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QUALITATIVE PHYTOCHEMICAL ANALYSIS OF ETHANOL LEAVES EXTRACT OF *JATROPHA TANJORENSIS* AND ITS EFFECTS ON LIVER FUNCTION OF MALE ALBINO WISTAR RATS

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ABSTRACT: *This research work focuses on the phytochemistry of Jatropha tanjorensis leaves extract, and its activities on specific serum liver enzymes. Phytochemical screening of the leaves extract was carried out following standard procedures. To check the effects of these phytochemicals on the serum liver enzymes biomarker (Aspartate amino transferase, Alanine amino transferase and Alkaline phosphatase), twenty (20) healthy and mature male Albino Wistar rats randomly distributed into four groups of five each were used. Group I received water and normal rat feed, Groups II, III and IV were given water, normal rat feed and in addition, were administered Jatropha tanjorensis leaves extract 100mg/kg, 250mg/kg and 500mg/kg respectively for 14 days. The results of the phytochemical study revealed the presence of flavonoids, alkaloids, tannins, saponins, terpernoids, cardiac glycosides and anthroquinones in the extract. Similarly, treatment with the leaves extract showed a noticeable decrease in all the three serum liver enzymes investigated, AST: 355.40 ± 2.18, 319.28 ± 2.18, 255.14 ± 2.66, ALT: 132.51 ± 1.29, 138.44 ± 1.38, 149.93 ± 1.95, ALP: 116.32 ± 0.57, 113.58 ± 1.77, 104.81 ± 0.29, as compared to the control group 410.24 ± 2.13, 159.22 ± 0.85 and 120.84 ± 1.45 respectively. This finding suggests that Jatropha tanjorensis leaves exhibit hepato-protective properties which could aid in the prevention of liver damage.*

KEYWORDS: *Jatropha tanjorensis* leaves extract, phytochemistry, alanine amino transferase, Aspartate amino transferase, alkaline phosphatase

INTRODUCTION

Plants have been used sinced the medieval times for medicinal purposes for the treatment of all kinds of ailments. Some plants apart from serving as food have also been known to exhibit medicinal properties. Over 400,000 species of tropical flowering plants have medicnal properties, (Akpulu *et al.,* 1994). An estimated 60% of antitumor and anti-infectious drugs already in the market or under clinical trials are of natural origins, (Sofowora, 2008).

The medicinal properties of these plants are suspected to be as a result of the antioxidant, antipyretic, antimicrobial effects of secondary metabolites of phytochemicals in them. (Soetan and Aiyelaagbe, 2009). Although plant based natural medicines are popularly acclaimed to be

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safe, scientists advocate for proper toxicological studies (Oyewole *et al.*, 2008), in order to ensure safety in the use of natural medicines. Toxicity is the undesirabe property of any drug or chemical capable of producing injurious or detrimental effects on a living organism. Whether or not these injuries occur depend on the amount of chemical absorbed.(Gossel and Bricker, 1990). The toxic effect caused by drugs is similar in man and other animals, hence the use of animal models in toxicological studies. Most toxic effects of drugs occur at a predictable time after administration. However, the target organ of toxicity is not neccesarily the site of accumulation.(Oboh and Masodje, 2009).

Jatropha tanjorensis belongs to the family Euphorbiaceae. It is commonly called Hospital Too Far, Catholic Vegetable (Iwalewa *et al.*, 2005). The leaf is commonly consumed in many parts of Southern Nigeria as it does not require any special conditions to grow in the Southern soil. It is popular as a medicinal plant and is commonly used as a natural remedy for diabetes, malaria, infection, hypertension and some other conditions in this region, (Olayiwola *et al.*, 2004). The roots of this plant have been documented to serve as booster of blood and immune system, (Ebe and Chukwuebuka, 2019).

