

PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITIES OF AQUEOUS AND METHANOL EXTRACTS OF *Jatropha curcas* LEAF

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Abstract. *Jatropha curcas* is an ornamental plant which is also employed to cure various infections in traditional medicine. It has been reported to have effective *in vitro* antimicrobial activity against bacteria. In this study, antimicrobial activities of aqueous and methanolic leaf extract of *Jatropha curcas* was investigated using microbiological and biochemical analysis. This activity was tested against eight different bacterial isolates comprising of Gram positive and Gram negative bacteria. Six (6) isolates out of eight were sensitive to both aqueous and methanolic extract of *Jatropha curcas* leaf. The extract also compared favourably with ampiclox and chloramphenicol which served as positive control in this work. The phytochemical analysis showed the presence of alkaloid (20.1%), saponin (2.60%), phenol(0.26%), flavonoid (4.7%), tannin(2.69%) and cyanide which was almost negligible at 3.62 ppm leaf extract.

Keywords: Phytochemicals; *E. coli*; aqueous; methanol; antibacterial; chloramphenicol; *Jatropha*; Ampiclox

INTRODUCTION

Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population especially in developing world [27]. It is a known fact that pharmaceutical companies produced a number of new antimicrobial in the last years, resistance to these drugs has increased and now become a global concern [2]. The global emergence of multi – drug resistant (MDR) bacteria is increasingly limiting the effectiveness of current drugs and significantly causing treatment failure [8]. The increasing prevalence of multi-drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raised the specter of 'untreatable' bacterial infections and adds urgency to the search for new infection-fighting strategies [23, 29]. Among the potential sources of new agents, plants have long been investigated because of the fact that they contain many bioactive compounds that can be of interest in therapeutics. Researches carried out by scientists into many plants showed that plants constitute a large reservoir of chemical substances that possess antimicrobial activity. Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many side effects that are often associated with synthetic antimicrobials [11]. *Jatropha curcas* variously known as physic nut, purging nut or pig nut [10] is used for treatment of various ailments such as skin infections, gonorrhoea, jaundice and fever [3]. Various parts of this plant such as the leaves, roots, seed, bark, fruit, flowers and immature pods act as cardiac and circulatory stimulants, possess anti tumor, Guevere [7] antipyretic, antiepileptic, anti inflammatory, antiulcer, Pal [21] antispasmodic, diuretic, Caceres [4], Morton [15] antihypertensive, cholesterol lowering, antioxidant, antidiabetic, hepatoprotective, antibacterial and antifungal activities, Nikkon [19] and are being employed for the treatment of different ailments in the indigenous system of medicine Mughal [16]. The seeds contain between 35 to 40% oil (50-55% oil on kernel basis) [12], which can be processed to produce a high

quality biodiesel fuel useable in a standard diesel engine [13, 14]. The oil which is similar to oil used for human consumption is not used for cooking purpose because it contains some toxic substances [24]. Besides biodiesel production, *J. curcas* has numerous other uses as it is a multipurpose plant. Among other benefits are health and environmental values. An escalating antibiotic resistance by pathogenic bacteria has been observed since the last decade and the adverse effect of conventional antibiotics calls for a friendly alternative. Therefore the aim of this study is to access the phytochemical constituents and the antibacterial potentials of aqueous and methanol leaf extracts of *Jatropha curcas* against selected bacteria isolates.

MATERIALS AND METHODS

Collection and Identification of Plants

Fresh healthy leaves of *Jatropha curcas* L. were collected from school of Agriculture and Agricultural Technology, Federal University of Technology Owerri, Imo State in July, 2014 and botanical identification was carried out in the Crop Science Technology Department of the Federal University of Technology, Owerri. The taxonomical identification of the plant was confirmed and authenticated by Dr. S.O. Ojiako, a plant taxonomist of the Department of Crop Science, Federal University of Technology Owerri, Nigeria. The leaves were washed, air – dried at room temperature for several days until well dried. The dried leaves were grounded to fine powder using laboratory mortar and the powder was then stored in an air – tight container until required. Chicken pie and beef burger were purchased from Sunic fast food in FUTO and was used for isolation of the bacteria organisms.

Preparation of media

The media used for the analysis were prepared according to the manufacturers' specification. MacConkey agar- MA (MLAB) and nutrient agar were prepared by 250g in 100ml of peptone water and were sterilized using an autoclave at 121psi for 15minutes. The media were allowed to cool a bit, then poured into sterile petri dish and allowed to set.

