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Phytochemical Content, Radical Scavenging and Antibacterial Properties of Aqueous Extract of *Jatropha curcas* Linn Leaves

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Abstract

This study investigated phytochemical content, radical scavenging and antibacterial activities of aqueous extract of leaves of *Jatropha curcas* Linn. Quantitative phytochemical analyses of alkaloids, phenols, tannins and flavonoids contents were carried out; radical scavenging activity was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and hydrogen peroxide inhibition assays while Disc diffusion and Agar well (ditch) diffusion methods were used for antibacterial activity against *Klebsiella pneumonia*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Proteus species* at 62.5, 125, 250 and 500 mg/mL. The extract contained alkaloids (1.600 ± 0.58 %), tannins (0.121 ± 0.00 mg/mL), phenols (0.463 ± 0.06 mg/mL) and flavonoids (0.672 ± 0.00 mg/mL), and showed radical scavenging activities against DPPH (IC₅₀ = 21.24) and hydrogen peroxide (15.67 mg/mL) which were less than that of Butylated hydroxyanisole (BHA); IC₅₀ = 3.92 (DPPH) and 6.19 mg/mL (hydrogen peroxide) respectively. It also showed antibacterial activity against *Klebsiella pneumonia*, *Escherichia coli*, and *Pseudomonas aeruginosa* at 250 and 500 mg/ml (MIC = 125 mg/mL), which were resistant to ampicillin, chloxacillin and erythromycin, and the extract was inactive against *Staphylococcus aureus* and *Proteus species* at these concentrations, though all were sensitive to gentamycin. This shows that the aqueous extract of leaves of *Jatropha curcas* Linn may possess some of the folkloric properties claimed.

Keywords: *Jatropha curcas*; Euphorbiaceae; aqueous extract; antibacterial activity; radical scavenging activity

Introduction

Jatropha curcas Linn (family Euphorbiaceae) is commonly called physic nut, purging nut or pig nut in English, *lapalapa funfun* in Yoruba, South-west Nigeria. It is cultivated in several tropical and subtropical countries, including Nigeria (Belewu *et al.*, 2010). It is a shrub of about 3 meters high. When matured its leaves are green in colour and its twig is very rich in latex. It is used against

erosion because of its drought-resistant nature (Ejelonu *et al.*, 2010).

J. curcas serves many purposes: its seeds contain oil used in the production of biofuel and for treatment of skin diseases (Okujagu *et al.*,

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2006; Belewu, 2008). The seeds are also used as livestock feeds, though, potentially toxic if untreated (Belewu and Ogunisola, 2010; Belewu *et al.*, 2010) while medicinally, various researches have reported that its latex contains jatrophine which is used in wound healing, cough, rheumatism and skin diseases (Uche and Aprioku, 2008; Ejelonu *et al.*, 2010). Previous studies have reported that it contains bioactive components which could cure sexually transmitted diseases, mouth odour and jaundice, and as antiseptic during child birth (Igbinsosa *et al.*, 2008; Namuli *et al.*, 2011). Rug and Ruppel (2000) also reported the molluscicidal and larvicidal activities of the seed extracts.

Different parts of the plant have been reported to contain phenolics, flavonoids, saponins, glycosides, tannins and alkaloids (Thomas *et al.*, 2008; Oskoueian *et al.*, 2011) and its bioactive components are said to have potentials as antimicrobial, anti-inflammatory, anticancer and antioxidant principles (Rathee *et al.*, 2009). Recently, Oskoueian *et al.* (2011) and James *et al.* (2011) showed that the methanol extracts of the leaves, stem bark, latex and roots exhibited antioxidant, anti-inflammatory, wound healing, antimicrobial and cytotoxic activities. Gbolade (2009) reported that the leaves served as antidiabetic when boiled with other materials in water.

El Diwani *et al.* (2009); Kalimuthu *et al.* (2010); Kamal *et al.* (2011); Oseni and Alphonse (2011) have all reported the antioxidant and antimicrobial activities of this plant.

Most of the published works on the phytochemical constituents, antioxidant and antimicrobial activities of *J. curcas* leaves were performed on the extracts using methanol, ethanol, hexane, petroleum ether, etc. Namuli *et al.* (2011) reported antimicrobial activity of the aqueous leaf extracts but this study investigates the composition, radical scavenging and antimicrobial activity of the aqueous extract of the Nigerian variety of the plant.

