and thoroughly washed with boiling water until the washings were no longer alkaline using red litmus paper. The crucible with its content was then dried in an oven at 105^{o}C overnight and cooled in desiccators and weighed. The crucible with its content was them ignited in a furnace at 600^{o}C for 30minutes, cooled and weighed. The loss in weight was expressed as a percentage of the initial weight of the sample (AOAC, 2000).

$$\%\text{Crude fibre} = \frac{(\text{wt. of crucible} + \text{sample before ignition}) - (\text{wt of crucible} + \text{ash}) \times 100}{\text{weight of fresh sample}}$$

$\ldots\ldots (4)$

Carbohydrate Content Determination

Total percentage carbohydrate (Nitrogen Free Extr
act) was determined by the difference method as reported by Amadi et al, (2004). This method involves adding the total values of crude protein, crude fat, crude fibre, moisture and ash constituents of the sample and subtracting it from 100. The value obtained is the percentage carbohydrate.

% Carbohydrate = 100 – (% moisture + % ash + % protein + % fat + % fibre).... (5)

Determination of Crude Lipid Content

5g of the sample was weighed into the extraction thimble, and about 50cm3 of petroleum ether (40 – 60^{o}C) range was added to the extraction flask. A condenser was fixed at the top of the extractor. The flask was fitted into the extraction unity and refluxed to about 60^{o}C for 6 hours. The ether extract was evaporated on an evaporating bath until the lipid was solvent free. This was dried in an oven at 100^{o}C for 1 hour, cooled in a desiccator and weighed. The lipid was stored in plastic containers for further analysis.

$$\%\text{Total nitrogen} = \frac{(\text{weight of beaker} + \text{lipid}) - (\text{weight of beaker}) \times 100}{\text{Sample weight}} \quad \ldots\ldots (6)$$

Phytochemical Analyses

Phytochemical analysis includes the Phenol determination, determination of Saponin, Alkaloids, Flavonoid, Tannin, Phytate and Oxalate.

Determination of Total Alkaloids

0.5g of the sample was dissolved in 96% ethanol -20% H2SO4 (1:1). 1ml of the filtrate was added to 5cm^3 of 60% tetraoxosulphate (VI), and allowed to stand for 5min. Then; 5cm^3 of 0.5% formaldehyde was added and allowed to stand for 3h. The reading was taken at absorbance of 565nm. The extinction coefficient (E296, ethanol {ETOH} = 15136M^{-1}cm^{-1}) of vincristine was used as reference alkaloid. (Harborne, 1976)

Determination of Saponins

0.5g of the sample was added to 20cm^3 of 1NHCl and was boiled for 4h. After cooling it was filtered and 50cm^3 of petroleum ether was added to the filtrate and either layer evaporated to dryness. 5cm^3 of acetone ethanol was added to the residue. 0.4cm^3 of each was taken into 3 different test tubes. 6cm^3 of ferrous sulphate reagent was added into them followed by 2cm^3 of concentrated H_2SO_4. It was thoroughly mixed after 10min and the absorbance was taken at 490nm. Standard saponin was used to establish the calibration curve. (Oloyed, 2005)

Determination of Tannin

0.2g of sample was measured into a 50cm^3 beaker. 20cm^3 of 50% methanol was added and covered with para film and placed in a water bath at 77-80^{O}C for 1hr. it was shaken thoroughly to ensure a uniform mixture. The extract was quantitatively filtered using a double layered Whatman No.41 filter paper into a 100cm^3 volumetric flask, 20cm^{3}water added; 2.5cm^3 Folin-Denis reagent and 10cm^3 of Na2CO3 were added and mixed properly. The mixture was made up to mark with water mixed well and allowed to stand for 20min for the development of a bluish-green color. The absorbencies of the tannic acid standard solutions as well as samples were read after colour development on a UV-spectrophotometer model 752 at a wavelength of 760nm. (AOAC, 2005)

Phytic Acid Content

The Phytic acid content was determined using a modified indirect colorimetric method of Wheeler and Ferrel (1971). The method depends on an iron t phosphorus ratio of 4:6 and is based on the ability of standard ferric chloride to precipitate phytate in dilute HCI extract of the sample. 5g of the sample was extracted with 20cm^3 of 3% trichloroacetic acid and filtered. 5ml of the filtrate was used for the analysis; the phytate was precipitated as ferric phytate and converted to ferric hydroxide and soluble sodium phytate by adding 5cm^3 of IM NaOH. The precipitate was dissolved with hot 3.2M HNO_3 and the absorbance and immediately at 480nm. Preparation of standard curve for phytic acid was done as follows: standard curve of different $Fe(NO_3)_3$ concentrations was plotted against the corresponding absorbance of spectrophotometer to calculate the ferric iron concentration. The phytate phosphorus was calculated from the concentration of ferric iron assuming 4:6 Iron: phosphorus molar ratio.