Microbiological Analysis

1ml of each sample was serially diluted in nine parts of peptone water in a total of six fold (10^6).

Sample inoculation and storage

An aliquot of 10^5 and 10^6 dilution factors from each of the samples were inoculated into the plates as described by Cheesebrough [5]. The inoculated plates were incubated at 37°C for 2 hours for bacteria. Acceptable plates were chosen from those with colony counts of between 30 - 300cfu/ml. Pure cultures were therefore obtained by streaking as described by Cheesbrough [5].

Characterization of isolates

The isolates were grouped according to their colonial morphology. Gram staining and biochemical analyses such as catalase, oxidase, indole, sugar fermenting tests were the methods used.

Extraction of plant materials

The powdered plant material (100g) was weighed and soaked in 200ml of methanol and aqueous (both hot and cold water) in 500ml conical flask. Methanol was added to the sample and shaken intermittently for at least three hours and left standing overnight. The mixture was filtered into a clean dry beaker, obtaining a dark green filtrate. The methanol was then gently evaporated by placing the beaker in an oven set at 75°C to obtain a thick dark green plant extract. The aqueous extraction was done using the same procedure and then the extracts were stored in sterile containers at 4°C in a refrigerator until required for use.

Antibacterial analysis of the methanolic and aqueous extract of *J. curcas*

The methanol and aqueous extracts were filtered using a Whatman No 1 filter paper cut into discs of 6mm diameter using an office perforator. The discs were placed in a glass petri dish and sterilized in hot air oven at 170°C for 1 hour. The paper disc was soaked in the extract (methanol, aqueous: both hot and cold water extracts) and was then placed on the culture plate. Also the paper disc was soaked in the antibiotics (used as positive control) and was also placed on the culture plate. It was allowed to stand for some minutes before inoculation for 24 hours. The zone of inhibition was measured using a ruler.

Analysis of phytochemicals

Phytochemical characterization of the leaves of *J. curcas* was carried out by analyzing for the presence and percentage concentrations of saponin, flavonoid, alkaloids, tannin, cyanogenic glycoside, and phenol using the methods described by Harborne [9].

Test for saponins

A quantity, (0.1g) of ethanolic extract of *J. curcas* was boiled with 5ml of distilled water for 5 minutes. The mixture was filtered while still hot. 1 ml of the filtrate was diluted with 4 ml of distilled water. The

mixture was shaken vigorously for two minutes and then observed on standing for a stable froth. Frothing which persisted on shaking was taken as evidence for the presence of saponins.

Test for alkaloids

A quantity, 0.2 g of the sample was boiled with 5ml of 2% HCl on a steam bath for twenty minutes, the mixture was filtered and 1ml aliquots of the filtrate were treated with 2 drops of the Mayer's reagent. A cream precipitate indicated the presence of alkaloid.

Test for tannins

A quantity, 2g of the sample was boiled with 5ml of 45% ethanol for 5 minutes. The mixture was cooled and then filtered and the filtrate was diluted with distilled water and then 2 drops of ferric chloride solution was added. A transient greenish to black colour indicates the presence of tannins.

Test for phenol

To a volume of 1ml of the extracts, 8ml of distilled was mixed with 6ml of ferric chloride in a test tube, A color change of light brown is an indication of a positive test.

Test for flavonoids

A quantity, 0.2g of the sample was heated with 10ml ethyl acetate in boiling water bath for 3 minutes. The mixture was filtered, and 4ml of the filtrate was shaken with 1 ml of 1% aluminium chloride solution and observed for light yellow colouration that indicates the presence of flavonoids.

Test for cyanogenic glycosides

To a volume of 3 ml of the ethanolic extract, 2ml of chloroform was added. Tetraoxiosulphate VI acid was carefully added to form a lower layer. A reddish brown color at interface is an indication of a positive test.

Statistical Analysis

Statistical analyses were carried out with graphpad prism 5.0 using one way Analysis of variance (ANOVA) * $P < 0.05$. Data were expressed as mean \pm SEM.