The liver is an essential organ, used for bile production and excretion, excretion of bilirubin, cholesterol, hormones, and drugs. The liver is also needed for metabolism of fats, proteins, and carbohydrates. Any condition that damages the liver and prevents it from functioning well is seen as liver disease. Liver disease can be inherited (genetic) or caused by a variety of factors that damage the liver, such as viruses and alcohol use. Obesity is also associated with liver damage. Cases of liver damage are alarming (Ebe, 2011). Over time, damage to the liver results in cirrhosis, this can lead to liver failure, a life-threatening condition.

The present work therefore studied the effects of ethanol extract of *Jatropha tanjorensis* leaves on liver function of male Albino Wistar rats, inoder to determine whether the leaves could have hepato-protective activities on the liver or not. The effect of the extract on liver function of the exprimental animals was assessed by testing for liver enzymes suchs as Aspartate amino transferase (AST), Alanine amino transferase (ALT), and Alkaline phosphatase (ALP).

MATERIALS AND METHODS

Fresh leaves of *Jatropha tanjorensis* were collected from an uncultivated land in Uyo Local Government Area of Akwa Ibom State, Nigeria. The leaves were authenticated at the department of Botany, University of Uyo, Nigeria. The leaves were washed and air dried at room temperature in the laboratory for three (3) weeks and grinded into powder form using a mechanical grinder. 450g of this sample was soaked in 1.5 litres of 80% ethanol in a large container, stirred, covered and left for 24 hours. This extract was filtered and placed in a water bath for 72 hours at 50°C to get rid of excess solvent (ethanol). The crude extract obtained was used for the research.

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Qualitative Phytochemical Analysis of Extracts

Standard procedures were adopted for qualitative phytochemical analysis of the leaves extract using High performance Liquid Chromatography (HPLC) and spectrophotometry. In addition, the steps described by Sofowora (2008) were followed.

Test for saponins: In a clean autoclaved test tube, 10ml of sterile water was taken and 0.5 g of the ethanol extract was added. The test tube was stirred vigorously; foaming was formed in the test tube which was an indication of the presence of saponins in the extract.

Test for tannins and phenolic: In a clean sterilized test tube was taken 0.5 g of the extract and 12 ml of sterile water was added. The mixture was stirred and then filtered. The filtrate was transferred to another test tube where 4 droplets of 6% $FeCl_3$ were supplemented to the filtrate. Blue black colouration indicated the occurrence of phenolic and tannins in the extract.

Test for steroids: In a clean and dried test tube was taken 0.5 g of the extract along with 4 drops of acetic anhydride and a drop of concentrated H_2SO_4. The combination was allowed to stand for 1 hour and neutralized with NaOH. A blue green colour indicated the presence of steroid in the extract.

Test for glycosides: In a clean and dried test tube was taken 0.5 g of the extract along with 3 ml of chloroform. Concentrated H_2SO_4 was carefully added to the test tube. A reddish brown colour at the border showed the existence of a steroidal molecule, which was a glycine portion of the cardiac glycosides.

Test for Flavonoids: In a clean and dried test tube was added 5 ml of ethanol extract along with concentrated H_2SO_4 (1ml) and 0.5 g of Magnesium. A pink colouration that disappeared on standing for 3 minutes showed the presence of flavonoids.

Test for alkaloids: In a 100 ml clean flask, 20 ml of ethanol extract solution was placed. The solution was evaporated by heating the beaker. The dried residue obtained was dissolved in 5 ml of HCl (2N) and filtered. 3 drops of Meyer's reagent were added; the presence of precipitate indicated the alkaloids.

Test for phylobatannins: In a clean test tube 3 ml of the extract solution was placed along with 3 ml of 2% HCl and the combination was allowed to bioleach. Appearance of a red aggregation was taken as an evidence for the occurrence of phylobatannins in the extract.

Test for anthraquinones: In a clean test tube 3 ml of ethanol extract solution was shaken and filtered and 6 ml of 12% ammonia was added to the filtrate. The combination was allowed to stand and the presence of a pink colour in the ammonical segment showed the existence of anthraquinones.

