INTRODUCTION

Traditional medicine using plant extracts continue to provide health coverage for over 80% of the world’s population, especially in the developing world\(^1\). It was reported that 60% - 85% of the population in every country of the developing world has to rely on traditional medicine or indigenous forms of medicine\(^2\).

*Jatropha curcas* (Euphorbiaceae) is believed to be a native of South America and Africa but later spread to other continents of the world\(^3\). It is used in traditional folklore medicine to cure various ailments in Africa, Asia and Latin America\(^4\). It is a wild growing hardy plant well adapted to acid and moisture demand and can come up in stony, gravelly and even calcareous soils. *Jatropha curcas* can be grown in habitat of tropical/subtropical areas with a suitable rainfall of 200-1500 mm/year\(^5\).

Anti-microbial agents are substances that kill microorganisms or inhibit their growth. They are widely employed to cure bacterial diseases. An antimicrobial agent that reversibly inhibits the growth of bacteria is called bacteriostatic whereas those with irreversible lethal action on bacteria are known as bactericidal\(^6\). The development of resistance in human pathogens against antibiotics commonly used necessitates research for new antimicrobial substances from other sources including plants\(^7\). Therefore, this study is aimed at evaluating the antibacterial and antifungal activities of ethanol stem bark extract of *Jatropha curcas* and investigate its phytochemical components.

MATERIALS AND METHODS

**Source of Plant Material, Collection and Identification**

The fresh stem bark of *Jatropha curcas* collected February, 2012 from Kaltungo Local Government Area of Gombe State was identified by Professor S. S. Sanusi a Taxonomist in the Department of Botany, University of Maiduguri, and a voucher specimen number PCG0026 was assigned and deposited in Department of Pharmacognosy Herbarium, University of Maiduguri.

**Preparation of the Stem Bark Extract**

The fresh stem bark of *Jatropha curcas* collected was air dried to a constant weight, pulverised in a mill (TYPE YC100L-4, China) and stored in an air tight container for further use. A 600g weight of the pulverised stem bark of *Jatropha curcas* was de-fatted using petroleum ether for 24 hours; the marc was dried and soaked in 1.5L of ethanol for 24 hours at room temperature with occasional mechanical shaking. The mixture was filtered and the marc was re-extracted with 150ml of ethanol. The filtrate obtained was concentrated using Rotary evaporator (R201D, U.S.A.) and the extract subsequently air dried. The weight of the ethanol extract obtained was 33.0g (5.5% Yield).

**Source of the Microorganisms**

Clinical isolates of the tests organisms (*Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* and *Candida albicans*) were obtained from the Department of Microbiology, University of Maiduguri Teaching Hospital (UMTH).

**Phytochemical analyses of the stem bark extract of *Jatropha curcas* Linn.**

The ethanol stem bark extract of *Jatropha curcas* was subjected to preliminary phytochemical analysis for secondary metabolites; saponins, tannins, cardiac glycosides, carbohydrates, reducing sugars, flavonoids and terpenoids. The extract also exhibited antimicrobial activities which were dose-dependent with zones of inhibition ranging from 10-15mm for *Staphylococcus aureus*, 11-17mm for *E. coli*, 10-18mm for *Klebsiella pneumoniae*, and 14-26mm for *Candida albicans*. The MIC of the extract on the clinical isolates (*Staphylococcus aureus, Escherichia coli, and Klebsiella pneumonia*) was 25mg/ml, 25mg/ml and 50mg/ml respectively. While the MBC of *Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae* was 50mg/ml, 50mg/ml and 100mg/ml respectively. The results obtained indicate that ethanol stem bark extract of *Jatropha curcas* has antibacterial and antifungal activity which can be attributed to the presence of some of the essential secondary metabolites.
Determination of the minimum inhibitory concentration (MIC)
The MIC of the ethanol stem bark extract of Jatropha curcas was carried out using broth dilution method as described by Ibekwe et al., 10
A set of 9 Bijou bottles were arranged serially and filled with 5ml of nutrient broth. The first bottle contained the double strength nutrient broth. A 1.0g ethanol stem bark extract of Jatropha curcas was dissolved in 5ml distilled water and added to the first bottle (i.e. the double strength nutrient broth), it was mixed thoroughly and 5ml was subsequently withdrawn and poured into the second bottle. This procedure was continued up to the 7th bottle where 5ml was withdrawn and discarded. The bottle number 8 contain only nutrient broth i.e. the negative control to examine the sterility of the media and bottle number 9 contains the organism (positive control). A loop full of the diluted overnight culture of a sensitive gram negative (Escherichia coli and klebsiella pneumoniae) and gram positive organism (staphylococcus aureus) were inoculated. All the bottles were incubated at 37°C for 24 hours in an electric incubator (DHG-9023A, China). The bottles were observed for turbidity of growth after 24 hours. The lowest concentration which showed no turbidity in the bottle was recorded as the MIC.

Determination of the minimum bactericidal concentration (MBC)
The broth dilution method as described by Iukun 11 was adopted. All the test bottles which showed no turbidity in the MIC assay were sub-cultured into a nutrient agar plate and incubated at 35°C for 24 hours and observed for colony growth.
The MBC was the plate with the lowest concentration of extract without colony growth.