Determination of Cyanide

Cyanide content was determined by alkaline picrate method according to Wang and Filled method as described by Onwuka, 2005. 5g of powdered sample was dissolved in 50ml of distilled water in a cooked conical flask and the extraction was allowed to stand over-night, filtered. 1cm^3 of sample filtered was mixed with 4cm^3 alkaline picrate in a corked test tube and incubated in a water bath for 5mins. After colour development (reddish brown colour) the absorbance was read at 490nm, the absorbance of the blank containing 1ml distilled water and 4ml alkaline picrate solution was also recorded. The cyanide content was extrapolated from cyanide standard curve prepared from different concentration of KCN solution containing 5-50µg cyanide in a 500cm^3 conical flask followed by addition of 25cm^3 of INHCI.

Determination of Oxalate

Oxalate in the sample was determined by permanganate titrimetric method as described by Oke, 1966. 2g of the sample flour was suspended in 190cm^3 of distilled water in 250cm^3 volumetric flask, 10cm3 of 6M HCI was added and the suspension digested at 100^{0}C for 1hr, cooled, then made to the mark before filtration. Duplicate portion of 125cm^3 of the filtrate were measured into beakers and 4 drops of methyl red indicator added. This is followed by the addition of cone. NH_4OH solution drop wise until the test solution changes from salmon pink colour to a faint yellow colour (pH 4-4.5). Each portion is then heated to 90^{0}C, cooled and filtered to remove precipitate containing ferrous ion. The filtrate is again heated to 90^{0}C and 10cm^3 of 5% $CaC1_2$ solution added while being stirred constantly. After heating, it was cooled and left overnight at 5^{0}C. The solution was then centrifuged at 2500rpm for 5mins, the supernatant decanted and the precipitate completely dissolved in 10cm^3 of 20% (v/v) H_2SO_4 solution. The total filtrate resulting from the digestion was made up to 300cm^3. Aliquots of 125cm^3 of the filtrate was heated until near boiling and then titrated against 0.05M standardized $KMnO_4$ solution to a faint pink colour which persisted for 30s. The calcium Oxalate content is calculated using the formula:

$$\frac{T \times (Vme)(Df) \times 10^5}{(ME) \times Mf} \quad \text{.......} \quad (18)$$

Where T is the titre of $KMnO_4$ (cm^3), Vme is the volume-mass equivalent 1cm^3 of 0.05M $KMnO_4$ solution is equivalent to 0.00225g anhydrous oxalic acid), Df is the dilution factor V_T/A (2.5 where V_T is the total volume of titrate (300ml) and A is the aliquot used (125ml $KMnO_4$ redox reaction), ME is the molar equivalent of $KMnO_4$ in oxalate () and Mf is the mass of flour used

Results

Table 1: Flavonoid Contents in the Root and Leaves Extracts of *Jatropha tanjorensis*

Extracts	Sample weight	Absorbance	% flavonoid
Roots	0.5001	1.530	0.306
Leaves	0.5000	2.040	0.405

Table 2: Tannins Contents in The Root and Leaf Extracts of *Jatropha tanjorensis*

Extracts	Sample weight	Absorbance	% tannins
Roots	0.5007	1.436	0.287
Leaves	0.5010	2.100	0.419

Table 3: Alkaloids Contents in the Root and Leaf Extracts of Jatropha tanjorensis

Extracts	Sample weight	Absorbance	% saponin
Roots	0.5010	0.129	0.026
Leaves	0.5036	0.168	0.033

Table 4: Saponins Contents in the Root and Leaf Extracts of Jatropha tanjorensis

Extracts	Sample weight	Absorbance	% alkanoid
Root	0.5027	0.134	0.027
Leaves	0.5019	0.145	0.029

Table 5.: Mineral Content of *Jatropha tanjorensis* Root Extract

Minerals	Compositions
Moisture	5.29 ± 0.14
Ash Content	1.15 ± 0.16
Crude Protein	8.82 ± 0.09
Crude Fat	2.67 ± 0.12
Crude Fibre	1.33 ± 0.07
Carbohydrate	80.04 ± 0.55

All values are the mean of triplicate determinations expressed in dry weight basis standard deviation c

Table6; Anti-nutritional contents of *Jatropha tanjorensis* Root

Anti-nutritional	quantity
Saponins	1.8 ± 0.07
Flavonoid	2.5 ± 0.31
Alkaloid	2.7 ± 0.24
Phytate	3.6 ± 0.17

Value represented as mean of triplicate values ± standard deviation.

The results from table 6 shows that Phytate had the highest concentration which is 3.6±0.17 and Alkaloid, Flavonoid, Oxalate, Tannin, Saponin, Alkaloid are low in concentrations which are 2.7±0.24,2.5±0.31,2.6±0.21,1.5±0.14 and 1.8±0.07 respectively.

Table 7; Mineral Contents of *J .tanjorensis* Root Extracts

Mineral Contents	**Value mg/ 100g**
Sodium (Na)	54.21 ± 0.01
Potassium (K)	142.61 ± 0.22
Phosphorus(P)	6.10 ± 0.14
Calcium (Ca)	22.4 ± 0.03
Iron (Fe)	31.40 ± 0.01
Zinc (Zn)	3.40 ± 0.01

Value represented as mean of triplicate values ± standard deviation The results from table 7 shows that Potassium, Sodium, Iron, Calcium and Phosphorus has the highest concentrations which are 142.61±0.22, 54.21±0.01, 31.40±0.01, 22.4±0.03 and 6.10±0.14 respectively and zinc is low in concentration which is 3.40±0.01.

