

Short communication

Phytochemical characterization, antimicrobial activity and reducing potential of seed oil, latex, machine oil and presscake of *Jatropha curcas*

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Abstract

Objective: This study aims to evaluate the antimicrobial activity, phytochemical studies and thin layer chromatography analysis of machine oil, hexane extract of seed oil and methanol extract of presscake & latex of *Jatropha curcas* Linn (family Euphorbiaceae).

Materials and Methods: *J. curcas* extracts were subjected to preliminary qualitative phytochemical screening to detect the major phytochemicals followed by its reducing power and content of phenol and flavonoids in different fractions. Thin layer chromatography was also performed using different solvent systems for the analysis of a number of constituents in the plant extracts. Antimicrobial activity was evaluated by the disc diffusion method, while the minimum inhibitory concentration, minimum bactericidal concentration and minimum fungicidal concentration were calculated by micro dilution method.

Results: The methanolic fraction of latex and cake exhibited marked antifungal and antibacterial activities against Gram-positive and Gram-negative bacteria. Phytochemical analysis revealed the presence of alkaloids, saponins, tannins, terpenoids, steroids, glycosides, phenols and flavonoids. Reducing power showed dose dependent increase in concentration compared to standard Quercetin. Furthermore, this study recommended the isolation and separation of bioactive compounds responsible for the antibacterial activity which would be done by using different chromatographic methods such as high-performance liquid chromatography (HPLC), GC-MS etc.

Conclusion: The results of the above study suggest that all parts of the plants possess potent antibacterial activity. Hence, it is important to isolate the active principles for further testing of antimicrobial and other biological efficacy.

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Introduction

In view of increasing resistance to existing antimicrobial agents, some research has been performed worldwide to identify herbal drugs, as they are very important sources for discovering some new agents for treating various ailments related to bacterial infections. (Pan et al., 2012). According to the World Health Organization (WHO), about 80% of world's population rely on herbal medicines for some aspect of their primary healthcare, and the worldwide annual market for herbal products approaches approx. US\$ 60 billion. With increasing resistance, also, antibiotics are occasionally associated with adverse side effects to the host, including hypersensitivity, immune-suppression and allergic reactions (Graul et al., 2009). These adverse reactions lead to discovering some natural antimicrobial agents which will be effective against pathogenic microorganism with minimal side effects. One possible strategy is the rational localization of bioactive products from folk medicines, with the hope that systematic screening of these will result in the discovery of novel moiety with potent and useful activities against microbes. With this respect, there is an increasing demand for plant-based therapeutics in both developing and developed countries due to a growing recognition that they are natural products, non-narcotic and, in most cases, easily available at affordable prices with no side effects (Winslow et al., 1998).

Jatropha species belong to the family Euphorbiaceae and are used in traditional folklore medicine to cure various ailments in Africa, Asia and Latin America. *Jatropha curcas* is commonly called physic nut, purging nut or pig nut. Previous researchers studies different parts of *Jatropha* plant and reported that leaf extract of *Jatrophacurcas* has significant wound healing activity (Shetty et al., 2006), antidiabetic (Mishra et al., 2010), Immunomodulatory effect (Abd-Alla et al., 2009) and antiparasitic/disinfectant