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Test for terpenoids: In a clean test tube 3 ml of the extract solution was dissolved in 3 ml of chloroform and vaporized to dryness. 3 ml of concentrated H_2SO_4 was added and heated for 3 minutes, grayish colour showed the existence of terpenoids

Experimental Animals and Extract Administration

Twenty (20) healthy and matured male Albino Wistar rats weighing between 150-262g were used in this study. The rats were housed in the Basic Medical Sciences animal house University of Uyo in wooden cages covered with net in a well-ventilated room under standard laboratory conditions. During the experimental period, animal feed (growers) and water were provided *ad libitum*. The animals were assigned into four (4) groups of five rats each. Group I served as the control group, Groups II, III and IV were administered *Jatropha tanjorensis* leaves extract 100mg/kg body weight, 250mg/kg body weight and 500mg/kg body respectively for 14 days. Administration was done orally using a fabricated cannula (constructed from syringe and feeding tube) and the doses were calculated based on the body weight of the animals. All experimental animals were treated in line with the guidelines of Institutional Animal Ethical Committee. At the end of the administration, the rats were fasted overnight (12 hours) and anaesthetized by putting each in glass chamber (desiccator) containing chloroform. The blood samples were obtained by cardiac puncture using sterile syringes and needles and placed into sterile plain tubes.

Sera samples were obtained from the clotted blood after centrifugation at 2000 rpm for 5 minutes and the supernatant serum was collected and stored in sample tubes and refrigerated for subsequent analysis.

Enzymes Assayed

Three enzymes assayed in this study were Aspartate amino transferase or Glutamic oxaloacetic transaminase (AST or GOT), Alanine amino transferase or Glutamic pyruvic transaminase (ALT or GTP), and Alkaline phosphatase (ALP) based on UV- kinetic method. All chemical kits used were of analytical grade, obtained from Randox Laboratories Limited, 55 Diamond road, Crumlin, Country Antrim, BT29 4QY, United Kingdom.

Estimation of Aspartate Amino Transferase (AST) Activity

Randox laboratories limited concentration reagents used were;

(i) phosphate buffer (100mmol/l, pH 7.4,
(ii) L-aspartate (100mmol/l),
(iii)α-oxoglutarate (2 mmol/l),
(iv)2,4-dinitrophenylthyldrazine (2mmol/l).

Principle

The enzymatic reaction sequence employed in the assay of aspartate amino transferase is as follows:

AST

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α-oxoglutarate + L-aspartate ⟶ L - glutamate + Oxaloacetate

Oxaloacetate + NADH + H^+ $\xrightarrow{MDH}$ Malate + NAD^+ + H_2O

Oxaloacetate formed in the first reaction is reduced to malate in the presence of malate dehydrogenase and NADH as cofactor. The activity of aspartate amino transferase is determined by measuring the rate of oxidation of NADH at 546nm.

Procedure

Twenty test tubes were secured in the test tube rack and 100µl of the test samples was pipetted into the test tubes. Five hundred (500) µl of buffer (reagent 1) was pipetted into the reagent blank and the test tubes. One hundred (100) µl of distilled water was pipetted into reagent blank. The solution was mixed gently for 10 seconds and incubated for 30 minutes at 37°C. Thereafter, 500µl of 2,4-dinitrophenyl hydrazine was pipetted into the reagent blank and test tube samples. The content was mixed gently and allowed to stand for 20minutes at 25°C. Five thousand (5000) µl of Sodium Hydroxide was pipetted into the reagent blank and test tube samples. This was mixed gently for 10 seconds. The absorbance of the sample was read against the reagent blank. The wavelength was set at 546nm and the reagent blank was used to zero the spectrophotometer. Each test sample was transferred carefully into cuvette and the absorbance was read against the reagent blank.

