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Free Radical Scavenging Activity of *Jatropha curcas* Leaves, Phytochemical and Antibacterial Analysis of Its Butanol Crude Extract

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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

The use of different plant parts for the prevention and cure of ailments, infections and diseases as an alternative to the use of orthodox medicine is gaining more attention. Moreso, resistance to orthodox drugs has been confirmed in various literature. The free radical scavenging activities of *Jatropha curcas (Euphorbiaceae)* leaves, phytochemicals present in its butanol crude extract as well as the antibacterial activities of its butanol crude extract were accessed. The leaves of *Jatropha curcas* were washed, air-dried and pulverized for active extraction of the composition of the plant in butanol. Fresh leaves of the plant were tested for its DPPH (2,2-Diphenyl-1-picrylhydrazyl) scavenging activity (indicator of antioxidant), while the butanol crude leaf extract

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was accessed for the presence or absence of phytochemicals and was also tested against some gram positive and gram negative bacteria all according to standard procedures. All analysis was carried out at the Chemistry Laboratory of the Federal College of Animal Health and Production Technology, Moor Plantation, Ibadan for four weeks. The result of the free radical scavenging activity of the leaves (74.73 mg/kg) was higher than that of ascorbic acid (31.01 mg/kg) (a standard antioxidant). The qualitative analysis showed the presence of alkaloids, tannins, saponin, flavonoids, steroid, phenols phlobatanins and cardiac glycoside. The quantitative analysis on the other hand showed the presence of alkaloids in an appreciable amount (0.567%), followed by saponin (0.316%) while others had relatively low values (below 0.19%). The average values of the antibacterial (inhibitory) activity as compared with standard antibiotics used had no significant difference except for *Pseudomonas sp.* which was very low to the antibiotics used. It could be concluded that *Jatropha curcas* is of great medicinal potential.

Keywords: Jatropha curcas; antibacterial; scavenging activity; phytochemicals.

1. INTRODUCTION

Ethnomedicine refers to the study of traditional medicinal practice which is concerned with the cultural interpretation of health diseases and illness and also addresses the health care seeking process and healing practices [1].

Ethnomedicinal plants have been used since ancient time for human healthcare and still remain the most widely used medication system in developing and least developed nations [2].

The reliance of people on ethnomedicine has been for reasons of cost-effectiveness, acceptability, biomedical benefits and accessibility. However, there has been a continuous growth of demand for herbal medicine globally [3].

Synthetic drugs have become expensive and not easily accessible by the less privileged. Moreso, resistance to most synthetic drugs are a serious health concern in the world today. In addition, people have returned to traditional medicine and natural plants are now used as drugs for various ailments based on their folkloric uses. Plant derived-bioactive compounds have received considerable attention due to their therapeutic potential as antimicrobial, anti-inflammatory properties and antioxidant activities [4].

Jatropha curcas (*J. curcas*) is a specie of flowering plant in the spurge family – *Euphorbiaceae* [5].

Jatropha curcas leaves have been used as cure for various ailments like; skin infection, diarrhea and cancer e.t.c. [6,7,8]. It has also been explored for alopecia, anasorea, ascites, burns, carbuncles, convulsions, cough, dermatitis, diarrhea, eczema, fever, rashes, sores, ache, and rheumatism [9].

Therefore, this research is targeted at evaluating the free radical scavenging activities of *Jatropha curcas* leaves, phytochemicals present in its butanol crude extract as well as the antibacterial activities of its butanol crude extract in order to ascertain some of its claims in traditional healing.

1.1 Free Radicals and DPPH Scavenging Activities

Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism [10]. Reactive oxygen (ROS) and reactive nitrogen species (RNS) are products of normal cellular metabolism. The most common ROS include superoxide anion Peroxy nitrite anion [11]. These reactive species play an important role in pathogenesis, cardiovascular diseases, and rheumatism. It is possible to reduce the risk of chronic diseases and prevent diseases progression by either enhancing the body's natural antioxidant defenses or by supplementing with dietary antioxidants [12]. Antioxidant offer resistance against oxidative stress by scavenging the free radicals inhibiting lipid peroxidation and by many other mechanisms and thus prevent disease progression. DPPH is a stable nitrogen centered free radical commonly used for testing radical scavenging activity of the compound or plant extracts. When stable DPPH radical accept an electron from the antioxidant compound the violet color of the DPPH reduce to yellow color or red. Diphenylpicrylhdrazyl radical which was measured colorimetrical. Substance which are able to perform this reaction can be

considered as antioxidant and therefore a radical scavenger [13].