RESULTS

The result on Table 1 shows that the sample for chicken pie had no significant effect on MacConkey agar plate, Nutrient agar, Salmonella – shigella agar plate and Sabouraud dextrose agar plate when compared with all pair of columns after 24 to 48 hours of incubation while the sample for beef burger still showed no significant effect on MacConkey agar plate, Nutrient agar plate, Salmonella – shigella agar plate and Sabouraud dextrose agar plate when compared with all pairs of columns after 24 to 48 hours of incubation.

Table 2 shows that the Gram positive bacteria that were sensitive include: *Micrococcus*, *Streptococcus*,

Lactobacillus plantarum, *Bacillus subtilis*, *Lactobacillus casei* and *Staphylococcus aureus* while the Gram negative bacteria were *Coccobacillus* and *E. coli*. The 3 positive catalase were *Micrococcus*, *Bacillus* and *Staphylococcus aureus* while the negative catalase were *Streptococcus*, *Coccobacillus*, *Lactobacillus casei* and *E. coli*.

From the table 3, the result show that at the end of 24 hours of incubation of Ampiclox culture in nutrient agar plate had no significant effect on bacteria isolate of *Micrococcus*, *Coccobacillus*, *Lactobacillus plantarum*, *Bacillus subtilis*, *Staphylococcus aureus* and *Lactobacillus casei* when compared with Chloramphenicol while bacteria isolates of *Streptococcus* and *E. coli* significantly decreased at (P<0.01) and (P<0.001) respectively when compared with Chloramphenicol.

At the end of 24 hours of incubation of Chloramphenicol cultured in nutrient agar plate, there was no significant effect on bacteria isolates of *Micrococcus*, *Coccobacillus*, *Lactobacillus plantarum*, *Bacillus subtilis*, *Staphylococcus aureus* and *Lactobacillus casei*, when compared with Ampiclox. While bacteria isolate of *Streptococcus* and *E. coli* significantly increased at (P< 0.01) and (P< 0.001) when compared with Ampiclox.

The hot water extract after 24 hours of incubation cultured with nutrient agar plate had no significant effect on bacteria isolates of *Streptococcus*, *Micrococcus*, *Lactobacillus casei* and *E. coli* when compared with Ampiclox. The bacteria isolate of *Lactobacillus plantarum*, *Bacillus subtilis* and *Staphylococcus aureus* significantly decreased at (P< 0.01) when compared with Ampiclox, while bacteria isolate of *Coccobacillus* significantly increased at (P< 0.01) when compared with Ampiclox. The bacteria isolate of *Micrococcus* showed no significance when compared with Chloramphenicol. The bacteria isolate of *Lactobacillus plantarum* significantly decreased at

(P< 0.05) when compared with Chloramphenicol and bacteria isolate of *Streptococcus*, *Coccobacillus*, *Bacillus subtilis*, *Staphylococcus aureus* and *E. coli* significantly decreased at (P< 0.01) when compared with Chloramphenicol.

The cold water extract after 24 hours of incubation cultured with nutrient agar plate had no significant effect on bacteria isolates of *Streptococcus*, *Micrococcus*, *Coccobacillus*, *Lactobacillus casei*, *Staphylococcus aureus* and *E. coli* when compared with Ampiclox. *Lactobacillus plantarum* and *Bacillus subtilis* bacteria isolate significantly decreased and increased at (P< 0.01) respectively when compared with Chloramphenicol while *Streptococcus* and *E. coli* significantly decreased at (P< 0.01) when compared with Chloramphenicol.

The methanol extract after 24 hours of incubation cultured with nutrient agar plate had no significant effect on bacteria isolate of *Streptococcus*, *Micrococcus*, *Coccobacillus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Lactobacillus casei* and *E. coli* when compared with Ampiclox while *Lactobacillus plantarum* bacteria isolate significantly decreased at (P< 0.01) when compared with Ampiclox. The bacteria isolate of *Coccobacillus*, *Bacillus subtilis*, *Staphylococcus aureus* and *Lactobacillus casei* had no significant effect when compared with Chloramphenicol. *Lactobacillus plantarum* bacteria isolate significantly decreased at (P< 0.05) when compared with Chloramphenicol while *Streptococcus* and *E. coli* bacteria isolates significantly decreased at (P< 0.001) when compared with Chloramphenicol.

The phytochemical analysis is outlined in Table 4 below, the results showed the presence of Alkaloid(20.1%), Saponin (2.60%), Phenol (0.26%), Flavonoid (4.7%), Tannins (2.69%) and Cyanide which was almost negligible at 3.62ppm leaf extract of *Jetropha curcas*.

