

Comparative Phytochemical and Antimicrobial Screening of Methanolic Extracts of *Jatropha Curcas* L. (*igi lapalapa*) from Two Ecological Zones

DAIRO Kunle Paul, FEMI-ADEPOJU Abiola Grace, OKE Christianah Oreoluwa & ADIO A. Abdulrasheed

School of Allied Health and Environmental Sciences, Kwara State University, Malete, Kwara State, Nigeria

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Abstract

Phytochemical analysis and antimicrobial screening of *Jatropha curcas* were carried out on Forest and Guinea savannah samples. Both quantitative and qualitative phytochemical screening was carried out on stems, seeds and leaves of *Jatropha* from Forest and Guinea savannah. Qualitative result revealed the presence of tannin, saponin, alkaloid, total phenol, flavonoid, combines and free anthraquinone in the plant from both zones. Quantitatively, Five (5) constituents were tested (i.e tannin, saponin, alkaloid, flavonoid and total phenol). It was revealed that the concentration of tannin was the highest with a range of 33.38mg/g and 25.21mg/g while the concentration of flavonoid was the lowest with a range of 8.08mg/g and 6.41mg/g in leaf extract of the plant collected from the guinea and forest zones respectively. However in the seed extract of the plant from the guinea and forest zones, it was recorded that alkaloid has highest concentration of 4.57mg/g and 3.77mg/g while saponin has the lowest concentration of 1.5mg/g and 1.22mg/g. 0.79mg/g and 0.86mg/g of total phenol was recorded as the highest concentration range while 0.18mg/g and 0.30mg/g of saponin was recorded as the lowest concentration range in stem extract of the plants from the two ecological zones. In general it was revealed that the seeds, leaves and stem extracts of the plant from the guinea savanna zone are significantly higher ($P < 0.5$) in the five constituents than the seed, stem and leaf extracts of the plant from the forest zone. The antimicrobial result revealed that extracts of *Jatropha curcas* collected from the guinea savanna has bioactive agents that contain antimicrobial properties against *Staphylococcus aureus* and *Streptococcus faecalis*.

Keywords: *Jatropha curcas*, Phytochemical, Antimicrobial, Methanolic extracts

Introduction

Medicinal plants have been used for centuries as remedies for human diseases and offer a new sources of biologically active chemical compounds as both antifungal (Femi-Adepoju *et al.*, 2014; Adepoju *et al.*, 2014) and antibacterial (Levy, 1994) agents. Medicinal plants are the richest bio-resources of drugs of traditional medicinal systems, modern medicines, nutraceuticals, food supplements, folk medicine, pharmaceuticals, intermediate and chemical entitles for synthetic drugs (Hammer *et al.*, 1999). Human beings have used plants for the treatment of diverse ailments for thousands of years (Sofowora, 1982). According to the World Health Organisation (WHO), most populations still rely on traditional medicines for their psychological and physical health requirement, since they cannot afford the products of Western pharmaceutical industries, together with their side effects and lack of health care facilities. Rural areas of many developing countries still rely on traditional medicine for their primary health care needs and have found a place in day to day life. These medicine are relatively safer and cheaper than synthetic or modern

medicine (Mann *et al.*, 2007). People living in rurals from their personal experience know that these traditional remedies are valuable source of natural products to maintain human health, but they may not understand the science behind this medicines, but know that some medicinal plants are highly effective only when used at therapeutical doses. Plant that contain substances which can be used for therapeutic purposes or which can be used as precursors for the synthesis of useful drugs is a medicinal plant (WHO, 1997; Sofowora, 1982). In spite of the millions of chemical structures currently available for screening for the therapeutic value, natural product particularly of plant origin remain a most important source of new drugs (Odugbemi and Akinsulire, 2006).

Jatropha curcas is a large shrub, 3-4m high occurring almost throughout world, leaves are alternatively arranged, 10-15cm x 7.5-12.5cm, they are broadly ovate, conate, acute usually palmately 3 or 5 lobed, glabrous, flowers in loose panicles of the cymes, yellowish, green fruits are 2.5cm long ovoid – oblong, dull brownish black. The seed resembles castor seed in shape but are smaller in size and dark brown in colour.

Materials and methods

Plants sample collection and processing

Fresh plant parts (stems, fruits and leaves) were collected in two ecological zone i.e. Forest zone (Ore, Ondo state) and Guinea savannah zone (Malete, Kwara State). After the collection of the fruits, stems and leaves, the leaves were then washed and air dried for two weeks, the fruits were de-capped and seeds were removed and also air dried for two weeks and the stems was also air dried for two weeks. When all the samples have dried, they were ground into powder for easy extraction.