.**Discussion**

Tannins and flavonoids have biological activities that are of benefit in the prevention and management of many ailments (James et al, 2007)) associated antimicrobial activity with presence of tannins and flavonoids therefore the presence of tannins and flavonoids in the leaf and root extract of j. tanjorensis is an indication of presence of essential secondary metabolites. The determination of the proximate constituents is necessary in assessing nutritional levels of plant parts of frequency consumed in traditional medicine (Namadina *et al*, 2019) the moisture content of the powdered plant material using loss on drying methods was found to be 8.0% and this values is within the permissible limits W H O (2011) recommended any the percentage moisture content in any crude drug to be within 12-14%. The high moisture content provide for greater activity of water soluble enzymes and co-enzymes needed for metabolic activities of these plants.

The presence of trace metals such as zinc, from manganese in the root and leaf extract of J. tanjorensis. These element are rich sources of macro and minor elements that aid in the growth of plants, and as well in human body functions such as muscle contraction, bone formation growth metabolic, osmotic balance, regulatory activities (Rabia et al., 2012). The concentration of element go hen from the study was within FAO/WH0 permissible limits for edible plants .Zinc (2n) is an element that aids in normal growth, reproduction, tissue repair and wound heading. Zinc deficiency causes growth retardation and skin lesions Saponins have different antimicrobial activities due to alkaloids on them and their presence save as an indicator towards possible antibacterial activities (Dangoggo et al, 2012) Tannins compound are also present in the extract and are compound which have the ability to react with proteins to form stable water insoluble components and since bacterial cell are made up of proteins, tannins are seen as active detoxitying agents by precipitating the proteins compounds and hence inhibiting their growth. . Aiyelaagbe et al. (2018) investigated the antimicrobial activity of the plant *Jatropha multifida*. Seth and Sarin (2010) studied the antibacterial activity of different solvents extracts of *Jatropha gossypifolia* against Escherichia coli and Bacillus subtilis.. Saetae and Suntornsuk (2010) studied the antifungal activities of ethanolic extract from *Jatropha curcas* seed cake. Purohit and Reena (2011) studied the antimicrobial activity of methanol and petroleum extracts of dried bark extracts of *Jatropha gossypifolia*. The methanol extracts of bark of the plant showed prominent antimicrobial activity in comparision to petroleum ether extracts at specific dose 200ug/100ul. Arekemase (2011) analysed the antimicrobial activity of the hexane, ethanolic and aqueous extracts of *Jatropha curcas* against different microorganisms responsible for various human infections. The extracts and latex displayed potent antimicrobial activity against *Staphylococcus aureus*, *Neisseria gonorrhea*, *Pseudomonas aeurginosa*, *Escherichia coli*, *Candida albicans* and *Aspergillus flavus*. The results confirmed the potency of this plant in treating human infections including sexually transmitted diseases. Omoregie and Sisodia (2012) evaluated the antiplasmodial activity of the extracts from *Jatropha tanjorensis* leaves .The antiplasmodial activity of the crude ethanolic extract was moderate when compared with the standard antimalaria drug chloroquine The antiplasmodial activity of the plant leaves supported the local claims on its efficacy in the treatment of malarial infection. Dhale and Birari (2013) studied the antimicrobial effects of petroleum ether, alcohol and chloroform extracts of *Jatropha gossypifolia* aganist gram-positive species *Staphylococcus spp*. and *Bacillus spp*. and gram negative species like *Escherichia spp.*and *Pseudomonas spp.* by agar disc diffusion method. The alcoholic extract of leaves showed maximum antibacterial activity. The significant antibacterial activity of active extract was compared with standard antibiotic Ampicillin. The extract showed highly significant antidiarrheal activity.

Alkaloids have a wide range of pharmacological activities including antimalarial (e.g., quinine), anticancer (Kittakoop et al., 2014) antibacterial (e.g., chelerythrine) (Cushnie et al., 2014), and antihyperglycemic activities (e.g., piperine) (Qiu et al., 1997). Tannin is one of the major active ingredients found in plant based medicines (Cushnie et al., 2014),they are used in the dyestuff industry as caustics for cationic dyes (tannin dyes), and also in the production of inks (iron gallate ink), textile dyes, antioxidants in beverages, and coagulans in rubber production aswell as possessing antiviral, antibacterial, and antitumor activity (Khanbabaee & Van Ree, 2001) Tannin has been reported to selectively inhibit HIV replication (Kashiwada *et al*, 1992

Conclusion

Jatropha tanjorensis leaves and root posses metabolites which include tannin, Saponins, The result obtained from this study contributes to the scientific validation for the use of this medicinal plant in traditional medicine which could be standardized culturally to as a broad spectrum for curing diseases.

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