Materials and Methods

Plant material

Fresh leaves of *J. curcas* were collected from

a local farm at Isale-Osun in Osogbo, Osun State, in the month of February, 2011. The plants were authenticated at the herbarium unit of the Forestry Research Institute of Nigeria, Ibadan, Oyo State, Nigeria with voucher number FHI. 108927.

Chemicals and Reagents

2, 2-diphenyl-1-picrylhydrazyl (DPPH), Quercetin, Folin-Ciocalteu's reagent, hydrogen peroxide and Butylated hydroxyanisole (BHA) were products of Sigma-Aldrich, St. Louis, USA, while tannic acid was a product of Kem Light Laboratories, Mumbai, India. Other chemicals and reagents used were of analytical grade and prepared with all-glass distilled water.

Test Microorganisms

The test microorganisms used in this study are *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Proteus species*.

Collection of Microorganisms

The already prepared broth cultures of the test organisms were obtained from the Microbiology Laboratory of the Department of Biological Sciences, Fountain University, Osogbo.

Preparation of Aqueous Extract of *J. curcas* leaves

The leaves were sun dried and pulverised using an electric blender. The powdered leaves were extracted in distilled water and placed on a flask shaker for 7 hours at 400 oscillations per minute and then filtered. The filtrate was then concentrated on water bath to get the powdered extract.

Determination of Alkaloids

This was done by the alkaline precipitation gravimetric method described by Harborne, (1973). 0.5g of the extract was dispersed in 5 mL of 10 % acetic acid solution in ethanol. The mixture was allowed to stand for 4 hours at 28°C. It was later filtered via Whatman No. 1 filter paper. The filtrate was concentrated to one quarter of its original volume by evaporation and

treated with drop wise addition of conc. aqueous NH_4OH until the alkaloid precipitate was received in a weighed filter paper, washed with 1% ammonia solution and dried in the oven at 80°C . Alkaloid content was calculated and expressed as a percentage of the weight of sample analyzed. The experiment was carried out in triplicate.

Determination of Flavonoids

The AlCl_3 method of Jagadish *et al.* (2009) was used for the determination of the total flavonoid content of the sample extract (*J. curcas*). 1.5 mL of extract was added to 1.5 mL of 2% methanolic AlCl_3 solution. The mixture was vigorously shaken on orbital shaker for 5 min at 300 rpm and the absorbance was read at 367 nm after 10 min of incubation. Quercetin was used as standard for the calibration curve. The flavonoid content was expressed as mg flavonoid/mg quercetin. The assay was carried out in triplicate.

Determination of Total Phenols

Total phenol content in the sample was determined using Folin-Ciocalteu's method as modified by Olajire and Azeez (2011). 0.5 mL of the extract was added to 10 mL of deionized distilled water and 2.5 mL of 0.2 N Folin-Ciocalteu's phenol reagent. The mixture was allowed to stand at room temperature for 5 min and then 2 mL of 2% sodium carbonate was added. The absorbance of the solution was measured at 780 nm after 10 mins. Quercetin was used as standard for calibration curve. This assay was carried out in triplicate.

Tannin Determination

Tannin content was determined by Folin-Denis colorimetric method described by Kirk and Sawyer (1998), with slight modification. 5 g of sample was dispersed in 50 mL of distilled water and shaken. The mixture was allowed to stand for 30 min. at 28°C before it was filtered through Whatman filter paper. 2 mL of extract was dispersed into a 50 mL volumetric flask. 2.5 mL of 10% Na_2CO_3 solution was added. The content of each flask was made up to 50 mL with distilled water and allowed to incubate at 28°C for 90 min. Tannic acid was used as standard. The absorbance

was measured in a UV/Visible spectrophotometer at 260 nm using the reagent blank. This assay was carried out in triplicate.

DPPH radical scavenging assay

The DPPH radical scavenging property was determined by the method described by Turkoglu *et al.* (2007). Briefly, 1 mL of various concentrations (0.2 - 1.0 mg/mL) of aqueous extract of *J. curcas* was added to 4 mL of 0.004% methanolic solution of DPPH. After 30 min incubation period in the dark at room temperature, the absorbance was read against a blank at 517 nm. Percentage inhibition (%I) of the DPPH radical was calculated using the expression:

$$I(\%) = \left[\frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \right] \times 100 \quad (1)$$

Where A_{control} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound. Linear regression analysis was used to calculate IC_{50} .