Estimation of Alanine Amino Transferase (ALT) Activity

Randox laboratories limited concentration reagent contents used were;

(i) Phosphate buffer (100 mmol/l. pH 7.4),
(ii) L- Alanine (200 mol/l).

Principle

The enzymatic reaction sequence employed in the assay of ALT is as follows:

α - Oxoglutarate + L – alanine ⟶ ALT L - glutamate + pyruvate

Pyruvate + NADH + H^+ $\xrightarrow{LDH}$ Lactate + NAD^+ + H_2O

Alanine amino transferase (ALT) catalyses the transfer of amino group from alanine to 2-oxoglutarate, forming pyruvate and glutamate. The pyruvate formed in the first reaction is reduced to lactate in the presence of lactate dehydrogenase and NADH (cofactor). The activity of alanine amino transferase is determined by measuring the rate of oxidation of NADH at 546nm. ALT is measured by monitoring the concentration of pyruvate hydrazine formed with 2, 4-dinitrphenyl hydrazine.

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Procedure

Twenty test tubes were secured in the test tube rack and 100µl of the test samples was pipetted into test tubes. Five hundred (500) µl of buffer (reagent 1) was pipetted into the reagent blank and the test tubes. One hundred (100) µl of distilled water was pipetted into reagent blank. The solution was mixed gently for 10 seconds and incubated for 30 minutes at 37°C. Thereafter, 500µl of 2,4-dinitrophenyl hydrazine was pipetted into the reagent blank and test tube sample. This was mixed and allowed to stand for 20 minutes at 25°C. Five thousand (5000) µl of sodium hydroxide was pipetted into the reagent blank and test tube samples. The content was mixed gently for 10 seconds. The wavelength was set at 546nm and the reagent blank was used to zero the spectrophotometer. Each test sample was transferred carefully into cuvette and the absorbance was read against the reagent blank.

Estimation of Alkaline Phosphatase Activity (ALP)

Alkaline phosphatase activity in serum was quantitatively determined by the use of Randox Laboratory reagent kit which employs the Kinetic procedure for its determination.

Randox laboratory Limited concentration reagents used were:

(i) Diethanilamine buffer (1mol/l, pH 9.5),
(ii) $MgCl_2$ (0.5 mmol/l),
(iii)p-nitrophenylphosphate (10 mmol/l)

Principle

The enzymes sequence employed in the assay of alkaline phosphatase involves the reaction steps below.

$$\text{P-nitrophenylphosphate} + H_2O \xrightarrow{\text{ALP}} \text{phosphate} + \text{P-nitrophenol}$$

The enzymes rate is determined using a 2-amino-2-methyl-1-propanol (AMP) buffer to measure activity of alkaline phosphatase in serum. Here, alkaline phosphatase catalyses the hydrolysis of colourless organic phosphate ester substrate, P-nitrophenyl phosphate, to yellow coloured product, P-nitrophenol and phosphate. The system monitors the rate of change of absorbance at 405nm over a fixed time interval. This rate of change in absorbance is directly proportional to the alkaline phosphatase activity in serum.

Procedure

Twenty test tubes were secured in the test tube rack and 100 µl of the test samples was pipetted into the test tubes. Two hundred and fifty (250) µl of buffer (reagent 1) was pipetted into the reagent blank and the test tubes. One hundred (100) µl of distilled water was pipetted into reagent blank. The solution was mixed gently for 10 seconds to ensure a complete mixture.

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Thereafter, 250µl of P-nitrophenyl phosphate was pipetted into the reagent blank and test tube samples. The mixture was maintained at 37°C for 60 seconds. This was mixed gently for 10 seconds. The wavelength was set at 405 nm using an empty cuvette with 1 cm light path to zero the spectrophotometer. Each test sample was transferred carefully into cuvette and the initial absorbance was read. The timer was started simultaneously and absorbance was read again after 1, 2 and 3 minutes. The average absorbance was calculated and used to determine the concentration. This was done by multiplying the average absorbance by 2760 to calculate U/I of alkaline phosphatase activity.