activities (Fagbenro-Beyioku et al., 1998). Bark extract of the plant was also reported for wound healing effects (Shetty et al., 2006), root isolated fraction has been reported for anti-diarrhoeal effect (Mujumdar et al., 2001), curcin from seeds reported for anti-tumour activity (Lin et al., 2003) and latex of the plant contains alkaloids reported for anti-cancerous properties. Many products from the seeds, especially the oil extracted from the seed, have multiple uses (Openshaw, 2000). After extraction of the oil, *Jatropha curcas* press cake has been used as a potential coagulating agent (Abidin et al., 2011), which can also be utilized as a substrate for biogas production (Schmidt, 2011). The presscake from seeds provides organic manure, and is rich in protein (60–63%) compared to soybean (45%). The presscake could be an ideal protein source with a high content of essential amino acids even higher (except lysine) than the Food and Agriculture Organization reference protein. Our previous research (Sharma et al., 2011) has shown that the seed cake of some *Jatropha* species possess antimicrobial activity, but there is no such comparative phytochemical and antimicrobial analysis of the seed oil, latex, machine oil and presscake of *Jatropha curcas*. Therefore, this study aimed to evaluate the potential of *Jatropha curcas* against various human pathogenic micro-organisms while it will help us to compare the activity of seed oil, machine oil, press cake and latex of the plant along with its preliminary phytochemical characterization. This research paper presents a consolidated account of comparison and scope of *Jatropha curcas* over the decades and focuses on contemporary information and trends of future research.

Materials and Methods

Plant material and extraction

The identification of the *Jatropha curcas* plants was carried out by Dr. K.N.

Dwivedi, Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India, with the reference number DG/KND/11-12/603. *Jatropha oil* was obtained from Suryapharmaceutical Company Ramnagar, Varanasi and termed as *Jatropha* machine oil (JCMO). Seeds of *Jatropha curcas* were collected from the Botanical Garden of the Institute of Agricultural Science, Banaras Hindu University, Varanasi (India) and dried in sun light and were processed chemically by an extractor in hexane solvent seeds and the seed powder was heated for half an hour. This process is again continued to heat the residual solution. This process is called reflux method and the total seeds of *Jatropha curcas* extracted with hexane by reflux method, were found oily in hexane fraction (JCHO).

Jatropha press cake collected from expeller machine was dried in an oven maintained at 50°C. One part of the cake was subjected to organic solvent extraction using reflux method and the methanolic extract of mechanically prepared seed-cake (MEMJC) was obtained.

Jatropha curcas latex was collected from the Botanical Garden of the Institute of Agricultural Science, Banaras Hindu University, Varanasi (India) and dried under sun light followed by extraction with methanol. For extract preparation, powdered drug was collected and weighed which was refluxed in round bottom flask for two hours on water bath. The solvent was filtered out and the process was repeated two times. The solvent from all the steps was collected and distilled in vacuum distillation plant. The solvent-free extract was prepared by drying the solvent on water bath and by desiccation in the vacuum desiccation till in constant weight and termed as the total methanol fraction of latex (JCML).

Preliminary phytochemical screening

Chemical tests were carried out using standard procedures to identify the

constituents in all of the extracts of plants (Metwally *et al.*, 2010; Gangwar *et al.*, 2011). The plant extracts were screened for the presence of alkaloids, glycosides, flavonoids, tannins, phenolic compounds, saponins, terpenoids and steroids by using the following standard test.

Tests for alkaloids

Mayer's test

One ml of Mayer's reagent was added to different extracts. The formation of creamy colour precipitate indicated the presence of alkaloids.

Tests for flavonoids

Shinoda test

To the extract samples little magnesium turnings/lead acetate and few drops of conc. hydrochloric acid were added after the appearance of crimson red colour in few minutes which indicated the presence of flavonoids.

Tests for cardiac glycosides

Keller-Killiani test (test for deoxy sugars)

This test was carried out by extracting the drug with chloroform and the extract was evaporated to dryness; then, 0.4 ml glacial acetic acid containing trace amounts of ferric chloride was added. After transferring it to a small test tube, 0.5 ml of conc. Sulphuric acid was added by the side of test tube. Appearance of blue colour of acetic acid layer indicated the presence of cardiac glycosides.

Tests for saponin glycosides

Froth formation test

Two ml of each extract sample was placed with water in a test tube and shaken well. Formation of stable froth (foam) indicated the presence of saponin glycosides.

Tests for tannins

Ferric chloride test

Different extract samples were treated with ferric chloride solution, in which the appearance of blue and green colors indicated the presence of hydrolysable and

condensed tannins respectively (Trease, 2002).

