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Antibacterial Activities of Leaf Extracts of *Jatropha tanjorensis* Ellis and Saroja (Euphorbiaceae)

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Abstract The need to substantiate the widely acclaimed antibacterial potentials of *Jatropha tanjorensis* leaf, informed the present study. Two extract types of the plant's leaf (an ethanolic, JELE, and aqueous extract, JALE) were prepared and analysed for phytochemical constituents following standard protocols. Three concentrations of each extract (30, 40 and 50 mg/ml) were assayed on two bacteria species, *Escherichia coli* and *Staphylococcus aureus.* Results revealed the presence of six bioactive compounds, which varied with extract type. The compounds include alkaloid, saponin, steroid, tannin, phenol and anthraquinone; five were found in JELE, while four in JALE. Antibacterial analyses showed a dose and bacterial-species dependent activities of the extracts. JALE showed higher activities on the organisms at all concentrations, while JELE had no activities on *E. coli* at lower concentrations. *Staphylococcus aureus* was susceptibility to all concentrations of JELE (range=8.67±3.6 to 15.03±0.95 mm) and JALE (12.80±0.98 to 20.33±1.53 mm). Minimum Inhibitory Concentration (MIC) of the JALE was 7.5 mg/ml for both species of bacteria, while JELE had MIC of 15 and 25 mg/L for *S. aureus* and *E. coli*, respectively, while Minimum Bactericidal Concentration (MBC) analyses showed the leaf extracts to be bacteriostatic in activity. These results suggest that the leaf extracts are active against the two bacteria studied and JELE more potent than JALE. These extracts could be of value in the management of disease conditions associated with these bacteria.

Keywords Bacteriostatic; Susceptibility; *Staphylococcus aureus*; *Escherichia coli*

Background

Green plants constitute a reservoir or storehouse of herbal medicines (Oyewole et al., 2007). In addition, herbal medicines have received greater attention as an alternative to clinical therapy and the demand of these remedies has currently increased (Ross and Brain, 1977; USDA, 2007). Their use is highly dependent on experimental screening to ascertain active components, safety, and efficacy of the plant products (Hara et al., 1998; Ozuola et al., 2006; Oyewole et al., 2007; Igbinaduwa et al., 2011). Among these screened plants is *Jatropha tanjorensis* Ellis & Saroja, which belongs to genus of flowering plants in the spurge family, Euphorbiaceae (MacIntyre, 2007; Oduola et al., 2007; Carlasabandar, 2010).

The plant is a common weed of field crops, in rainforest zones of West Africa including Nigeria (Iwalewa and Agbani, 2005) and a natural hybrid between *J. curcas* and *J. gossypifolia* (Prabakaran and Sujatha, 1999). Its primary use is for fencing, and secondarily as a source of edible leafy vegetables and natural medicinal remedy against diabetes in many parts of Southern Nigeria; where it is commonly referred to as "Hospital too far", "Catholic vegetable", "Iyana-ipaja", "Lapalapa" (Iwalewa et al., 2005). It is prepared locally in most parts of Southern Nigeria by collecting the leaves and squeezing out the juice (Prabakaran and Sujatha, 1999). In view of the common use of *J. tanjorensis*, there is need to evaluate these widely acclaimed activities of the plant.

However, studies have shown that variations in activities of a plant extract exist due to solvents of extracts and plant part. Thus, in addition to determining the efficacies of the leaf of the plant, comparative analyses of the

activities of extract types of this plant part is imperative in increasing knowledge on the effectiveness of the plant, hence, this study.

1 Materials and Methods

1.1 Collection, authentication, preparation, and extraction of plant materials

Fresh leaves of *Jatropha tanjorensis* were collected and authenticated by a Botanist at the Department of Biological Sciences, Federal University of Technology, Minna, where voucher specimens of the leaves were deposited (VN: JT/83/16). The leaves were dried under shade in the Laboratory of Department of Microbiology for 14 days after which they were ground to powder using an electric blender (Model no: QASA QLB – 20L40).

