

Comparative *in vitro* Antimicrobial and Phytochemical Evaluation of Methanolic Extract of Root, Stem and Leaf of *Jatropha curcas* Linn

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ABSTRACT

Background: Earlier researchers have reported antibacterial activity of different specific parts, but none of the reports show the comparative microbial and phytochemical studies of root, stem and leaf extract. **Objective:** To compare and investigate antimicrobial, qualitative phytochemical studies, phenol, flavonoid and TLC analysis of root, stem bark, leaf extracts of *Jatropha curcas* Linn family Euphorbiaceae. **Methods:** The dried plant powder was subjected to Soxhlet extraction with methanol. These solvent extracts were subjected to a preliminary phytochemical screening to detect the different chemical principles present viz., carbohydrates, proteins, amino acids, steroids, glycosides, alkaloids, flavonoids, tannins and phenolic compounds, fixed oils. Antimicrobial activity was evaluated by disc diffusion method and minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), minimum fungicidal concentration (MFC) was calculated by micro dilution method. Thin layer chromatography was also performed using solvent system chloroform, benzene, hexane, and ethyl acetate for the analysis of a number of constituents in the plant extract. The content of the total phenolics in the extract was determined spectrometrically according to the Folin-Ciocalteu procedure and calculated as catechol equivalent. The content of total flavonoids in the extract was determined and calculated as quercetin equivalent. **Result:** These extracts showed antibacterial, antifungal activities against gram-positive and gram-negative bacteria with varying magnitudes. The phytochemical analysis showed the presence of alkaloid, saponin, tannins, terpenoids, steroids, glycosides, phenols and flavonoids. Maximum phenolic content (38.8) was found in leaf extract and flavonoid content (18.14) in latex of plant. **Discussion:** It is concluded that the antimicrobial activity showed by the plant was due to the presence of these phytochemicals. Further studies are highly needed for drug development.

Key words: phytochemical, microdilution, TLC, folin-ciocalteu, quercetin, MBC, MFC.

INTRODUCTION

Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being.

Their role is two fold in the development of new drugs. They may become the base for the development of a medicine, a natural blue print for the development of new drugs or a phytomedicine to be used for the treatment of diseases.^[1] Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in the developing world.^[2] *Jatropha* species belongs to the family Euphorbiaceae and is used in traditional folklore medicine to cure various ailments in Africa, Asia and Latin America.^[3] *Jatropha curcas* Linn is commonly called physic nut, purging nut or pig nut. Previous studies have reported that the plant exhibits bioactive activities as wound healing,^[4] antidiarrhoeal,^[5] antidiabetic,^[6] antitumor effects,^[7] immunomodulatory activity^[8] and used

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in treatment of rheumatism.^[9,10] Fagbenro-Beyioku (1998) investigated and reported the anti-parasitic activity^[11] of the sap and crushed leaves of *J. curcas*. The water extract of the branches also strongly inhibited HIV-induced cytopathic effects with low cytotoxicity.^[12] Previous works have shown that many *Jatropha* species possess antimicrobial activity^[13] and antifungal activity of *J. curcas* seed cake.^[14] Many useful products from seeds, especially the oil which is extracted from the seed is of multipurpose use.^[15,16] In addition, PEs with molluscicidal/antimicrobial activities could be utilized for agricultural and pharmaceutical^[17] use with antihelminthic activity.^[18] After extraction of oil, *Jatropha* presscake strongly supports its use as a potential coagulant agent,^[19] which can also be utilized as a substrate for biogas production.^[20] Defatted *Jatropha curcas* L. (*J. curcas*) seed kernels contained a high percentage of crude protein (61.8%) and relatively little acid detergent fiber (4.8%) and neutral detergent fiber (9.7%), recommending its use as a source of anticancer therapeutic agent toward breast cancer cells.^[21] Several studies have confirmed the antimicrobial efficacy of different *Jatropha* species. Whatever limited information is available on the medicinal properties of *Jatropha curcas* is mostly on the leaf extracts of the plant.

Therefore this study was planned to evaluate comparative antibacterial and antifungal activity of crude extracts of the stem bark, root and leaf of *J. curcas* and also investigate preliminary phytochemical analysis including phenolic and flavonoid content.