Tests for steroids and triterpenoids

Salkowski test

Different extract samples were treated with few drops of concentrated sulphuric acid. Appearance of red and yellow colour at the lower layer indicated the presence of steroids and triterpenoids respectively (Harborne, 1998; Kokate, 2006).

Liebermann-burchard's test

The extract (2 mg) was dissolved in 2 ml of acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulfuric acid was added along the side of the test tube. A brown ring formation at the junction and the turning of the upper layer to dark green color confirmed the test for the presence of phytosterols.

Quantitative estimation

Estimation of total phenolic content

The total phenolic content in the plant material was determined according to the Singleton and Rossi method, with some modifications (Singleton, 1965). Estimation was done by folin-ciocalteu method, where phenolic compounds form a blue complex. The gallic acid was used as a standard solution. 0.5ml of the test extract solution was mixed with 2.5ml of 1N folin-ciocalteu reagent and was incubated for 5 minutes and then 2ml of 75 g/L sodium carbonate was added followed by distilled water. After incubation at room temperature for 2 hours, absorbance of reaction mixture was measured at 760nm against blank as methanol. The total phenolic content was expressed in μg of gallic acid equivalent (GAE) of dry plant material. The linearity range for this assay was determined as 0.5-5.0mg/1 GAE ($R^2=0.999$), giving an absorbance range of 0.050-0.555 absorbance units (Kajaria et al., 2012; Sharma et al., 2012).

Estimation of total flavonoid content

Total flavonoid content in plant material was determined colorimetrically

according to the method described by Lamaison and Carret (Lamaison, 1990) by using quercetin as the standard. Here, 5ml of 2% aluminum chloride in methanol was mixed with the same volume of test solution. After incubation of 10 minutes, absorbance was measured at 415nm against blank sample. The total flavonoid content was determined using a standard curve of quercetin at 0-50 $\mu\text{g}/\text{ml}$. The average of three readings was used and then expressed in μg quercetin equivalent to flavones per mg of extract.

Reducing power

Reducing power was determined according to the developed method (Kajaria et al., 2012). A 2.5 ml solution of the extract (100-800 mg/ml) was mixed with equal volume of phosphate buffer (0.2 M, pH 6.6) and 1% potassium ferricyanide and placed in water bath at 50°C for 20 min. Then, it was cooled rapidly and 2.5 ml of 10% trichloroacetic acid was added and vortexed. This incubation mixture was centrifuged at 3,000 rpm for 10 min and its 5 ml supernatant was mixed with equal volume of distilled water and 1 ml of 0.1% ferric chloride. It was further incubated at room temperature for 10 min and the absorbance was read at 700 nm. The reducing property of the test sample was standardized against quercetin and expressed as the difference in optical density (OD) from control as well as the test as 0.1, and expressed as $\mu\text{g}/\text{ml}$. A higher degree of absorbance indicates the stronger reducing power.

Thin layer chromatography

Thin layer chromatography (TLC) was used to separate the different parts of *J. curcas* extract into different spots on the chromatoplate. The chromatograms were developed on the microscope slide, dried and observed visually for the various parts of the plant extract components. The developing solvents used in the different extracts are hexane, chloroform, and

benzene with a ratio of 9:1 (Kajaria *et al.*, 2011, Sharma *et al.*, 2012).

The retention factor was calculated using the following equation:

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

Screening for antibacterial activity

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

Preparation of sample extract for microbiological assay

About 1 g 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 1:10 to be equivalent to 100 mg mL⁻¹ and 1:5 to make a 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 for antibacterial (Singh *et al.*, 1999; Kajaria *et al.*, 2012) and antifungal activity (Aladag *et al.*, 2009). Muller Hinton Agar (Hi-media, Mumbai) (MHA) plates were prepared by pouring 15 mL of molten media into sterile petriplates. The freshly grown bacteria were suspended in sterile saline to achieve a 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 mg/mL, 200 mg/mL) were placed on 6-mm sterile discs of Whatman filter paper no.1. The discs were then placed on the surface of the medium where the compound was allowed to diffuse for 5 min while the plates were incubated at 37°C for 24 hours for bacteria and 48 hours at 25°C for fungal agents. At the end of incubation, inhibition zones were examined around the discs which, if present, were measured with a transparent ruler in mm. This study was performed in triplicate.