To obtain Ethanolic Leaf Extract, (JELE), of the plant, powdered material of the leaf measuring 197.05 grams (g) were introduced into extraction chamber of sohxlet extractor and extraction done for 48 hours with temperature maintained at 70°C using ethanol as solvent. For Aqueous extract, (JALE), the pulverised material was soaked in distilled water for 72 hours and agitated until a deep colour of the extract was obtained. At the end of the period, the residue were dried in a laboratory oven at 40°C to constant weight. The dried residue weighed 154.04 g, thus, representing a yield of 21.8%. The extracts produced were concentrated to dryness on water bath and then weighed. These were according to methods of Daniyan and Muhammad (2011).

1.2 Sources of chemicals and reagents

All chemicals used were of analytical grade and were products of BDH Chemicals Ltd, Poole, England.

1.3 Qualitative phytochemical screening of leaf extracts of *Jatropha tanjorensis*

Qualitative test were carried out on the ethanolic (JELE) and aqueous (JALE) leaf extracts of the plant using standard procedures described by AOAC (1984), Sofowara (1993), Harborne (1998), Oloyed (2005), and Trease and Evans (2008).

1.4 Antibacterial assay of extracts of leaf of *Jatropha tanjorensis*

1.4.1 Reconstitution of extracts

The extracts were dissolved in DMSO and distilled water to get extract concentrations of 30, 40, and 50 mg/ml. These concentrations were prepared by weighing 1.5, 2.0 and 2.5 g of each extract and dissolved in 1 ml of DMSO and 4 ml of distilled water.

1.4.2 Sources of microorganisms

The clinical bacteria isolates assayed in this study were *Escherichia coli* and *Staphylococcus aureus.* These organisms were obtained from the Centre for Genetic Engineering and Biotechnology, Federal University of Technology, Minna, Niger State. Nutrient Broth was used to confirm the organisms by sub-culturing at 37°C for 18 hours; and the organisms were maintained on Nutrient Agar (NA) slant and Sabouraud Dextrose Agar (SDA) slant in a refrigerator at 4°C until required as described by Igbinosa et al. (2009).

1.4.3 Preparation of media

These media were prepared following procedures in the manufacturer's instructions. Twenty-eight grams of NA standard measurement was poured into sterile conical flask; to which 1 Litre of distilled water was added. For SDA, 11 g was poured into sterile conical flask; to which 0.5 g of chloramphenicol was added. The prepared media were cork-fitted with cotton wool, foil paper, and masking tape. This was done to avoid removal of the cork when autoclaving. The media was autoclaved for 15 minutes at 121°C, removed and cooled to 47°C. Subsequently, these were mixed properly before pouring into sterile petri-dishes.

1.4.4 Standardization of inoculum

Three colonies of test organisms were picked, inoculated into 5 ml of sterile nutrient broth and incubated (18-24 hours) for bacterial growth. Turbidity was adjusted to match 0.5 McFarland's standard (Coyle, 2005).

1.5 *In vitro* evaluation of antibacterial activity

The extracts of the leaf extracts (JALE and JELE) were evaluated *in vitro* for antibacterial activity against *Staphylococcus aureus*, and *Escherichia coli.* Nutrient agar (28 g) was added to 1 Litre distilled water in a conical flask. This was stirred and autoclaved at 121°C and then cooled to 50°C. A portion of the medium (19 ml) was poured into a sterile Petri dish and allowed to solidify. The sterility of the medium was confirmed by allowing it to stay for 8 hours and ensuring no contamination (Daniyan et al., 2011).

An isolate colony of each test organism was sub-cultured on Nutrient agar and incubated at 37°C for 8 hours. This was spread on the entire plate medium to ensure uniform growth. Sterilized crude extract for agar well diffusion method. It was placed inside the bored holes on the agar plates containing the test organisms. The plates were, subsequently, incubated for 24 hours at 37°C (Wakirwa et al., 2013). Zones of inhibition were observed using a hand lens for proper magnification and measured. Antimicrobial assays were carried out in quadruplicate.