1.2 Antibacterial

Antibacterial also known as antibiotics is a type of antimicrobial drug used in the treatment and prevention of bacterial infection. They may kill or inhibit the growth of bacteria. They are not effective against viruses such as common cold or influenza. Antibiotics may be given as a preventive measure (prophylactic) and this is usually limited to at risk populations such as those with a weakened immune system (particularly in HIV cases to prevent pneumonia) [14].

2. MATERIALS AND METHODS

2.1 Experimental Material

Some of the experimental apparatus used for this work included; Petri dishes, 25 ml conical flasks, boiling tubes, Bunsen burner, 5 ml, 10 ml and 20 ml measuring cylinders. Experimental material included; *J. curcas* leaves, while the reagents utilized included; Butanol, Ethanol, Ethyl acetate, Chloroform used were all BDH general purpose chemicals and distilled prior to use. 2,2-diphenyl-1-picrylhydrazyl (DPPH), Ascorbic acid were obtained from Sigma Chemical Co. (St. Louis, MO). HCl, Conc. H_2SO_4, Potassium iodide, Mayer's reagent, Dilute ammonia, Potassium ferrocyanide, Acetic acid, Olive oil, Ferric chloride solution MacConkey agar, Nutrient agar were all BDH general purpose chemicals. Some of the Instruments used in this research included; Mettler analytical balance H80 (UK), Water Bath (Gallenkamp), UV-Visible spectrophotometer (Unico1200 & Perkin Elmer Lambda 25 models).

2.2 Experimental Procedure

Sample collection: Fresh plant sample (leaves) was collected around the quarters of the Federal college of Animal Health and Production Technology, Moor Plantation, Ibadan (around September, 2016). They were then taken to the Botany unit of the Institute of Agricultural Research and Training, Moor Plantation, Ibadan for proper identification. 1 kg of fresh leaves were harvested, the fresh leaves were used for the DPPH scavenging activities while the rest were air-dried to preserve some phytochemical constituent which are thermolabile and could be denatured by heat and also to prevent physiological change of the leaves.

The air-dried samples were pulverized, using an electric blender in order to reduce them to fine particles for effective extraction of the bioactive compounds.

Exactly, 100 g of the dried pulverized leaves were weighed into a 1000 ml beaker and excess butanol was added until the samples were fully immersed, the extraction was carried out by decanting the butanol (supernatant) every 24 hours and were filtered through a muslin. This was replaced with fresh volumes of butanol and was repeated for 10 days.

The samples were concentrated using rotary evaporator until all the butanol had evaporated and the extract was obtained.

2.3 DPPH Free Radical Scavenging Activity

The DPPH scavenging activity of the leaves was carried out according to the methods as decribed by [15,16].

100 g of fresh sample was weighed into a beaker, 100 ml of ethanol was added and shaked vigorously for 2 min, it was stirred with a magnetic stirrer for 15 min, it was allowed to stand for 2 hours for proper extraction, then centrifuged at 2500 rpm for 10 min. The supernatant was poured into another beaker and concentrated by evaporating in a water bath at 80°C. The concentrated extract was kept for further assay.

1 mM DPPH was prepared in ethanol (394.32 mg DPPH dissolved in 1 litre of ethanol. It was dissolved with 10 mg of the concentrated sample extract in 10 ml of ethanol (1 mg/ml) 1.5 ml of the extract was pipette into a test tube. 1.5 ml DPPH solution prepared was added into the test tube. The spectrophotometer was zeroed with ethanol as blank. Thereafter the absorbance\optical density of the control (DPPH solution) and that of the sample was read at 517 nm.

$$\text{DPPH Scavanged \%} = \frac{absorbance\ of\ control - absorbance\ of\ test\ sample \times 100}{absorbance\ of\ control + sample\ weight} \quad (1)$$

This procedure was also carried out for Vitamin C (ascorbic acid) a standard antioxidant.