Collection of microbial isolates

The test organisms which include *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus faecalis* were carefully identified and labeled as pure isolate on nutrient agar slants respectively in McCartney bottles from the Department of Microbiology and Parasitology, University of Ilorin Teaching Hospital and taken for Refrigeration at 4°C before use. However, further sub-culture was done to keep the organisms viable. After then and before the antimicrobial sensitivity assay, these isolates were sub-cultured into Nutrient Broth at 37°C overnight the organisms were at their exponential phase of growth before carrying out the sensitivity analysis (Abah and Egwari, 2011).

The extraction process

The extraction was done using methanol as the extracting solvents in the ratio 1:5 *Jatropha* seed, leaves, and stems:solvent. 50g of the powdered seed, stem and leaf each was extracted in 300ml of methanol. The extraction was done using soxhlet apparatus. The extractions were done at boiling temperature of the solvents respectively for 8 hours (Adegoke et al, 2010; Lin et al, 1999; Orji et al, 2012). After the extraction, the filtrates were separately concentrated at 40°C in the oven. The semi solid concentrates of the extracts were then collected in sterile pre-weighed screw capped bottles and labeled accordingly (Ogunjobi et al, 2007). The extracts were stored at refrigeration temperature of 4°C till they were needed for further work.

Phytochemical screening

Chemical tests for the screening and identification of bioactive chemical constituents in the medicinal plants under study were carried out in extracts using the standard procedures as described by Okwu (2001) and Schmidt (1979).

Qualitative analysis

1. **Detection of alkaloids:** Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

For the filtrates Mayer's reagent (Potassium Mercuric Iodide) is added. Formation of a yellow coloured precipitate indicates the presence of alkaloids.

2. **Detection of Tannins:** 0.5g of powdered sample of each plant is boiled in 20ml of distilled water in a test tube and filtered 0.1% FeCl₃ is added to the filtered samples and observed for brownish green or a blue black colouration which shows the presence of tannins.
3. **Detection of Saponin:** 2g of powdered sample of each plant is boiled together with 20ml of distilled water in a water bath and filtered. 10ml of the filtered sample is mixed with 5ml of distilled water in a test tube and shaken vigorously to obtain a stable persistent froth. The frothing is then mixed with 3 drops of olive oil and for the formation of emulsion which indicates the presence of saponins.
4. **Detection of Flavanoids:** A few chop of 1% NH₃ solution is added to the extract of each plant sample in a test tube. A yellow coloration is observed if flavonoids compound are present.
5. **Detection of Terpenoids:** 5ml of each plant sample is mixed with 2ml of CHCl₃ in a test tube 3ml of concentrated H₂SO₄ is carefully added to the mixture to form a layer. An interface with a reddish brown coloration is formed if terpenoids constituent is present.
6. **Detection of Glycosides:** 1ml of concentrated H₂SO₄ is prepared in test tube of 5 ml extract from each plant sample and mixed with 2ml of glacial CH₃CO₂H containing 1 drop of FeCl₃. The above mixture is carefully added to 1ml of concentrated H₂SO₄ so that the concentrated H₂SO₄ is underneath the mixture. If cardiac glycoside is present in the sample, a brown ring will appear indicating the presence of the cardiac.
7. **Detection of Phenolic compounds:** The extract was dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green colour indicated the Phenolic compound.
8. **Detection of Phlobatannins:** Boil 2ml of extract with 1ml of aqueous HCL. Formation of red precipitate indicates presence of phlobatannins.
9. **Detection of Free Antraquinones:** 5ml of chloroform is added to 0.5g of extract. Shake for 5min and filter, to the filtrate 10% ammonia solution. Formation of bright pink colour.
10. **Detection of Combined Antraquinones:** Boil 1g of extract for 15min and add 2ml of 10% hydrochloric acid. Filter to cool then divide the filtrate into two. Add chloroform to a part and transfer chloroform layer into clean dry test tube using a clean pipette. Add 10% ammonia solution to chloroform layer. Formation of rose pink.

Quantitative determination of phytochemicals Preparation of Methanolic extract

Methanolic extract of the seeds was prepared following the method of Chan et al. (2007), by adding 25 ml of

methanol to 0.5g of sample contained in a covered 50 ml centrifuge tube, and shaking continuously for 1 h at room temperature. The mixture was centrifuged at 3,000 rpm for 10 min, and then the supernatant (subsequently referred to as methanolic extract) was collected and stored at -4°C for further analysis.