1.6 Minimum inhibitory concentration (MIC) of extracts of the extracts

The MIC of extracts against the test organisms were determined using the broth dilution method as described by the NCCLS (2003). Briefly, 2.5 ml of fresh Nutrient broth (NB) was dispensed into three test tubes and autoclaved for 15 minutes. Two point five millilitre of stock extracts at the concentration of 30 mg/ml was added to the first test tube, and serial diluted to obtain concentrations of 15 mg/ml and 7.5 mg/ml. A loopful of standardized test organisms adjusted to 0.5 McFarland standard was inoculated into each tube. Positive controls were equally set up by using the NB alone and the extract the test tubes were sealed with foil paper, cotton wool, and masking tape. The tubes were then incubated at 37°C for 18-24 hours. Tube (s) with least concentration or with no detectable growth when checked visually for turbidity was recorded as the MIC (Abalaka et al., 2011).

1.7 Minimum bactericidal concentration (MBC) of extracts of the extracts

Sample from the tubes used in MIC determination, which did not show any visible growth after period of incubation, were streaked out on Nutrient Agar for 24 hours to determine the minimum bactericidal concentration of the extract that will kill the test organisms. The lowest concentration of the extract indicating a bactericidal effect after incubation was regarded as the Minimum Bactericidal Concentration (MBC) (Aboada et al., 2006).

1.8 Data analysis

One-way analysis of variance (ANOVA) was used to compare the mean differences between the zones of inhibition of the extracts and controls. Significant difference between means were separated by Duncan multiple range test (DMRT). All results were expressed as mean±SD, while all statistical decisions were taken at 95% level of significance.

2 Results

2.1 Phytochemical screening of the crude extracts of leaf of *J. tanjorensis*

Phytochemical analyses of crude Ethanolic (JELE) and Aqueous (JALE) leaf extracts of the plants revealed the presence of six (6) medically active components (Table 1). However, their presence was dependent on the solvent extraction type. Five (5) compounds namely, alkaloids, saponins, tannin, phenols and anthraquinones were detected in JELE, while four (4), namely, alkaloids, steroids, tannin and phenols were detected in JALE (Table 1).

Alkaloids, tannin and phenols were found in all extract types. While Saponins and Anthraquinones were found only JELE, steroids were found exclusively in JALE (Table 1). Between the extract types, Alkaloid was more in JALE than JELE.

2.2 Antibacterial screening of crude ethanolic leaf extracts of *J. tanjorensis*

The Antibacterial screening of crude ethanolic leaf extracts of *J. tanjorensis* is shown in Table 2. Ethanolic (JELE) showed activity on *E. coli* only at the highest concentration tested (50 mg/ml) with zone of inhibition of 9.10±0.85 mm, JALE showed activities at all concentrations tested ranging from 10.07±1.68 to 16.03±2.70 mm, respectively at 30 and 50 mg/ml (Table 2).

Both extract types had significant effects on *S. aureus*, with JELE having range of values of 8.67±3.06 to 15.03±0.95 mm and JALE, 12.80±0.98 to 20.33±1.53 mm, respectively, at 30 and 50 mg/ml.

2.3 Minimum inhibition (MIC) and bactericidal concentration (MBC) of crude extract of *J. tanjorensis*

The values of MIC for JELE were higher than those of JALE for both species of bacteria. JELE had values of 25 and 15 for *E. coli* and *S. aureus*, respectively, while JALE has a value of 7.5 mg/ml for both species of bacteria. Interestingly, both extract types were bacteriostatic in action (Table 3).

Table 1 Phytochemical Components of Crude extracts of *Jatropha tanjorensis*

Components	JELE	JALE
Alkaloids	+	++
Saponins	+	-
Steroids	-	+
Cardiac glycosides	-	-
Tannin	+	+
Flavonoids	-	-
Phenols	+	+
Terpenes	-	-
Anthraquinones	+	-
Total	5	4

Note: - Not detected, + fairly present, ++ moderately present; JELE = *Jatropha* Ethanolic Leaf Extract; JALE = *Jatropha* Aqueous Leaf Extract

Table 2 Zones of inhibition (mm) of Crude ethanolic leaf extracts of *Jatropha tanjorensis*