Determination of minimum inhibitory concentration, minimum bactericidal concentration and minimum fungicidal concentration

The minimum inhibitory concentration (MIC) was determined by micro-dilution method (Wiegand *et al.*, 2008) using serially diluted (2-fold) plant extracts according to the National Committee for Clinical Laboratory Standards (NCCLS, 2000). The MIC of the extracts was determined by dilution of methanolic extract with various concentrations. Equal volumes of each extract and nutrient broth were mixed in wells of a microtiter plate. Specifically, 0.1 mL of standardized inoculum (1–2 × 10⁷ cfu/mL) was added to each well of the microtiter plate. The plates were incubated aerobically at 37°C for 18–24 hours for bacteria and 48 hours at 25°C for fungal growth. Two control wells were maintained for each test batch. These included positive control (containing extract and growth media without inoculum) and organism control (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 was regarded as the MIC. However, the minimum bactericidal concentration (MBC) and minimum fungicidal

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concentration (MFC) were determined by sub-culturing the test dilution from wells of titerplate on to a fresh drug-free solid medium and incubated further for culture. The highest dilution that yielded no bacterial or fungal colony was taken to be the MBC and MFC, respectively.

Media

MHA plus Luria broth (Hi-media, Mumbai, India), and Sabouraud dextrose agar pH 7.3 ± 0.2 (Hi-media, Mumbai, India) plus RPMI 1640 culture media (Roswell Park Memorial Institute medium) were used for antibacterial and antifungal activity, respectively.

Statistical analysis

The number and size of tumor were compared by Paired t-test.

Results

The preliminary phytochemical screening of the alcoholic extract of various parts of *J. curcas* Linn revealed the presence of alkaloids, phenolic groups, flavonoids, saponins, steroids, tannins, cardiac glycosides, and terpenoids by using standard methods (Table 1). The total phenolic content was expressed in µg of gallic acid equivalent. Phenolic compounds may contribute directly to antioxidative action. and the total phenolic content was found maximum in MEMJC followed by JCML i.e. 38.40 ± 0.84 mg/g of gallic acid equivalent per extract. All values are expressed in mean ± SEM (n= 4). The total flavonoid content was also found maximum in MEMJC, followed by JCHO, JCMO and JCML, where the values are 20.70 ± 0.35, 19.23 ± 0.67,

18.14 ± 0.24 and 16.34 ± 0.89 mg/g quercetin equivalent per mg of the plant extract respectively. All values are expressed in mean ± SEM (n= 4). Reducing Potential of JCMO, JCHO, MEMJC and JCML showed dose dependent increase in concentration compared to standard Quercetin (Figure 1). Activities of different extracts against the test organisms were expressed as zone of inhibition (in mm) (Figures 2 & 3) compared to standard antibiotic dick used in the study (Table 5).