Table 1. Variation of microbial count for food samples using culture media

Samples	MA	NA	SSA	SDA
Chicken Pie	0.00± 0.00 ^{ns}	28.00±28.00 ^{ns}	0.00± 0.00 ^{ns}	0.6667± 1.155 ^{ns}
Beef Burger	4.000± 4.000 ^{ns}	5.333± 5.333 ^{ns}	12.67±12.67 ^{ns}	1.000±1.000 ^{ns}

Values are expressed as Mean ± SEM, n= 3, *P < 0.05 vs compared all pairs of column, ns = no significant. MA - MacConkey Agar, NA – Nutrient Agar, SSA – Salmonella – Shigella Agar, SDA – Sabouraud Dextrose Agar

Table 2. Results for Microbiological and Biochemical Analysis

Sample code	Colonial morphology	Gram reaction	Catalase	Isolate suspected
BB(MA)	Pinkish colony	+ve cocci	-ve	<i>Streptococcus spp</i>
BB(MA)C	Round milky flat colony	+ve	+ve	<i>Micrococcus spp</i>
BB (MA) B	Milky flat colony	-ve cocci	-ve	<i>Coccobacillus spp</i>
BB (NA)A	Milky wavy flat colony	+ve rods	-ve	<i>Lactobacillus plantarum</i>
CP (NA) A	Milky flat smooth shiny colony	+ve rods	+ve	<i>Bacillus subtilis</i>
CP (NA) B	Whitish colony	+ve rods	+ve	<i>Staphylococcus aureus</i>
BB (NA) B	Milky large circular flat colony	+ve rods	-ve	<i>Lactobacillus casei</i>
BB (NA) C	Yellowish big circular flat colony	-vr rods	-ve	<i>E.coli</i>

Table 3. Variation of antimicrobial sensitivity pattern of methanolic, aqueous and antibiotics (positive control) of leaf extracts of *Jatropha curcas*

Bacterial isolates	Ampiclox (control I)	Chloramphenicol (Control II)	Hot water	Cold water	Methanol
<i>Streptococcus spp</i>	3.267±0.1202 ^b	20.00 ± 5.774 ^{**}	0.0 ± 0.0 ^{cns}	0.0 ± 0.0 ^{cns}	0.0 ± 0.0 ^{cns}
<i>Micrococcus spp</i>	5.000±2.887 ^{ns}	0.0 ± 0.0 ^{ns}	0.0 ± 0.0 ^{ns}	0.0 ± 0.0 ^{ns}	12.00 ± 3.464 ^{cns}
<i>Coccobacillus spp</i>	0.0 ± 0.0 ^{ns}	0.0 ± 0.0 ^{ns}	11.73 ± 3.166 ^{c***}	0.0 ± 0.0 ^{ns}	3.150 ± 1.114 ^{ns}
<i>Lactobacillus plantarum</i>	13.30 ± 3.868 ^{ns}	10.00 ± 2.887 ^{ns}	1.000 ± 0.2887 ^{a**}	0.0 ± 0.0 ^{a**}	0.0 ± 0.0 ^{ns}
<i>Bacillus subtilis</i>	0.700±2.31 ^{ns}	0.0 ± 0.0 ^{ns}	7.00 ± 1.00 ^{c**}	5.300 ± 1.599 ^{b**}	0.0 ± 0.0 ^{ns}
<i>Staphylococcus aureus</i>	0.0±0.0 ^{ns}	6.700 ± 1.905 ^{ns}	16.67 ± 4.821 ^{c**}	0.0 ± 0.0 ^{ns}	3.300 ± 0.9815 ^{ns}
<i>Lactobacillus casei</i>	8.700 ± 2.300 ^{ns}	14.00 ± 4.041 ^{ns}	0.0 ± 0.0 ^{bns}	0.0 ± 0.0 ^{bns}	6.700 ± 1.905 ^{ns}
<i>E.coli</i>	0.0 ± 0.0 ^c	20.00 ± 5.774 ^{**}	0.0 ± 0.0 ^{cns}	0.0 ± 0.0 ^{cns}	0.0 ± 0.0 ^{cns}

Values are expressed as Mean ± SEM, n = 3, *P< 0.05 vs Am, a, b, c represents P< 0.05, P< 0.01 & 0.001 respectively vs CH. Am = Ampiclox, CH = Chloramphenicol, ns = no significance.