MATERIAL AND METHODS

Plant Material and Extraction

The plant *Jatropha curcas* Linn was collected from Botanical Garden of the Institute of Agriculture Science, Banaras Hindu University, Varanasi (India). The identification of the plants was done by Dr. K.N. Dwivedi, Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi (India). Reference number "DG/KN/D/11-12/603" was given to the plant sample.

Preliminary Phytochemical Screening

Chemical tests were carried out on the alcoholic extract and on the powdered specimen using standard procedures to identify the constituents.^[22,23] The plant extract was assayed for the presence of alkaloids, glycosides, flavonoids, tannins, phenolic compounds, saponins, terpenoids and steroids.

Testing for alkaloids

Each extract (0.5 g) was stirred with 5 mL of 1% HCL on a steam bath. The solution obtained was filtered and one mL

of the filtrate was treated with a few drops of Mayer's reagent. The turbidity of the extract filtrate on addition of Mayer's reagent was taken as evidence of the presence of alkaloids in the extract.

Testing for tannins and phenolics

Each extract (0.5 g) was separately stirred with 10 ml of distilled water and then filtered. A few drops of 5% FeCl₃ reagent were added to the filtrate. Blue-black or blue green colouration or precipitation was taken as an indication of the presence of phenolics and tannins.

Test for saponin

About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered, 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, and observed for the formation of emulsion.

Test for steroids

Two ml of acetic anhydride was added to 0.5 ethanolic extract of each sample with 2 ml H₂SO₄. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for terpenoids (Salkowski test)

Five ml of each extract was mixed in 2 ml of chloroform, and concentrated H₂SO₄ (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face formed to show positive results for the presence of terpenoids.

Test for cardiac glycosides (Keller-Killani test)

Five ml of each extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayered with 1 ml of concentrated sulphuric acid. A brown ring of interface indicated the deoxysugar characteristic of cardenolides. A violet ring could appear below the brown ring, while in the acetic acid layer, a greenish ring formed just gradually throughout the thin layer.

Screening of Antibacterial Activity

Test Microorganism

A total of 4 bacterial strains viz. *S. flexneri* ATCC 12022, *E. coli* ATCC 25922 (Gram-negative), *S. aureus* ATCC 25323, *E. faecalis* (gram-positive), and four fungal strains namely *Candida albicans* ATCC 90028, *Candida krusei* ATCC 6258, *Candida tropicalis* ATCC 750, *Candida parapsilosis* ATCC 22019 were used in the investigation. All cultures were obtained from the American Type Culture Collection (ATCC), MTCC, clinical strains preserved at Department of Microbiology, Institute of Medical Sciences, BHU, Varanasi, India. Fresh bacterial broth cultures were prepared before the screening procedure.

Preparation of sample extract for microbiological assay

About 1g of each extract was dissolved in 10 ml (100 mg ml⁻¹) of peptone water to obtain a stock solution and the working solution was prepared. The extract was diluted as 1:10 equivalent to 100 mg ml⁻¹ and 1:5 dilution equivalent to 50 mg ml⁻¹, from which 5 µl was dispensed on a sterile disc of Whatman's filter paper no. 1 of 6 mm diameter for susceptibility testing.

Antimicrobial Susceptibility Test

The disc diffusion method was used to screen the antibacterial activity^[24,25,26] and antifungal activity.^[27,28] Muller Hinton agar (MHA) plates were prepared by pouring 15 ml of molten media into sterile petriplates. The fresh grown bacteria was suspended in sterile saline to achieve concentration of 10⁷ cfu/ml. This suspension was spread on the surface of MHA agar plates. The plates were allowed to dry for 5 min. The different concentrations of extract (100, 200 mg/ml) were put on 6 mm sterile disc of Whatman filter paper no. 1. The disc was then placed on the surface of the medium and the compound was allowed to diffuse for 5 min and the plates were kept for incubation at 37 °C for 24 hr for bacteria and 48 hr at 25 °C for fungal agents. At the end of incubation, inhibition zones were examined around the discs, which if present, were measured with transparent ruler in millimeters. This study was performed in triplicate.