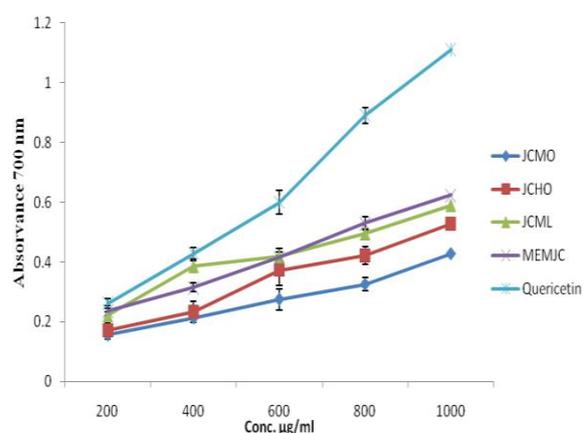


Figure 1. Reducing potential of JCMO, JCHO, JCML and MEMJC of *J. curcas*

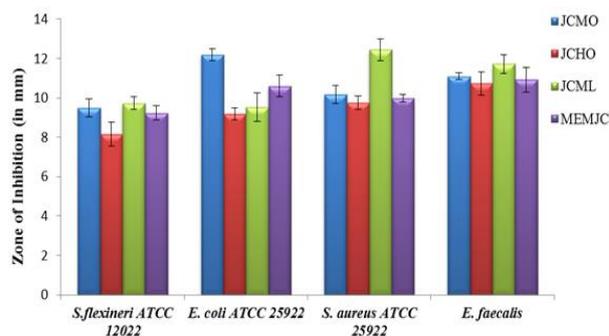


Figure 2. Antibacterial activity (in mm) of JCMO, JCHO, JCML and MEMJC of *J. curcas* against Human pathogens.

Table 1. Phytochemical constituents in different extracts of *Jatropha curcas*

| S. No. | Constituents | Tests | JCMO | JCHO | JCML | MEMJC |
|--------|-------------------|------------------------------|------|------|------|-------|
| 1 | Alkaloids | Mayer's reagent | + | - | + | + |
| 2 | Saponin | Foam test | + | - | + | + |
| 3 | Tanin | Extract+5% FeCl ₃ | - | + | + | - |
| 4 | Terpenoid | Salkowski test | + | + | - | - |
| 5 | Steroid | Liebermann Burchard reaction | + | + | - | - |
| 6 | Glycosides | Keller-kilani test | + | + | + | + |
| 7 | Phenolic compound | Extract+5% FeCl ₃ | - | - | + | + |
| 8 | Flavonoid | Residue+Lead acetate soln | - | - | + | + |

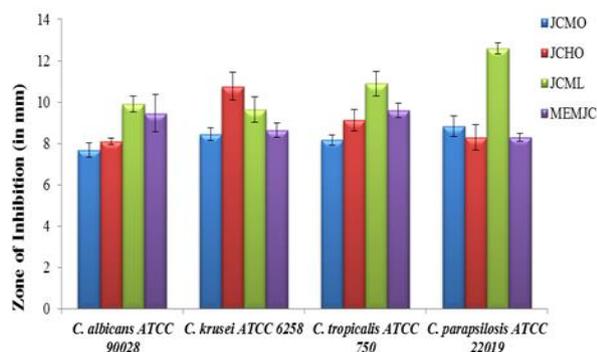


Figure 3. Antifungal activity (in mm) of JCMO, JCHO, JCML and MEMJC of *J. curca* against *Candida* species

The MICs of JCMO, JCHO, MEMJC and JCML for the different organisms ranged between 4 mg/mL and 25 mg/mL. JCML extract showed the minimum MIC value against the Gram negative (*S. aureus* ATCC 25922) bacteria compared with the

other Gram positive bacteria. The MBC and MFC of the various extracts for different microbes ranged between 4 mg/mL and 30 mg/mL (Tables 2 and 3). In vitro antibacterial test results (Figure 1)

revealed that all the above extracts showed significant antibacterial activity against Gram positive and negative bacteria with strong antifungal activity (Figure 2). The result of TLC analysis using the benzene, hexane, chloroform solvent mixture (Table 4) revealed four spots for JCML, five spots for JCHO and MEMJC, and six spots for JCMO. The methanol extract i.e. JCML and MEMJC of plant parts had better activity than the other extracts. This may be due to the presence of soluble phenolic and polyphenolic compounds (Kowalski and Kedzia, 2007).