Statistical Analysis

Data were analyzed using mean ± standard deviation, and comparison between the test groups and control group was performed using student's T' test. Means were considered significantly different at P = 0.05.

RESULTS

Table 1 below shows the results of the phytochemical screening of the ethanol extract of *Jatropha tanjorensis* leaves. Phytochemical analysis of the extract revealed the presence of flavonoids, alkaloids, tannins, saponins, terpernoids, cardiac glycosides and anthroquinones. Phylobatannins and steroids were absent.

TABLE 1: PHYTOCHEMICAL SCREENING OF ETHANOL LEAVES EXTRACT OF *Jatropha tanjorensis*

PHYTOCHEMICALS	INDICATIONS
Flavonoids	+
Alkaloids	+
Tannins	+
Phylobatannins	-
Steroids	-
Terpernoids	+
Saponins	+
Cardiac glycosides	+
Anthroquinones	+

Key:
+ = present
- = absent

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The biochemical assessment of the liver enzymes of Albino Wistar rats treated with ethanol extract of *Jatropha tanjorensis* leaves are presented on table 2. The results obtained for AST were 410.24 ± 2.13, 355.40 ± 2.18, 319.28 ± 2.18 and 255.14 ± 2.66 for groups I (control), II, III and IV respectively. For ALT determination, the results showed 159.22 ± 0.85, 132.51 ± 1.29, 138.44 ± 1.38 and 149.93 ± 1.95 for groups I, II, III and IV respectively. For ALP, the results gave 120.84 ± 1.45, 116.32 ± 0.57, 113.58 ± 1.77 and 104.81 ± 0.29 for groups I, II, III and IV respectively.

TABLE 2: EFFECT OF ETHANOL LEAVES EXTRACT OF *Jatropha tanjorensis* ON LIVER FUNCTION OF MALE ALBINO WISTAR RATS

Groups	Concentration mg/kg body weight	AST (µl)	ALT (µl)	ALP (µl)
I	Control	410.24 ± 2.13	159.22 ± 0.85	120.84 ± 1.45
II	100	355.40 ± 2.18	132.51 ± 1.29	116. 32 ± 0.57
III	250	319.28 ± 2.18	138.44 ± 1.38	113.58 ± 1.77
IV	500	255. 14 ± 2.66	149.93 ± 1.95	104.81 ± 0.29

Values are given as Mean ± Standard Deviation (SD), n = 5.
Values are significantly different from Control at P = 0.05.

DISCUSSION

This study was conducted to determine the phytochemical composition of ethanol extract of *Jatropha tanjorensis* leaves and its effect on liver function of male Albino Wistar rats. The results indicated the presence of flavonoids, alkaloids, tannins, saponins, terpernoids, cardiac glycosides and anthroquinones in the extract. This finding is consistent with previous work by Ehimwenma and Osagie (2007).

Flavonoids have antioxidant activity which has many beneficial effects on the cardiovascular system, also, epidemiological studies have illustrated that heart diseases are inversely related to flavonoid intake ((Evans, 1989).). This implies that taking flavonoid containing foods reduced the chances of developing heart disease. Flavonoids prevent the oxidation of low-density lipoprotein, lowers the blood levels of cholesterol and triglycerides thereby reducing the risk for

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the development of atherosclerosis (Subramani and Casimir, 2002). Flavonoids have also been reported to have vaso-dilatory and inhibitory effects on platelet aggregation thereby preventing coronary heart diseases (Okwu, 2004).

Saponins have been reported to have beneficial effects on blood cholesterol levels by preventing its re-absorption. The non-sugar part of saponins also has antioxidant activity which may help to reduce risk of heart diseases (Oakenfull and Sidhu, 1990). Cardiac glycosides which were also found in the ethanol extract of *Jatropha tanjorensis* leaves have been variously used from time immemorial as diuretics and heart tonics due to their beneficial effects on the heart. Cardiac steroids are widely used in the modern treatment of congestive heart failure and for treatment of atrial fibrillation. They increase the force of contraction of the heart and are very useful for heart failure patients. They act by affecting the availability of intracellular Ca^{2+} for myocardial contraction or increasing the sensitivity of myocardial contractile proteins (Walker *et al.*, 2002).