Determination of total phenol content

The total phenol content of samples methanolic extracts was determined according to the Folin–Ciocalteu method reported by Chan *et al.* (2007). Briefly, 300 µL of extract was dispensed into test tube (in triplicates). To this was added 1.5 ml of Folin–Ciocalteu reagent (diluted 10 times with distilled water), followed by 1.2 ml of Na₂CO₃ solution (7.5% w/v). The reaction mixture was shaken, allowed to stand for 30 mins at room temperature before the absorbance was measured at 765 nm against a blank prepared by dispensing 300 µL of distilled instead of sample extract. Total phenol content was expressed as gallic acid equivalent in mg/g material.

Determination of tannin content

Tannin content of samples was determined according to the method of Padmaja (1989) as follows. Sample (0.1g) was extracted with 5 mL of acidified methanol (1% HCl in methanol) at room temperature for 15 minutes. The mixture was centrifuged at 3,000rpm for 20minutes. 0.1 mL of the supernatant was added with 7.5 ml of distilled water, 0.5 ml of Folin-Denis reagent, 1 ml of 35% sodium carbonate solution and diluted to 10 ml with distilled water. The mixture was shaken well, kept at room temperature for 30 mins and absorbance was measured at 760 nm. Blank was prepared with water instead of the sample. Tannin content was expressed as tannic acid equivalent in mg/g material.

Determination of total flavonoid content

Total flavonoid content was determined using aluminum chloride method as reported by Kale *et al.* (2010). 0.5 ml of methanolic extract was dispensed into test tube, followed by 1.5 ml of methanol, 0.1 ml of aluminum chloride (10%), 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. The reaction mixture was shaken, allowed to stand at room temperature for 30 minutes, before absorbance was read at 514 nm. Total flavonoid content was expressed as quercetin equivalent in mg/g material.

Determination total saponin content

Total saponin was determined by the method described by Makkar *et al.* (2007). 0.5 g of sample was extracted with 25 ml of 80% aqueous methanol by shaking on a mechanical shaker for 2 h, after which contents of the tubes were centrifuged for 10 min at 3,000 rpm. In a test

tube an aliquot (0.25 ml) of the supernatant was taken to which 0.25 ml vanillin reagent (8% vanillin in ethanol) and 2.5 ml of 72% aqueous H₂SO₄ were added. The reaction mixtures in the tubes were heated in a water bath at 60°C for 10 min. Then tubes were cooled in ice for 4 min and then allowed to acclimatize to room temperature. Subsequently, the absorbance was measured in a UV/Visible spectrophotometer at 544 nm. Diosgenin was used as a standard and the results obtained were expressed as mg diosgenin equivalent per g of sample dry matter.

Determination total alkaloid content

The total alkaloid content of the samples was measured using 1,10-phenanthroline method described by Singh *et al.* (2004). 100mg sample powder was extracted in 10ml 80% ethanol. This was centrifuged at 5000rpm for 10 min. Supernatant obtained was used for the further estimation total alkaloids. The reaction mixture contained 1ml plant extract, 1ml of 0.025M FeCl₃ in 0.5M HCl and 1ml of 0.05M of 1,10-phenanthroline in ethanol. The mixture was incubated for 30 minutes in hot water bath with maintained temperature of 70 ± 2°C. The absorbance of red coloured complex was measured at 510nm against reagent blank. Alkaloid contents were calculated with the help of standard curve of quinine (0.1mg/ml, i.e. 10mg dissolved in 10ml ethanol and diluted to 100ml with distilled water). The values were expressed as mg/g of dry weight.

Sterility test for the extracts

The extracts were checked for sterility on Mueller-Hilton agar by streaking method after sterilization by employing pasteurization temperature at 70°C for 30 minutes.

Antimicrobial sensitivity assay

The antibacterial activity of the crude extracts was determined in accordance with the agar-well diffusion method described by Irobi *et al.* (1994). The bacterial isolates were first grown in a nutrient broth for 18 h before use and standardized to 0.5 McFarland standards (10⁶ cfu/ml).

200 µl of the standardized cell suspensions were spread on a Mueller-Hinton agar. Wells were then bored into the agar using a sterile 6 mm diameter cork borer. Approximately 100 µl of the crude extract at 10 mg/ml were introduced into the wells, allowed to stand at room temperature for about 2 hour and then incubated at 37°C. Controls were set up in parallel using the solvents that were used to reconstitute the extract. The plates were observed for zones of inhibition after 24 hour. A zone of clearance round each well signified inhibition and the diameter of each zone were measured in millimeter (mm) with a transparent ruler (Abah and Egwari, 2011).