2.4 Qualitative Phytochemical Analysis

The crude extract was subjected to qualitative and quantitative phytochemical tests for tannins, alkaloids, saponins, flavonoids, steroids 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 [17,18,19,20].

2.4.1 Test for alkaloids

0.5 g of the plant sample was dissolved in 5ml dilute HCl in a steam bath and filtered. 1 ml of the above filterate was treated with few drops of Mayer's reagent. A creamy white precipitate was observed which indicated the presence of alkaloids.

2.4.2 Test for tannin

Exactly 0.5 g of each plant extract was stirred with about 20 ml of distilled water and then filtered. Few drops of 0.1% ferric chloride solution were added to the filtrate. A dirty green precipitate was observed, indicative of the presence of tannin.

2.4.3 Test for saponin

2 gram of each portion was boiled with 20 ml of distilled water filtered. To the filterate about 3 ml of distilled water was further added and shake vigorously for about 5 min frothing which persisted on warming was observed. This was a positive test for saponin.

2.4.4 Test for flavonoids

A portion of the powdered plant sample was heated with 10 ml ethyl acetate over a steam bath for 3 min. it was filtered and 4 ml of the filterate was shaken with 1 ml of dilute ammonia. A yellow coloration indicating the presence of flavonoids was observed.

2.4.5 Test for steroids

About 0.2 g of the plant extract, 2 ml of acetic acid was added and the solution was cooled well, Conc. H_2SO_4 was added to the solution. A violet to blue color indicates the presence of a steroidal ring.

2.5 Quantitative Phytochemical Analysis

2.5.1 Test for alkaloids

2 g of the extracted sample was weighed into a 100 ml beaker and 20 ml of 80% absolute alcohol added to give a smooth paste. The mixture was transferred to a 250 ml flask and more alcohol added to make up to 100 ml and 1 g magnesium oxide added. The mixture was digested in boiling water for 1.5 hours under a reflux air condenser with occasional shaking. The mixture was filtered while hot through a small bucher funnel. The residue was returned to the flask and redistilled for 30 minutes with 50 ml hot water to replace the alcohol lost. When all the alcohol had been removed, 3 drops of 10% HCl was added. The whole solution was later transferred into a 250 ml volumetric flask 5ml of zinc acetate solution and 5ml of Potassium Ferro cyanide solution was added thoroughly mixed to give homogenous solution.

The flask was allowed to stand for a few minutes filtered through a dry filter paper and 10 ml of the filtrate was transferred into a separatory funnel and alkaloids present were extracted vigorously by shaking with five succession portions of chloroform. The residue obtained was dissolved in 10 ml hot distilled water and transferred into a kjeldalh tube with the addition of 0.20 g sucrose and 10ml Conc. H_2SO_4 and 0.02 g selenium for digestion to colorless solution to determine %N by kjeldahl distillation method. % N got was converted to % total alkaloid by multiplying with a factor 3.26 i.e

$$\% \text{ total alkaloid} = \%N + 3.26 \quad (2)$$

2.5.2 Test for flavonoids

Exactly 0.50 g of the extracted sample was weighed into a 100ml beaker and 80ml of 95% ethanol added and stirred with a glass rod to prevent lumping. The mixture was filtered through a whatman NO 1 filter paper into a 100 ml volumetric flask and made up to mark with ethanol. 1ml of the extract was pipette into 500 ml volumetric flask , four drops of conc. HCl added via a dropping pipette after which 0.5 g of magnesium turning was added to develop a magenta red coloration . Standard solution was read on digital labomed 200 spectrophotometer at a wavelength of 520

nm. The percentage flavonoids was calculated using the Formula

% flavonoid =

$$\frac{absorbance\ of\ sample \times average\ gradient \times dilution\ factor}{weight\ of\ sample \times 10000} \quad (3)$$

2.5.3 Test for tannin

Exactly 0.20 g extracted sample was measured into a 500 ml of beaker, 20 ml of 50% methanol was added and covered with paraffin and placed in water bath at 77-80°C for 1 hour. It was shaked thoroughly to ensure uniform mixing. The extract was quantitavely filtered using a double layered whatman NO. 41 filter paper into a 100 ml volumetric flask, 20 ml water added, 2.5 ml folin-denis reagent and 10 ml of 17% Na_2CO_3 were added and mixed properly . the mixture was made up to mark with water mixed well and and allow to stand for 20 mins, the bluish-green color which developed at the end of range 0-10 ppm were treated similarly as 1 ml sample above.