Tannins have been reported to be responsible for decreases in feed intake, growth rate, feed efficiency, net metabolisable energy, and protein digestibility in experimental animals. Therefore, foods rich in tannins are considered to be of low nutritional value. However, it has been suggested that consumption of tannin containing beverages, especially green teas and red wines can cure or prevent a variety of illness including heart related diseases (Van-Burden and Robinson, 1981). Terpenoids occur in nearly every natural food (including *Jatropha tanjorensis*) and have been associated with protection from and treatment of heart disease due to their antioxidant properties (Wagner and Elmadfa, 2003).

Liver function test (LFT) is commonly used in clinical practices to screen for liver diseases, monitor the progress of a known disease and determine the effects of potentially hepatotoxic drugs (Dufour *et al*., 2000). It also helps in detecting hepatocellular necrosis but is considered a less specific biomarker enzyme for hepatocellular injury (Ozer *et al*., 2008) as it can also signify abnormalities in heart, muscle, brain or kidney (Dufour *et al*., 2000). Data accumulated from this study showed that the ethanol leaves extract of *Jatropha tanjorensis* showed a significant decrease ($P = 0.05$) in serum AST in groups II, III and IV as compared to the control. The ratio of serum AST to ALT can be used to differentiate liver damage from other organ damage (Nathwani *et al*., 2005). Inflamed or injured liver cells release higher than normal amounts of specific chemicals including liver enzymes into the blood stream resulting in elevated levels of these liver enzymes after serum examination. The extracts of *Jatropha tanjorensis* leaves showed a significant decrease on the serum levels of AST, indicating that the leaves extract has no negative or toxic interaction on the hepatocytes. ALT activity in the liver is much higher than that of the serum. This implies that in the case of hepatocellular injury or death, release of ALT from the damaged liver cells increases measure ALT in the serum. Elevated level of this enzyme is released during liver damage. The estimation of this enzyme is a more specific test for detecting liver abnormalities since it is primarily found in the liver (Dufour *et al*,. 2000) However, lower enzymatic activities are also found in skeletal muscles and heart tissue. This enzyme detects hepatocellular necrosis. An elevated ALT level is therefore an indicator of

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hepatocellular damage and has been used as a marker of liver injury. From the results, it was discovered that ALT decreased significantly (P = 0.05) in groups II, III and IV when compared to the control. ALP is another biomarker employed to assess liver function. It is often employed to assess the integrity of the plasma membrane. ALP also may be elevated if bile excretion is inhibited by liver damage. Hepatotoxicity leads to elevation of the normal values due to the body's inability to excrete it through bile due to the congestion or obstruction of the biliary tract. In humans, increased ALP levels have been associated with drug induced cholestasis (Dufour *et al.*, 2000).

The decrease in liver enzymes concentration in the serum of the experimental rats studied is an indication that administration of *Jatropha tanjorensis* leaves extract may not cause liver damage. This result shows no apparent toxic effects of the extract on the liver of the experimental animals and could therefore be effective as hepato-protective agent.

CONCLUSION

Phytochemical screening of *Jatropha tanjorensis* leaves revealed the presence of bioactive compounds such as flavonoids, alkaloids, tannins, saponins, terpernoids, cardiac glycosides and anthroquinones.

This research also showed that the plant leaves extract was able to alter the concentration of certain liver enzymes in the serum, leading to the decrease of AST, ALT and ALP in the serum of the Albino Wistar rats. Based on the results, the plant leaves extract may have the ability to protect the liver; this could reduce the risk of liver damage or disease.

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