Table 1: Qualitative analysis of phytochemicals in Guinea Savanna Zone of *J. curcas*

Phytochemicals	Leaves extract	Seeds extract	Stems extract
Alkaloids	+	+	+
Tannins	+	+	+
Saponins	+	+	+
Glycoside	+	-	-
Flavonoids	+	+	+
Phlobatanins	-	-	-
Terpenoids	+	-	+
Phenol	+	+	+
Free anthraquinone	+	+	+
Combined anthraquinone	+	+	+

+ = present and - = absent

Table 2: Qualitative analysis of phytochemicals in Forest Zone of *J. curcas*

Phytochemicals	Stems extract	Seeds extract	Leaves extract
Alkaloids	+	+	+
Tannins	+	+	+
Saponins	+	+	+
Glycoside	-	-	+
Flavonoids	+	+	+
Phlobatanins	-	-	-
Terpenoids	+	-	+
Phenol	+	+	+
Free anthraquinone	+	+	+
Combined anthraquinone	+	+	+

+ - present and - - absent

Table 3: Quantitative analysis (mg/g) for *J. curcas* of the Guinea and Forest zone

Data represent the mean \pm standard deviation of triplicate readings; values with the different superscript letter along the same column are significantly different ($P > 0.05$)

Samples	Total Phenol	Tannin	Flavonoids	Saponins	Alkaloids
Guinea leaves	23.09 \pm 0.21 ^a	33.38 \pm 0.04 ^a	8.08 \pm 0.21 ^a	22.44 \pm 0.75 ^a	16.98 \pm 0.42 ^a
Forest leaves	18.56 \pm 0.11 ^b	25.21 \pm 0.26 ^b	6.41 \pm 0.21 ^b	16.10 \pm 0.20 ^b	13.14 \pm 0.12 ^b
Guinea stems	2.55 \pm 0.03 ^c	2.22 \pm 0.09 ^c	2.76 \pm 0.11 ^c	1.53 \pm 0.05 ^c	4.57 \pm 0.08 ^c
Forest stems	2.44 \pm 0.01 ^c	2.05 \pm 0.04 ^c	1.95 \pm 0.02 ^d	1.22 \pm 0.14 ^c	3.77 \pm 0.04 ^d
Guinea seeds	0.79 \pm 0.01 ^d	0.34 \pm 0.02 ^d	0.54 \pm 0.04 ^e	0.18 \pm 0.01 ^d	0.67 \pm 0.01 ^e
Forest seeds	0.86 \pm 0.01 ^d	0.41 \pm 0.01 ^d	0.67 \pm 0.02 ^e	0.30 \pm 0.01 ^d	0.74 \pm 0.01 ^e

Results

Qualitative Phytochemical Analysis of *Jatropha curcas*

The present study carried out on the *Jatropha curcas* revealed the presence of medicinal active constituents. The Phytochemical compounds of *Jatropha curcas* were qualitatively analyzed in stems, seeds and leaf extracts in two ecological zones i.e. Forest and Guinea zone separately and the results are presented in Tables 1 and 2. In these screening process alkaloids, tannins, saponins, flavonoids, terpenoids, glycosides, phenols, phlobatanines, free anthraquinone and combine anthraquinone are present in some of the parts extracted and some are not present, as shown in the tables above.

Quantitative Phytochemical Analysis of *Jatropha curcas*

It is as shown in table 3

Sterility test

Table 4: Extract Sterility Testing Result

Extracts	Sterility result
Guinea leaves	No growth
Forest leaves	No growth
Guinea seeds	No growth
Forest seeds	No growth

Table 5: Antibacterial activities profile of four extracts of *J. curcas* in two ecological zones

Test Bacteria	ZONE OF INHIBITION (mm) (Mean)			
	Forest leaves	Forest seeds	Guinea leaves	Guinea seeds
<i>Escherichia coli</i>	-	-	7	-
<i>Staphylococcus aureus</i>	-	-	7	5
<i>Streptococcus feacalis</i>	6	5	7	14

Antibacterial activity

All four extracts of the plant i.e. Seeds and Leaves from both Forest and Guinea Zone tested showed varying degree of antibacterial activities against the test bacterial species (Table 5). The inhibition zone for *Escherichia coli* was much less than as compared to the other two bacteria.