Hydrogen peroxide scavenging assay

The ability of aqueous extract of *J. curcas* leaves to scavenge hydrogen peroxide was determined according to the method described by Ruch *et al.* (1989). Briefly, 4 mM of hydrogen peroxide was prepared in phosphate buffered saline (PBS; pH 7.4). 4 mL of various concentrations (0.2 - 1.0 mg/mL) of aqueous extract of *J. curcas* was added to 0.6 mL of hydrogen peroxide solution. The absorbance of hydrogen peroxide at 230 nm was read 10 minutes later against a blank solution containing *J. curcas* extract without hydrogen peroxide.

Preparation of Experimental Discs

The discs were prepared from Whatman No. 1 filter paper using the office paper perforator to produce about 220 well-rounded standard 0.6 cm diameter discs. The filter papers were punched into the sterile glass Petri dish and sterilized at 160°C . 10 discs from the glass Petri dish were placed in 4 plate Petri dishes. Various concentrations of the extract were prepared (500 mg/mL, 250 mg/mL, 125 mg/mL and 62.5 mg/mL). 0.3 mL of each of the concentrations was

added to the plates and then transferred to the oven to dry at 37 °C for 24 hours.

Preparation of Agar Solution

Nutrient Agar was used for the sensitivity testing. The agar was prepared following the manufacturer's instruction and autoclaved at 121°C for 15 min.

Sensitivity Test

This was done using different concentrations. About 20 mL of the molten sterile agar solution after autoclaving were poured aseptically into six Petri dishes, covered and allowed to cool at room temperature. The Petri dishes were then inverted to prevent the condensing liquid from returning and destroying the agar film formed. While the Petri dishes were still in the inverted position, the bottom of each plate was marked. This assay was carried out by the Disc and Ditch (Bore hole) methods.

Disc diffusion Method

The method described by Cheesebrough (2000) was used. Briefly, the broth culture of the test organisms was rubbed on the surface of the agar film using sterile swab sticks. The dried extract discs were then placed on each of the concentrations labelled on the agar film using a sterile pin and then incubated at 37°C for 24 hours.

Agar well (ditch) diffusion Method

This was done by using the modified agar well diffusion (ditch) method described by Nair and Chando (2005). A sterile 8 mm cork borer was used. Concentrations of 500 mg/mL, 250 mg/mL, 125 mg/mL and 62.5 mg/mL were used. The prepared nutrient agar plates were rubbed with the test organisms using sterile swab sticks. The sterile 8 mm cork borer was used to bore a hole on the agar film in the Petri dish and filled with each of the concentration prepared. This was then transferred to the incubator at 37 °C for 24 hours. Gentamycin, Ampicillin, Chloxacillin and Erythromycin were used as control.

Results

Table 1 shows some of the phytochemicals present in the aqueous extract of *J. curcas* leaves. The alkaloids have the highest concentration (1.600± 0.58%), followed by flavonoids (0.672± 0.00 mg/mL), phenols (0.463 ± 0.06 mg/mL) and then tannins (0.121 ± 0.00 mg/mL).

Table 1: Some of the phytochemicals present in aqueous extract of *J. curcas* leaves

Phytochemicals	Concentration
Flavonoids (mg/mL)	0.672 ± 0.00
Phenols (mg/mL)	0.463 ± 0.06
Tannin (mg/mL)	0.121 ± 0.00
Alkaloids (%)	1.600 ± 0.58

Each value is a mean of 3 determinations± SD

Figure 1 shows the values obtained for the antioxidant activity of aqueous extract of *Jatropha curcas* leaves. The extract produced concentration dependent increase in its ability to inhibit DPPH radical, though, the response compares poorly with BHA, a standard antioxidant with IC₅₀ of 21.24 and 3.92 mg/mL, respectively (Table 3).

Figure 2 shows the hydrogen peroxide scavenging activity of the extract compared with BHA. The extract showed scavenging activity against hydrogen peroxide with IC₅₀ of 15.67 mg/mL, while BHA has IC₅₀ of 6.19 mg/mL (Table 3).