Determination of MIC, MBC and MFC

MIC was determined by micro-dilution method^[29,30] using serially diluted (2 fold) plant extracts according to the National Committee for Clinical Laboratory Standards (NCCLS) (National Committee for Clinical Laboratory Standards, 2000).^[31] MIC of the extracts was determined by dilution of methanolic extract with various concentrations. Equal volume of each extract and nutrient broth were mixed in wells of microtiter plate. Specifically 0.1 ml of standardized inoculums (1-2 × 10⁷cfu/ml) was added in each tube. The plates were incubated aerobically at 37 °C for 18-24 h for bacteria and 48 h at 25 °C for fungal growth. Two control wells were maintained for each test batch. These included antibiotic control (containing extract and growth media without inoculum) and organism control (tube containing the growth medium, saline and the inoculum). The lowest concentration (highest dilution) of the extract that produced no visible bacterial growth (no turbidity) when compared with the control were regarded as MIC. However, the MBC and MFC were determined by sub-culturing the test dilution on to a fresh drug free solid medium and incubated further. The highest dilution that yielded no bacterial or fungal colony was taken as MBC and MFC.

Media used

Muller-Hinton agar, Luria broth (Hi-media, Mumbai, India), and Sabouraud dextrose agar pH 7.3 ± 0.2 (Hi-media), RPMI 1640 were used for antibacterial and antifungal activity respectively.

Determination of Total Phenols (Tp) by Spectrophotometric Method

Total Phenolic concentration in different fractions of alcoholic extract was measured by Folin Ciocalteu assay. Briefly, 5 ml of distilled water, 0.5-1.0 ml of sample, and 1.0 ml of Folin Caiocalteu reagent was added to a 25 ml flask. The content was mixed and allowed to stand for 5-8 min at room temperature. Next 10 ml of 7% sodium carbonate solution was added followed by distilled water. Solution was mixed and allowed to stand at room temperature for 15 min, and then absorbance was recorded at 750 nm. TP content was standardized against gallic acid and expressed as milligram per liter of gallic acid equivalents (GAE). The linearity range for this assay was determined as 0.5-5.0 mg/l GAE (R²=0.999), giving an absorbance range of 0.050-0.555 absorbance units.^[32,33]

Determination of Total Flavonoid Content

Total flavonoid content was measured by using aluminium chloride (2%) in which it was mixed with a solution of the test samples. Absorbance reading at 415 nm (Elico SL 177) was taken after 10 min against a blank sample consisting of 5 ml of sample solution and 5 ml of methanol without aluminium chloride. The total flavonoid content was determined using a standard curve of quercetin at 0-50 µg/ml. The average of three readings was used and then expressed in µg quercetin equivalent flavones per mg extract.^[33]

Thin layer chromatography (TLC)

Thin layer chromatography (TLC) was used to separate the different part of *Jatropha curcas* extract into different spots on the chromatplate. The chromatograms developed on the microscope slide, were dried and observed visually for the different parts of plant extract components. The developing solvents used in different extracts were hexane, chloroform, and benzene with ratio 9:1.

The retention factor was calculated using the following equation:

$$R_f = \frac{\text{Distance move by the substance (cm)}}{\text{Distance move by the solvent (cm)}}$$

RESULT

Ancient Indian system of medicine (Ayurveda) is mainly based on herbal treatment. The root, stem, bark, leaf of *J. curcas* Linn are extensively used in the management of

various infections and allergic diseases. The preliminary phytochemical screening of alcoholic extract of various parts of *J. curcas* Linn is presented in Table 5, showing the presence of alkaloids, phenolic groups, flavonoids,

saponins, steroids, tannins, cardiac glycosides and terpenoids. In addition to the phytochemical screening, antimicrobial efficacy was determined on the basis of number of secondary metabolites. Activities of extracts

Table 1: Determination of MIC, MBC (mg/ml) of *Jatropha Curcas* Linn

Strains	<i>E. coli</i> ATCC 25922		<i>S. aureus</i> ATCC 25323		<i>E. faecalis</i>		<i>S. flexineri</i> ATCC 12022	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
ROOT	20	25	6.25	6.25	10	12.5	12.5	25
LEAF	12.5	12.5	12.5	25	6.25	6.25	6.25	12.5
STEAM	10	12.5	6.25	12.5	10	12.5	10	12.5

Table 2: Determination of MIC, MFC (mg/ml) of *Jatropha curcas* Linn

Strains	<i>C. albicans</i> ATCC 90028		<i>C. tropicalis</i> ATCC 750		<i>C. krusei</i> ATCC 6258		<i>C. parapsilosis</i> ATCC 22019	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
ROOT	12.5	25	12.5	25	25	30	20	25
LEAF	10	12.5	12.5	30	12.5	20	12.5	12.5
STEAM	12.5	25	25	30	12.5	25	20	25