Discussion and conclusion

The quantitative phytochemical results of *Jatropha curcas* (seeds, leaves and stems) from Guinea zone and forest zone of five constituents (total phenol, saponin, flavonoids, alkaloid and tannin) revealed that Guinea leaves had significantly higher ($P < 0.05$) phytochemical contents than other samples. This may be due to the climatic condition of guinea zone as indicated by its greenish pigment than in the leaves of Forest zone. Also from table 3, it shows that Guinea *Jatropha curcas* (seeds, leaves and stems) generally had significantly higher ($P < 0.05$) phytochemical compounds than Forest *Jatropha curcas* (seeds, leaves and stems) which had significantly lower ($P > 0.05$) compounds. The richness of the five constituents in the samples of Guinea zone maybe due to the environmental conditions like lower rainfall pattern and higher temperature range. Population growth of plant is rapid and there is also outstripping improvements in agricultural output in Guinea.

Qualitatively, phytochemical screening of *J. curcas* stems, seeds and leaves from both Guinea and Forest zone extracts revealed the presence of saponins,, tannins, alkaloids, flavonoids, free anthraquinone and combine anthraquinone. These compounds are known to be biologically active and therefore aid the antimicrobial activities of *J. curcas*. These secondary metabolites exert antimicrobial activity through different mechanisms. Tannins have been found to form irreversible complexes with prolinerich protein (Shimada, 2006) resulting in the inhibition of cell protein synthesis. Parekh and Chanda (2007) reported that tannins are known to react with proteins to provide the typical tanning effect which is important for the treatment of inflamed or ulcerated tissues. Herbs that have tannins as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery (Dharmananda, 2003). These observations therefore support the use of *J. curcas* in herbal cure remedies. Li and Wang (2003) reviewed the bio-logical activities of tannins and observed that tannins have

anticancer activity and can be used in cancer prevention, thus suggesting that *J. curcas* has potential as a source of important bioactive molecules for the treatment and prevention of cancer. The presence of tannins in *J. curcas* supports the traditional medicinal use of this plant in the treatment of different ailments.

Another secondary metabolite compound observed in the Forest and Guinea zone extract of *J. curcas* was alkaloid. One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms. These activities have been widely studied for their potential use in the elimination and reduction of human cancer cell lines (Nobori et al., 1994). Alkaloids which are one of the largest groups of Phytochemical in plants have amazing effects on humans and this has led to the development of powerful pain killer medications (Kam and Liew, 2002). Just et al. (1998) revealed the inhibitory effect of Saponin on inflamed cells. Saponin was found to be present in *J. curcas* extracts and has supported the usefulness of this plant in managing inflammation. Steroidal compounds present in *J. curcas* extracts are of importance and interest due to their relationship with various anabolic hormones including sex hormones (Okwu, 2003). Flavonoids, another constituent of *J. curcas* Forest and Guinea zone extracts exhibited a wide range of biological activities like antimicrobial, anti-inflammatory, anti-angionic, analgesic, anti-allergic, cytostatic and antioxidant properties (Hodek et al., 2002). Different parts of *J. curcas* contain the toxic alkaloids curcin and phorbol ester which prevent animals from feeding on it. Hence, the presence of these compounds in *J. curcas* corroborates the antimicrobial activities observed. It is concluded that *J. curcas* stem bark could be a potential source of active antimicrobial agents, and a detailed assessment of its *in vivo* potencies and toxicological profile is ongoing.

From the result of the antimicrobial screening, it was revealed that Guinea *Jatropha curcas* leaves had the ability and had bioactive agents that contain antimicrobial properties against *Staphylococcus aureus* and *Streptococcus feacalis*

Mousumi performed antimicrobial studies of *Jatropha curcas* leaf extract (distillation method of extraction in methanol, chloroform and distilled water) with certified strains of fungi and bacteria. The microorganism were *Scleristoria minor*, *Curvularia lunata*, *Alternaria alternate*, *Aspergillus niger*, *Microsporium gypisum*, *Rhizopus spp* and *Escherichia coli* (MTCC 41), *Bacillus subtilis* (MTCC 441), *Streptococcus mutan* (MTTC 497), *Staphylococcus aureus* (MTTC 737) obtain from R.D. University, jabalpur,

india and IM tech, chandigarh, india. Petri-dishes for testing the antimicrobial activity for each type of pathogen were prepared by inoculating 100ml of microorganism to 20ml of 2% agar. After 24 hours, the growth of the microorganism was observed in each petridish and the zone of inhibition was measured. The chloroform extract of *Jatropha curcas* leaves showed a strong inhibitory activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*. *Jatropha curcas* extracts also inhibit a broad range of fungi viz. *Scleristoria minor*, *Alternaria alternate*, *Curvularia lunata* and *Aspergillus niger*. *Jatropha curcas* showed significant antifungal as well as antibacterial activity and *Staphylococcus aureus* was the most susceptible bacteria.

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