Table 2 shows the results obtained for antimicrobial activities of the aqueous extract of *J. curcas* leaves. The extract showed significant activity, which was better than Chloxacillin, Ampicillin and Erythromycin against *Klebsiella pneumonia*, *Escherichia coli* and *Pseudomonas aeruginosa* at 250 and 500 mg/mL with MIC of 125 mg/mL (Table 3), but was inactive against *Staphylococcus aureus* and *Proteus species* at all the doses used.

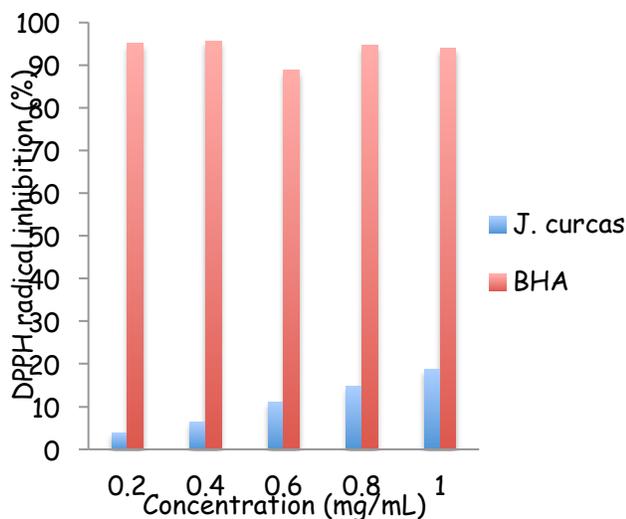


Fig. 1: DPPH radical inhibition activity of aqueous extract of *J. curcas* leaves

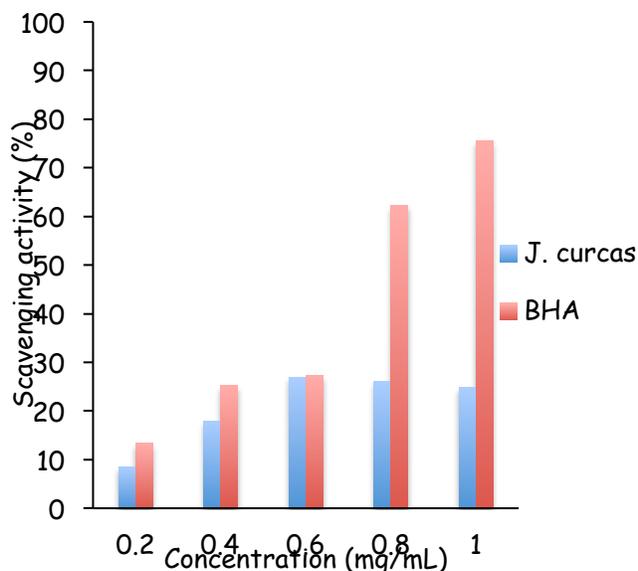


Fig. 2: Hydrogen peroxide scavenging activity of aqueous extract of *J. curcas* leaves

Table 2: Antimicrobial activity of aqueous extract of *J. curcas* leaves

Conc. (mg/mL)	<i>S. aureus</i>	<i>K. pneumonia</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>Proteus spp.</i>
62.5	-	-	-	-	-
125	-	-	-	-	-
250	-	+	+	+	-
500	-	+	+	+	-
Ampicillin	-	-	-	-	-
Erythromycin	-	-	-	-	-
Chloxacillin	-	-	-	-	-
Gentamycin	+	+	+	+	+

S. aureus = *Staphylococcus aureus*; *K. pneumonia* = *Klebsiella pneumonia*; *E. coli* = *Escherichia coli*; *P. aeruginosa* = *Pseudomonas aeruginosa*; *Proteus spp.* = *Proteus*

Table 3: Minimum inhibitory concentration (MIC) and IC₅₀ for aqueous extract of *J. curcas* leaves

	<i>J. curcas</i>	BHA
MIC (mg/mL)	125	NA
DPPH IC ₅₀ (mg/mL)	21.24	3.92
H ₂ O ₂ IC ₅₀ (mg/mL)	15.67	6.19

BHA= Butylated hydroxyanisole, NA= not applicable

Discussion

Phytochemicals confer pharmacological activities on natural products derived from plants. Many drugs in common use in modern medicine today were isolated and purified from plants. These natural products have been found to possess a range of activities, which include antimicrobial and antioxidant (Olajire and Azeez, 2011, Upadhyay, 2011). This study, thus investigates the phytochemical composition, radical scavenging and antimicrobial activities of the aqueous extract of *Jatropha curcas* Linn leaves.