Table 2. MIC and MBC values (mg/mL) of various parts of *Jatropha curcas* Linn against different bacterial species

| Strains | <i>E. coli</i> ATCC 25922 | | <i>S. aureus</i> ATCC 25323 | | <i>E. faecalis</i> | | <i>S. flexneri</i> ATCC 12022 | |
|---------|---------------------------|------|-----------------------------|------|--------------------|------|-------------------------------|------|
| | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC |
| JCMO | 25 | 25 | 12.5 | 25 | 12.5 | 25 | 12.5 | 25 |
| JCHO | 12.5 | 12.5 | 12.5 | 25 | 6.25 | 12.5 | 12.5 | 12.5 |
| JCML | 12.5 | 12.5 | 3.125 | 6.25 | 12.5 | 25 | 25 | 25 |
| MEMJC | 12.5 | 12.5 | 6.25 | 12.5 | 12.5 | 25 | 25 | 50 |

Table 3. MIC and MFC values (mg/mL) of various parts of *Jatropha curcas* Linn against different fungal species

| 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 |
| JCMO | 25 | 25 | 12.5 | 25 | 25 | 25 | 25 | 50 |
| JCHO | 25 | 25 | 12.5 | 25 | 12.5 | 25 | 25 | 25 |
| JCML | 12.5 | 25 | 25 | 25 | 6.25 | 12.5 | 6.25 | 12.5 |
| MEMJC | 25 | 50 | 12.5 | 25 | 25 | 25 | 12.5 | 25 |

Table 4. TLC profiling of extracts of *Jatropha curcas* Linn

| Extracts | Solvent system | Number of components | Distance of spot (cm) | Solvent front (cm) | R _f value |
|----------|--|----------------------|-------------------------------|--------------------|------------------------------------|
| JCMO | Hexane+chloroform+few drop acetic acid (8:2) | 6 | 10.5, 5.7, 2.9, 2.3, 1.9, 1.3 | 12.5 | 0.84, 0.45, 0.23, 0.18, 0.15, 0.10 |
| JCHO | Hexane: Ethyl acetate (9:1) | 5 | 11.2, 9.2, 5.7, 3.9, 2.9 | 13.1 | 0.85, 0.70, 0.43, 0.29, 0.21 |
| JCML | Benzene:chloroform:few drop glacial acetic acid(8.5+1.5) | 4 | 8.9, 7.9, 6.9, 3.4 | 12.5 | 0.71, 0.63, 0.55, 0.27 |
| MEMJC | Hexane:chloroform:few drop acetic acid (9:1) | 5 | 9.1, 7.3, 6.1, 5.1, 4.2 | 11.9 | 0.76, 0.61, 0.51, 0.49, 0.37 |

Antimicrobial screening of *Jatropha curcas*

Table 5. Antimicrobial activities of the different extracts of *Jatropha curcas*

| Microorganism | Zone of inhibition (in mm) | | | | |
|-----------------------------------|-----------------------------------|--------------|--------------|--------------|-------------------------------|
| | Extract Concentration (200 mg/mL) | | | | Standard drugs |
| | JCMO | JCHO | JCML | MEMJC | |
| <i>S. flexineri</i> ATCC 12022 | 9.5 ± 0.45 | 12.2 ± 0.60 | 10.18 ± 0.33 | 11.10 ± 0.36 | 25.73 ± 1.09 (Ciprofloxacin) |
| <i>E. coli</i> ATCC 25922 | 8.16 ± 0.30 | 9.20 ± 0.30 | 9.76 ± 0.73 | 10.73 ± 0.55 | 26.66 ± 0.77 (Norfloxacin) |
| <i>S. aureus</i> ATCC 25922 | 9.73 ± 0.46 | 9.53 ± 0.35 | 12.45 ± 0.56 | 11.73 ± 0.20 | 24.93 ± 0.15 (Ampicillin) |
| <i>E. faecalis</i> | 9.24 ± 0.18 | 10.60 ± 0.60 | 9.98 ± 0.47 | 10.93 ± 0.63 | 28.12 ± 0.17 (Ciprofloxacin) |
| <i>C. albicans</i> ATCC 90028 | 7.67 ± 0.34 | 8.10 ± 0.30 | 9.90 ± 0.26 | 9.46 ± 0.49 | 16.03 ± 0.70 (Fluconazole) |
| <i>C. krusei</i> ATCC 6258 | 8.45 ± 0.15 | 10.76 ± 0.67 | 9.63 ± 0.50 | 8.63 ± 0.61 | 18.10 ± 0.91 (Fluconazole) |
| <i>C. tropicalis</i> ATCC 750 | 8.16 ± 0.38 | 9.12 ± 0.61 | 10.90 ± 0.60 | 9.60 ± 0.26 | 16.93 ± 0.90 (Amphotericin B) |
| <i>C. parapsilosis</i> ATCC 22019 | 8.83 ± 0.90 | 8.29 ± 0.35 | 12.60 ± 0.36 | 8.30 ± 0.20 | 15.85 ± 0.81 (Amphotericin B) |