The absorbance of the tannic acid standard solutions as well as samples were read after color development on a spectrophotometer at a wavelength of 70 mm. %tannin was calculated using the formula:

% tannin =

$$\frac{absorbance\ of\ sample + average\ gradient\ factor + dilution\ factor}{Weight\ sample \times 10000} \quad (4)$$

2.5.4 Test for saponin

1 g of sample was weighed into a 250 ml beaker and 10 ml of butyl alcohol was added. The mixture was shaken on a UDY shaker for 5 hrs to ensure uniform mixing. Thereafter the mixture was filtered through a whatman NO.1 filter paper into a 100ml beaker and 20 ml of 40% saturated solution of magnesium carbonate was added. The mixture obtained with saturated $MgCO_3$ was again filtered through a whatman NO. 1 filter paper to obtain a clear colorless solution was pipette into a 500 ml volumetric flask and 2 ml of 5% $FeCl_3$ solution was added and made up to mark with distilled water . It was allowed to stand for 30 min for blood red color to development in a Jenway V6300 spectrophotometer at a wavelength of 380 nm.

% saponin =

$$\frac{absorbance\ of\ sample + average\ gradient + dilution\ factor}{Weight\ sample \times 10000} \quad (5)$$

2.5.5 Test for steroid

0.5 g of sample extract was weighed into a 100 ml beaker 20 ml of chloroform- methanol (2:1) mixture was added to dissolve the extract upon shaking for 30mins on a shaker. The whole mixture was filtered through a whatman NO. 1 filter paper into another dry clean 100 ml conical flask /beaker.

The resultant residue was repeatedly treated with chloroform- methanol mixture until it was free of steroids. 1 ml of the filterate was pipette into a 300 ml test tube and 5 ml of alcoholic KOH was added and shaken thoroughly to obtain a homogenous mixture. The mixture was later placed in a water bath set at 37°C- 40°C for 90 mins. It was cooled to room temperature and 10 ml of petroleum ether was added followed by the addition of 5ml of chard reagent to residue in dry bottle and absorbance taken at a wavelength of 620nm on spectronic 20D spectrophotometer. Stand steroids of concentration of 0.4mg/l were prepared from 100 mg/ml stock steroid was calculated using equation 6:

$$\frac{absorbance\ of\ sample + average\ gradient + dilution\ factor}{Weight\ sample \times 10000} \quad (6)$$

2.5.6 Test for glycosides

10 ml of extract was pipetted into a 250 ml conical flask. 50 ml chloroform was added and shaken on a vortex mixer for 1hour,the mixture was filtered into 100 ml conical flask and pyridine, 2 ml of 2% sodium nitroprusside were added and shaken thoroughly for 10 minutes. 3ml of 20% NaOH was later added to give a brownish yellow color. The absorbance of the sample and the standards were read on a spectronic 20D digital spectrophotometer at a wavelength of 510 nm. Percentage glycoside was calculated using equation 7:

$$\frac{absorbance\ of\ sample + average\ gradient + dilution\ factor}{Weight\ sample \times 10000} \quad (7)$$

2.6 Antibacterial Susceptibility Test

Agar well diffusion method was used in the assessment of the antibacterial activity of the extract as described by [16,21]. Tested bacteria 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.

2.6.1 Preparation of culture media

MacConkey agar of 24.00 g and 14.00 g of nutrient agar were weighed into two different conical flasks (500 ml each), covered with aluminum foil and shaken thoroughly and then left to disperse. They were then sterilized at 121°C for 15 mins in autoclave. They were then poured into petri dishes and left to solidify. After which it was incubated for 24 hrs at 37°C.

2.6.2 Isolation of organism

The inoculating loops were sterilized and were used to pick the samples. They were then used to streak the surface of each labeled petri-dishes containing the prepared media. The streaked media were then kept for the bacterial susceptibility test.