Table 3: TLC Result of root, stem and leaf methanolic extract of *Jatropha curcas* Linn

Extracts	Solvent system	Number of components	Distance of spot (cm)	Solvent front (cm)	Rf value
Root	Benzene:chloroform:few drop acetic acid (9:1)	4	8.9, 7.3, 6.9, 5.4,	10.9	0.81, 0.66, 0.63, 0.49
Stem	Hexane:chloroform:few drop acetic acid(9:1)	5	8.8, 8.1, 7.3, 4.5, 2.9	10.2	0.86, 0.79, 0.70, 0.44, 0.26
Leaf	Hexane:chloroform:few drop acetic acid (9:1)	4	11.5, 9.3, 7.1, 3.4	12.5	0.92, 0.74, 0.56, 0.27

Table 4: Antimicrobial activity of the different extract of *Jatropha curcas* Linn plant

Microorganism	Zone of inhibition (in mm)						Standard drugs
	<i>J. curcas</i> extract concentration (mg/ml)						
	Root		Leaf		Steam		
	100	200	100	200	100	200	
<i>S. flexineri</i> ATCC 12022	7 ± 0.19	9 ± 0.29	12 ± 0.53	14 ± 0.73	10 ± 0.32	12 ± 0.52	25 (Ciprofloxacin)
<i>E. coli</i> ATCC 25922	6 ± 0.11	9 ± 0.21	8 ± 0.11	10 ± 0.32	9 ± 0.59	12 ± 0.79	26 (Norfloxacin)
<i>S. aureus</i> ATCC 25323	9 ± 0.12	11 ± 0.24	11 ± 0.17	13 ± 0.37	8 ± 0.35	10 ± 0.55	24 (Ampicilin)
<i>E. faecalis</i>	9 ± 0.33	11 ± 0.55	10 ± 0.11	12 ± 0.27	8 ± 0.25	10 ± 0.81	28 (Ciprofloxacin)
<i>C. albicans</i> ATCC 90028	8 ± 0.36	10 ± 0.56	8 ± 0.42	10 ± 0.62	7 ± 0.50	9 ± 0.43	16 (Fluconazole)
<i>C. krusei</i> ATCC 6258	6 ± 0.54	8 ± 0.74	10 ± 0.25	12 ± 0.45	8 ± 0.15	10 ± 0.27	18 (Fluconazole)
<i>C. tropicalis</i> ATCC 750	10 ± 0.27	12 ± 0.47	9 ± 0.26	11 ± 0.36	7 ± 0.73	9 ± 0.41	16 (Amphotericin B)
<i>C. parapsilosis</i> ATCC 22019	7 ± 0.18	9 ± 0.38	10 ± 0.24	12 ± 0.44	7 ± 0.36	9 ± 0.39	15 (Amphotericin B)

Table 5: Phytochemical analysis of various parts of *Jatropha curcas* Linn using methonolic extract

S. No.	Constituents	Tests	Leaf extract	Root extract	Stem extract
1	Alkaloids	Mayer's reagent	+	+	+
2	Saponin	Foam test	+	+	+
3	Tanin	Extract + 5% Fecl3	+	+	+
4	Terpenoid	Salkowski test	+	-	+
5	Steroid	Liebermann-Burchard reaction	+	+	+
6	Glycosides	Keller-kilani test	+	+	+
7	Phenolic Compound	Extract + 5% Fecl3	+	+	+
8	Flavonoid	Residue + Lead acetate soln.	+	+	+

against test organism were expressed in the form of diameter of zone of inhibition around extract (Table 4). The inhibition zone increases with increase in concentration. The MIC of root, leaf and stem extract for different organism ranged between 6.25 mg/ml and 25 mg/ml. The MBC and MFC of the various extracts for different microbes ranged between 6.25 mg/ml and 30 mg/ml (Tables 1 and 2). The results TLC analysis using benzene: hexane: chloroform solvent mixture as shown in Table 3 revealed four spots for root, five spots for stem and four spots for leaf extracts.

Estimation of total phenolic and total flavonoid content showed that the leaf extract was having the maximum phenolic content (38.8 ± 2.14) in μg Gallic acid equivalents (GAE) followed by the root extract (26.15 ± 3.84) with a very less flavonoid content in root (1.88 ± 1.00) and leaf (1.72 ± 2.08) extracts in μg of quercetin equivalents (QE).