Discussion

Among the different extracts of the plant used, JCML was observed to be more effective against human pathogens and fungus. The MICs reported above (Tables 2 and 3) correlate with previous reports that bacteria vary widely in the degree of their susceptibility (Emeruwa, 1982; Willey et al., 2008). The antimicrobial results presented above from the JCHO, MEMJC and JCML indicated that the whole plant has a broad spectrum of activity against bacteria and fungi, justifying their traditional use as a medicine. Sofowora (1982) reported that, as some active metabolites in the plant extract are traditionally used in treating wound infections, this indicates potential antimicrobial activity. Plant-based medicines used as antimicrobials have enormous therapeutic potential with fewer side effects than synthetic drugs (Iwu et al., 1999).

This inhibitory effect of extracts from different parts of the plant against human pathogens introduces this plant as a potential candidate for development of new drugs against pathogenic microbes. Previous studies show that the latex of *Jatropha* possesses antifungal activity (Fagbenro et al., 1998), which was confirmed in the current study by the inhibition of fungal growth. The presence of antimicrobial activity has been found to be due to specific metabolites, such as alkaloids, tannins, phenols, steroids, glycosides, volatile oils, gums, saponins, and flavonoids (Sofowora, 1993).

The variation in potential of different extracts of *Jatropha* used in the study as compared to the standard might be due to differences in growth rate of pathogenic microbes, its inoculum size and test methods themselves. Also, it might be the fact that the plant collected may vary in their phytoconstituent, due to the habitat, different seasons, and the environment, also, may be a factor which will contribute to the difference in antimicrobial activity.

Most secondary metabolites, such as alkaloids, saponins, tannins, anthraquinones and flavonoids, are known to possess activity against several pathogens, and therefore aid the antimicrobial activity of *J. curcas* and justifies its traditional use for the treatment of various illnesses (Hassan et al., 2004). *J. curcas* is rich in tannins, which might be responsible for the inhibition of cell protein synthesis, as they form irreversible complexes with proline-rich proteins (Shimada, 2006). The methanolic extract exhibited considerable inhibition of the test organisms, which could be 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 drugs used to treat bacterial and fungal infections.

Due to the complex nature of Phytochemical, a single method to evaluate the Phytochemical cannot be evaluated. In this context, standard methods were used to validate the content of phenol and flavonoids in plant extracts.

The results indicate that JCMO, JCHO, MEMJC and JCML contain significant amounts of flavonoids and phenolic compounds. Both these classes of compounds have good antioxidant potential and their effects on human nutrition and health are considerable. The mechanism of action of flavonoids is through scavenging or chelation. It supports its use in stress related diseases or uses in wound dressing, cuts, and curing infections against micro flora. Flavonoids, due to actions by its anion radicals, serve as health promoting compounds fascinating its microbial activity. Phenolic compounds are also very important plant constituents because their hydroxyl groups confer antimicrobial and other biological ability. Reducing power of the different extracts was compared with standard Quercetin. As shown in figure 1, the extracts exhibits significant reducing power as it possess various mechanisms such as prevention of chain initiation, decomposition of peroxides and reducing capacity.

Conflict of interest

The authors declare that they have no conflict of interest.

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