Aqueous leaf extract of *J. curcas* contains flavonoids, phenols, tannins and alkaloids (Table 1). This agrees with reports by James *et al.* (2011); Oseni and Alphonse (2011) and Oskoueian *et al.* (2011). Flavonoids are secondary metabolites, which are the most common group of polyphenolic compounds that are found ubiquitously in plants (Upadhyay, 2011). They have been reported to exhibit activity against gram-positive bacteria (Meyer *et al.*, 1997) and *Streptococcus mutants* (He *et al.*, 2006). Phenols or phenolics are widely distributed organic compounds in the plant kingdom, which help in defence against predators and pathogens; they have been reported to be active against a wide range of organisms (Upadhyay, 2011). Tannins are polyphenolic compounds used for tanning or colouring leather (Gajendiran and Mahadevan, 1990) and possess sufficient hydroxyls and other suitable groups (such as carboxyls) to form strong complexes with proteins and other macromolecules. Tannins have been reported to possess activity against *A. naeslundii* and Methicillin Resistant *S. aureus* (MRSA) (Liu *et al.*, 2006) as well as gram-negative rods, *Klebsiella spp.*, *Escherichia coli* and *Enterobacter spp.* (Min *et al.*, 2008). Alkaloids are heterocyclic secondary plant metabolites containing basic nitrogen atoms and act as local anaesthetic and stimulant. Common examples are caffeine, nicotine, morphine and the antimalarial drug quinine (Upadhyay, 2011). The presence of these phytochemicals in plant extracts has been linked to antioxidant capacity (Potchoo *et al.*, 2008a, 2008b; Ajiboye *et al.*, 2010). James *et al.* (2011) attributed antioxidant, cytotoxic and wound healing activities to the phytochemicals present in extracts of *J. curcas* leaves.

Measurement of antioxidant capacity gives information about the health benefits and functions of foods and can be determined by measuring DPPH radical and hydrogen peroxide scavenging ability. DPPH, superoxide ion and ABTS are very stable free radicals widely used to evaluate *in vitro* antioxidant activities within a relatively short time (Ajiboye *et al.*, 2010). The radical scavenging activity shown by the extract (Figure 1) could be as a result of the phytochemicals present in the extract (Table 1).

The ability of *J. curcas* leaf extract to scavenge free radicals *in vitro* is in line with reports by Kamal *et al.* (2011). The extracts showed scavenging activity against DPPH and hydrogen peroxide with IC₅₀ values higher than the standard BHA (Figures 1 and 2; Table 3), indicating that the activity is less than that of the standard. The ethanolic extract reported by El Diwani *et al.* (2009) has higher antioxidant activity than the aqueous extract reported in this study.

The extract exhibited antimicrobial activity against organisms that were resistant to standard antibiotics like ampicillin, chloxacillin and erythromycin at varying concentrations (Table 2). The aqueous extract was active against the Gram negative bacteria used while the Gram-positive bacterium studied (*Staphylococcus aureus*) was resistant to the extract.

The activity of *J. curcas* against *Klebsiella pneumonia*, *Escherichia coli* and *Pseudomonas aeruginosa* has also been reported by Kalimuthu *et al.* (2010) and Kamal *et al.* (2011). Oseni and Alphonse (2011) reported that the aqueous extract shows very little activity compared with the solvent extracts. That explains why the MIC reported in this study is as high as 125 mg/mL. The antimicrobial properties shown may not be unconnected with the phytochemicals present in the aqueous extract.

The antioxidant activity shown was less than the standard antioxidant (BHA) compared and the antimicrobial activity shown was at relatively high concentrations (250 and 500 mg/mL). This shows that the aqueous extract may possess some of the folkloric properties claimed but at high concentrations. The leaves can also serve as source of active principles for drug manufacturing.

In conclusion, the results obtained from this study indicate that aqueous extract of *J. curcas* leaves exhibit lower antioxidant activity compared to BHA though it shows antimicrobial activity against *Klebsiella pneumonia*, *Escherichia coli*, and *Pseudomonas aeruginosa* at 250 and 500 mg/mL, but inactive against *Staphylococcus aureus* and *Proteus species* at these concentrations. Further studies are required to

Ascertain the particular phytochemicals associated with these activities.

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