DISCUSSION

These classes (alkaloids, saponins, tannins, anthraquinones and flavonoids) of compounds are known to have activity against several pathogens and therefore aid the antimicrobial activities of *J. curcas* and suggest their traditional use for the treatment of various illness.^[34,35] In all the three extracts, tannins were present resulting in the inhibition of cell protein synthesis as it forms irreversible complexes with prolinerich protein.^[36] Tannin containing herbs have been reported in treating intestinal disorders such as diarrhea and dysentery,^[37] treatment of inflamed or ulcerated tissues.^[38] *In vitro*

antibacterial test results presented in Figure 1 show antibacterial activity against gram-positive and negative bacteria with strong antifungal activity (Figure 2). The methanolic extracts exhibited considerable level of inhibition against the entire test organism compared to the standard drug. This is suggestive of the presence of some compounds or groups in the extract with similar mechanism of action to that of the standard drug used in bacterial and fungal activity. It has been 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. These observations therefore support the use of *J. curcas* in herbal cure remedies.

Various workers have already shown that gram-positive bacteria are more susceptible towards plants extracts as compared to gram negative. These differences may be attributed to the fact that the cell wall in gram-positive bacteria is of a single layer, whereas the gram negative cell wall has a multilayered structure. Alternatively, the passage of the active compound through the gram negative cell wall may be inhibited. Although *J. curcas* leaf, root and stem extracts show less MIC against gram positive as compared to gram negative bacteria. It is thought that the observed differences may result from the doses used in this study. In addition, microorganisms show variable sensitivity to chemical substances related to different resistance levels between strains. Mujumdar et al. (2001) also reported that the crude methanol extract from the root of *J. curcas*

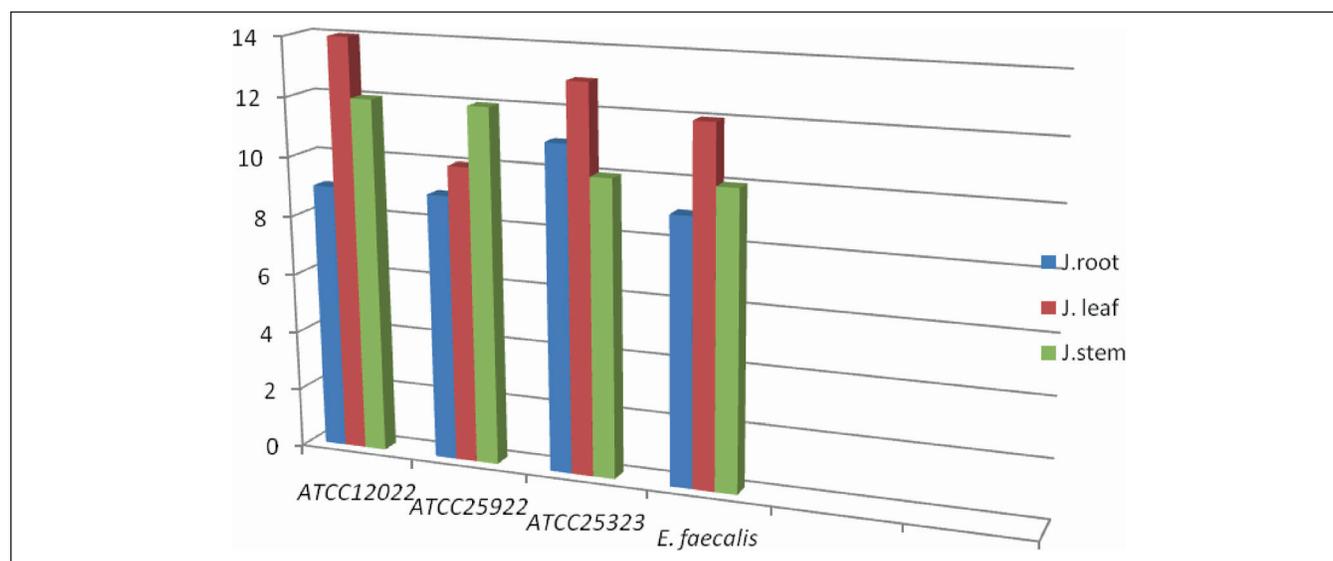


Figure 1: Antibacterial effect (in mm) of root, stem and leaf extract

Graph showing antibacterial activity with various strains, among root, stem and leaf. Leaf extract of *J. curcas* is comparatively more active in all the microorganisms as leaf extract has a higher zone of inhibition than the root and stem bark.