2.6.3 Bacterial susceptibility test

24 hrs old standardized culture of bacteria was subcultured into distilled water and 1 ml of the broth was used to flood the surface of the media prepared and allowed to dry. A sterile cork borer of 1 cm was used to make holes and 1 ml of the extract was dropped into each hole of the labeled dishes. Antibiotics were also placed on the surface of the media containing the cultured bacterial which were then incubated at 37°C for 24 hrs, the minimum zone of inhibition of the extract and antibiotics were compared and records were taken.

3. RESULTS AND DISCUSSION

Table 1 reveals the DPPH scavenging activity (indicator of the antioxidant) in fresh *Jatropha curcas* leaves in which the value was relatively high compare to the antioxidant ability of ascorbic acid a standard antioxidant which proves that the leaves have the ability of scavenging free radicals and the oxidation process in the body. These free radicals are responsible for some diseases and therefore *Jatropha curcas* has the ability to cure disease since it has the ability to inhibit the oxidation processes in human body that are responsible for these diseases.

The qualitative and quantitative phytochemical analysis of the butanol extract of the leaves of *J. curcas* (Table 2), showed the presence of most phytochemicals like alkaloid, tannin, saponin, flavonoid, glycoside and phenol. This corroborates the findings of [22].

The medicinal value of this plant lies in its phytochemical constituents since each phytochemical is said to have a definite physiological and pharmacological action on the human body. Different classes of phytochemicals have been found to possess wide range of activities which helps in prevention and protection against diseases. The presence of alkaloid in the butanol extract could make the plant active against malaria, asthma and support its use as an analgesic.

Phytochemicals such as flavonoids, phenol are effective as antioxidant while saponin and glycosides are effective as anti-inflammatory and antibiotics respectively [12].

Antibacterial activity of the extract (Table 3) revealed that the minimum zones of inhibition (mm) values were not significantly different when compared with the antibiotics used which shows that the leaves could be used as antibacterial agents with the exception of *pseudomonas sp.* in which the its inhibitory zone value was relatively low to that of the antibiotics used.

[23] reported that presence of biologically active principle (as confirmed in Table 2) may aid the antimicrobial activities of *J. curcas* as these secondary metabolite exerts antimicrobial activity through different mechanisms.

Table 1. DPPH free radical scavenging activity of fresh *Jatropha curcas* leaves

Parameter	Fresh leaves (mg/kg)	Ascorbic acid (mg/kg)
Antioxidant	74.73	31.01

Table 2. Qualitative and quantitative analysis of butanol crude extract of *Jatropha curcas* leaves

Parameters	Qualitative	Quantitative (%)	Inference
Alkaloids	+	0.5670	Present
Phlobatannins	+	0.0005	Present
Tannins	+	0.0018	Present
Saponin	+	0.3160	Present
Flavonoids	+	0.0020	Present
Steroids	+	0.0037	Present
Cardiac glycosides	+	0.1880	Present
Phenol	+	0.0760	Present

Table 3. Average value of the antibacterial activity of butanol extract of *Jatropha curcas* leaves

Isolates	Zone of inhibition (mm)	
	Antibiotics	Butanol extract
Gram +ve		
Staphylococcus sp.	32(gen), 29(ofl)	30
Bacillus cereus	22(gen), 20(ofl)	20
Clostridium sp.	15 (gen), 20(ofl)	15
Gram –ve		
Escherichia coli	28(gen), 25(ofl)	41
Proteus sp.	20(ch), 20(ch)	17
Pseudomonas sp	40(cpx), 35(pef,ofl)	22

Key: Cpx-ciprofloxacin; Pef-pefloxacin; Ofl-ofloxacin; Ch-chloraphenicol; Gen –gentamycin

This indicates that the plant extract is effective against most of the bacteria used in this study. Therefore, the plant extracts have medicinal properties which support its use in the treatment of various forms of infections.

4. CONCLUSION

It can therefore be concluded from these research that *Jatropha curcas* leaves has bioactive compounds (phytochemicals) as well as a high free radical scavenging activity (indicator of antioxidant activity). This is also evident in its ability to inhibit the growth of some bacteria. Therefore, *Jatropha curcas* has great medicinal potential and this could justify the use of its leaves in folklore therapy.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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