Concentration	*E. coli*		*S. aureus*	
(mg/ml)	JELE	JALE	JELE	JALE
Water (0.1 ml)	N. I	N. I	N. I	N. I
30	N. I	10.07±1.68^{a}	8.67±3.06^{a}	12.80±0.98^{a}
40	N. I	13.40±1.22^{b}	12.33±1.53^{b}	15.67±2.89^{b}
50	9.10±0.85^{a*}	16.03±2.70^{c}	15.03±0.95^{c}	20.33±1.53^{c}
Amoxicillin (1.3)	16.33±2.52^{b}	22.67±5.51^{d}	20.03±1.79^{d}	16.00±2.65^{d}

Note: *Values followed by same superscript alphabets in a column are not significantly different at P=0.05; N. I. = No Inhibition; JELE = *Jatropha* Ethanolic Leaf Extract; JALE = *Jatropha* Aqueous Leaf Extract

Table 3 Minimum Inhibitory (MIC) and Bactericidal (MBC) Concentrations of crude extracts of *Jatropha tanjorensis* Leaf

Test Organism	MIC (mg/ml)		MBC (mg/ml)	
	JELE	JALE	JELE	JALE
E. coli	25	7.5	I. N.	I. N.
S. aureus	15	7.5	I. N.	I. N.

Note: I. N. = Inhibitory or Bacteriostatic; JELE = *Jatropha* Ethanolic Leaf Extract; JALE = *Jatropha* Aqueous Leaf Extract

3 Discussions

Phytochemical analysis of the two leaf extract types revealed the presence of six different components, namely, alkaloid, saponins, steroids, tannin, phenols and anthraquinones. There was differential composition in two extract types; ethanolic leaf extract (JELE) had five, while the aqueous counterpart had four. This difference in phytochemical constituents of extracts from the same plant could be due to the solvent of extraction (Amos-Tautua et al., 2011). Earlier studies by Oyewole and Akingbala (2011) using methanol, and Arun and Brindha (2012) using ethanol as solvents of extraction have reported the presence of flavonoids, terpenoids, and cardiac glycosides.

The presence of these secondary metabolites in the extracts of this plant part (leaf) could probably be responsible for its ethnomedical importance. Plants rich in alkaloids have been reported to possess antimalarial, analgesic and/or stimulant and antidiabetic effects (Costantino et al., 2003; Egunyomi et al., 2009; Erdemoglu et al., 2009), those with saponins, are useful in the treatment of hypercholesterolemia (Malinow et al., 1997; Amos-Tautua et al., 2011). While plants rich in Tannins, are useful in combating diarrhoea, headache, poor appetite, haemorrhoids, bactericides (Gonzalez et al., 2002; Katie and Thorington, 2006; Doss et al., 2009; Yisa, 2009). Phenol, when present in a plant, helps the plant to act as an oral analgesic/ anaesthetic and to temporarily treat pharyngitis and prevention of UV-induced skin damage (Svobodová et al., 2003).

The JALE appeared to be more active against the bacteria than JELE. The difference in the phytochemical composition of JALE over JELE, especially, in the higher presence of alkaloids, and exclusive presence of steroids could be responsible for the greater activities of former over the latter extract. *Staphylococcus aureus* was more susceptible to the plant extracts than *Escherichia coli*, with JELE being more active against the former species than the latter. Notably, JELE was only active against *E. coli* at the highest concentration tested (50 mg/ml). JALE, on the other hand, was very active against both bacterial species.

Although, MBC analyses revealed inhibitory or bacteriostatic effects of both extract types on the bacterial organisms, JALE have, proven, further to be more potent over JELE, as lower MIC values were obtained in the former extract type than the latter for both bacterial species.

Author's contributions

DSY and UCC conceived and designed the experiment. UCC, OOJ and CPO performed the experiments. DSY, UCC and UAC analyzed the data. UCC and UAC wrote the first draft of the manuscript. UCC contributed to writing of the manuscript. All Authors agreed with manuscript results and conclusion. All Authors made critical revisions and approved final version of the manuscript.

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