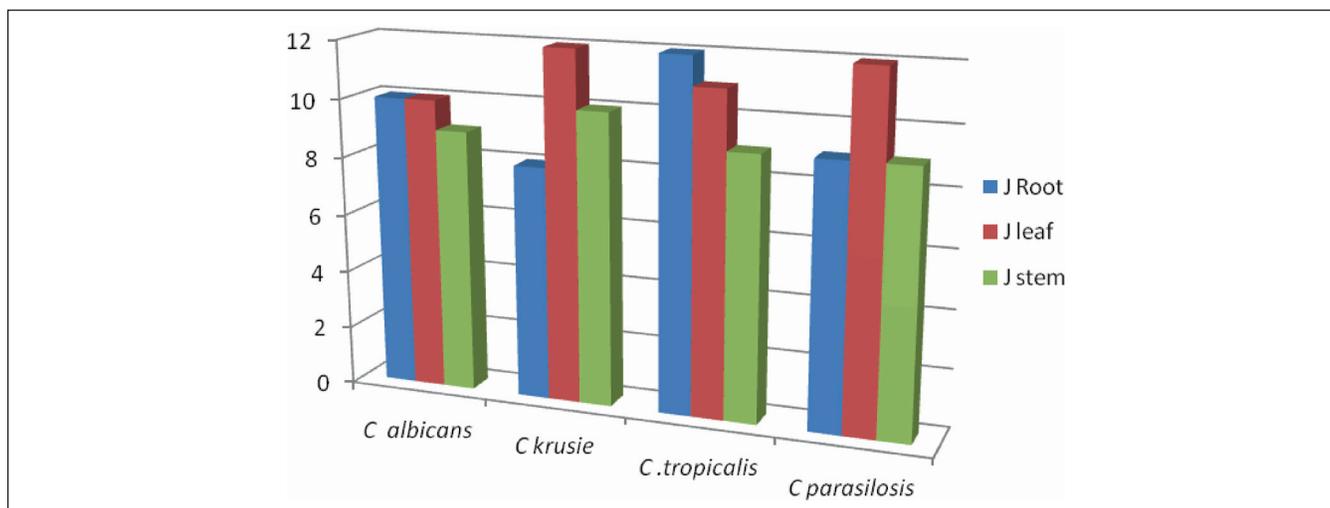


Figure 2: Antifungal effect (in mm) of root, stem and leaf extract

Graph showing antifungal activity with various strains, among root, stem and leaf. Leaf extract of *J. curcas* is comparatively more active in all candida species as leaf extract has a higher zone of inhibition than root and stem bark.

exhibited anti-diarrhea activity^[39] in mice through the inhibition of prostaglandin biosynthesis and the reduction of osmotic pressure. Recently, Aiyelaagbe et al. (2007) reported that the presence of some secondary metabolites in the root extract of *J. curcas* inhibited some microorganisms isolated from sexually transmitted infections.^[40] This may be attributed to the presence of soluble phenolic and polyphenolic compounds.^[41] However, it may be suggested that plant extracts exhibiting diameters of zones of inhibition larger than 10 mm are considered active.^[35] Thus it is believed that the extract is a better antimicrobial agent for various pathogenic fungus and bacteria. Among these, leaf extract was showing better antibacterial and antifungal properties compared to stem and root extract of *J. curcas*. The pharmacological activities of the drug may be contributed to the presence of secondary metabolites.

Hence, the presence of some metabolites in *J. curcas* suggests its activities against microbes. It is concluded that leaf, stem and root of *J. curcas* could be a potential source of active antimicrobial agents, and a detailed assessment of its *in vivo* potencies and toxicological profile is ongoing.

CONCLUSION

The inhibitory effect of the extract of *J. curcas* against pathogenic bacterial strains can introduce the plant as a potential candidate for drug development for the treatment of ailments caused by human pathogens. The ability of the extracts to inhibit the growth of several bacterial and fungal species is an indication of the broad spectrum antimicrobial potential of various parts of *J. curcas*, which makes the complete plant a candidate for bioprospecting for antibiotic and antifungal drugs.

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CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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