Table 4. Phytochemical analysis of the leaf extract of *Jatropha curcas*

Phytochemicals	%/ppm Extracts
Tannins	2.69%
Saponin	2.60%
Alkaloid	20.1%
Flavonoid	4.7%
Cyanide	3.62ppm
Phenol	0.26%

DISCUSSION

The antibacterial activity of aqueous and methanol leaf extract of *J. curcas* was determined against eight(8) bacterial isolates comprising of both Gram positive and Gram negative bacteria. Pathogenic microorganisms isolated and identified in this study include *Streptococcus*, *Micrococcus*, *Coccobacillus*, *Lactobacillus*, *Bacillus subtilis*, *Staphylococcus aureus* and *E. coli*.

Table 3 shows that six (6) bacteria were sensitive to the extract. The Gram positive that were sensitive include *Micrococcus*, *Lactobacillus plantarum*, *Bacillus subtilis*, *Lactobacillus casei* and *Staphylococcus aureus* while the only sensitive Gram negative bacteria was *Coccobacillus*.

The cold aqueous extract of the leaf extract did not exhibit antimicrobial activity against all the test organisms except for *Bacillus subtilis*. The reference drugs exhibited higher antimicrobial activity against all bacteria isolates except *Coccobacillus*. The aqueous extract of *J. curcas* leaf particularly hot extract had higher antimicrobial activity against *Staphylococcus aureus* and *Coccobacillus* amongst other test isolates while the antimicrobial of the methanol extracts of *J. curcas* leaves exhibited higher antimicrobial activity against *Microoccus*.

The apparent resistance of Gram negative bacteria to the extracts could be attributed to the fact that the Gram negative bacteria have an outer phospholipids membrane with the structural lipo-polyssaccharide components, which make their cell wall impenetrable to antimicrobial agents [20] while the Gram positive bacteria seemed to be more susceptible having an outer peptidoglycan, which is not an effective permeability barrier [26]. The methanol extract of *J. curcas* exerted a broader spectrum of inhibitory activity on Gram positive bacteria than Gram negative bacteria strains.

However, *Streptococcus* and *E. coli* were found to be resistant to the extract, a result similar to the one obtained by Namuli *et al* [18] who suggested that this might be due to the outer membrane of the bacteria. However, the results of this study is in agreement with Akinpelu *et al* [3] who investigated the antibacterial of the methanolic extract of the leaves of *J. curcas* against 13 bacterial species including *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The extract showed appreciable inhibitory activity against these organisms.

Phytochemical screening helps to reveal the chemical nature of the constituents of the extracts and the one that predominates over the others [6]. It may also be used to search for bioactive lead agents that could be used in the partial synthesis of some useful drugs [28]. Alkaloids, glycosides, saponins, tannins and phenol were present in all extracts, which is in agreement with a previous report by Rahila *et al* [22], who reported that plants contained components which were active against microorganisms. Achten *et al* [1] reported the high concentration of phorbol esters (phorbol-12-myristate-13-acetate) present in *Jatropha* seed which has been identified as the main toxic agent responsible for *Jatropha* toxicity. Phytochemical screening of *J. curcas* leaf extract revealed the presence of Alkaloids as major active secondary metabolite while Cyanide is present as minor constituents. This is similar to the phytochemical study of aerial parts of *J. curcas* carried out by Naeem *et al* [17]. Many researchers have also shown the presence of flavonoids in the leaf extract of *J. curcas* [25]. Saponins are characterized by their surface – active properties and they dissolve in water to form foamy solutions and because of surface activity, some drugs containing saponins have a very high long history of usage. The leaf extract of *J. curcas* was found to inhibit six (6) out of eight (8) bacterial isolates. The extract also compared favourably with ampiclox and

chloramphenicol which served as positive control. The leaf extracts have been shown to have bactericidal properties. Multiple drug resistant microorganisms are one of the recent and major concerns in public health. Hence, further intensive studies on this plant might provide possible solutions to this problem. Future research should be directed towards molecular taxonomy of *J. curcas*, the new drug discovery based on natural products in different parts of the plant and investigations on toxicity and natural product chemistry of different taxonomy of *J. curcas*. More research on the toxicology of *J. curcas* would give direction on how clinically utilizable the extracts are, in order to enhance proper health delivery.

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