Determination of activity index
The activity index of the ethanol stem bark extract of Jatropha curcas was calculated according to Arya et al., 12
Activity index (A.I.) = Mean of zone of inhibition of the test

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Concentration (mg/ml)</th>
<th>Negative control</th>
<th>Positive control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>S. aureus</td>
<td>-</td>
<td>18.00±0.00</td>
<td>15.00±1.06</td>
</tr>
<tr>
<td>E. coli</td>
<td>-</td>
<td>54.33±2.08</td>
<td>17.33±0.58*</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>-</td>
<td>30.67±3.21</td>
<td>18.33±0.58*</td>
</tr>
<tr>
<td>C. albicans</td>
<td>-</td>
<td>36.67±1.53</td>
<td>26.67±2.08*</td>
</tr>
</tbody>
</table>

*indicates a significant difference with positive control at p-value < 0.05. (T- Test) Negative control = Distilled water. Positive control = Ciprofloxacin and Ketoconazole for bacterial and fungal isolates respectively. S. aureus = staphylococcus aureus, E. coli = Escherichia coli, K. pneumoniae = Klebsiella pneumoniae, C. albicans = Candida albicans.

Table 1: Qualitative phytochemistry of ethanol stem bark extract of Jatropha curcas Linn.

<table>
<thead>
<tr>
<th>Secondary metabolites</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>Reducing sugars</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = present, - = absent

Table 2: Antimicrobial analyses of ethanol stem bark extract of Jatropha curcas Linn. Showing the zones of inhibition (mm) (n=3)
RESULTS

Qualitative phytochemistry of ethanol stem bark extract of Jatropha curcas Linn.

Details of the various phytochemical constituents present or absent in the ethanol stem bark extract of *Jatropha curcas* is shown in Table 1. The extract showed the presence of saponins, tannins, and cardiac glycosides, carbohydrates, reducing sugars, flavonoids and terpenoids.

Antimicrobial analyses of ethanol stem bark extract of Jatropha curcas Linn.

Table 2 shows a dose dependent effect of the extract on bacterial growth with a better activity against *Klebsiella pneumoniae* at 100mg/ml concentration but significantly less than positive control (ciprofloxacin), no inhibition of growth was observed at 6.25mg/ml. It is however important to note that there was no significant difference (P-value < 0.05) in the zone of inhibition of the extract (100mg/ml) against *Staphylococcus aureus* compared to the positive control (ciprofloxacin). Growth inhibition against *Escherichia coli* was also dose dependent and significantly less than positive control at all the concentrations used. The inhibition of growth *Candida albicans* was also dose dependent with significantly lower zone compared to positive control (Ketoconazole) as shown in Table 2.

**Table 3: MIC, MBC and Activity index (A.I.) of ethanol extract of *Jatropha curcas* stem bark**

<table>
<thead>
<tr>
<th>Organism</th>
<th>MIC (mg/ml)</th>
<th>MBC (mg/ml)</th>
<th>Activity index (A.I.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em></td>
<td>25</td>
<td>50</td>
<td>0.76</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>25</td>
<td>50</td>
<td>0.27</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>50</td>
<td>100</td>
<td>0.47</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>Not tested</td>
<td>No effect</td>
<td>0.55</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The phytochemical analysis of the ethanol stem bark extract of *Jatropha curcas* showed the presence of some secondary metabolites as shown in Table 1. Alkaloids and anthraquinones were found to be absent. This result contradicts the findings of Igbionosa. However, the difference in geographical location of the plant collection could justify such difference. It is important to note also that contrary to the findings of Obasi et al., who studied the methanol stem bark extract of *Jatropha curcas*, the ethanol stem bark extract of *Jatropha curcas* contains tannins and flavonoids which according to James et al., reported that tannins and flavonoids have biological activities that are of benefit in the prevention and management of many ailments. Erah et al., had reported earlier that antimicrobial activities are associated with the presence of tannins and flavonoids.

The presence of other preformed compounds like saponins which is in concordance with the findings of Obasi et al., also justifies the antifungal property of the extract. Therefore the presence of this essential secondary metabolites in the extract used explains the broad spectrum antimicrobial activity in this study.

The observations that emanated in the present study as shown in Table 2 indicated that the extract used demonstrated a dose dependent activity against the bacteria (both Gram positive and Gram negative) and fungal isolates used. There was no activity with the negative control (distilled water). The positive control (Ciprofloxacin 50mg/ml and Ketoconazole 50mg/ml for bacterial and fungal isolates respectively) showed significant higher zone of inhibition. Compared to the extract studied at various concentrations employed (P-value < 0.05). The difference may be attributed to the fact that positive controls employed are pure compounds compared with the doses of the crude ethanol stem bark extract of *Jatropha curcas* used. Also, El-Mahmood and Ameey reported that crude extracts having a mixture of plants constituents interfered with antimicrobial activity via degradation and decomposition especially on long term storage. The results obtained also demonstrated that the extract used has a better activity against *E. coli* compared with both *S. aureus* and *K. pneumoniae* except at the highest concentration of the extract (100mg/ml) where activity against *K. pneumoniae* is better than *E. coli*. This result shows that the ethanol stem bark extract of *Jatropha curcas* has a better activity against Gram negative compared to Gram positive bacteria. These findings are supported by the report of Gupta et al.,. The activity of the extract against *C. albicans* was only observed at higher concentrations (25, 50 and 100mg/ml) which was dose dependent. This could be an indication that the extract has better antifungal activity at higher concentration. This is also in line with the findings of Igbionosa.

High MIC value is an indication of low activity while low MIC value is an indication of high activity. MBC is the lowest concentration of antibiotic required to kill a particular bacterium. From the results shown in Table 3, the extract used has a better activity against *S. aureus* and *E. coli* compared to *K. pneumoniae*.

CONCLUSION

Conclusively, based on the findings of this study, the ethanol stem bark extract of *Jatropha curcas* exhibited activities against bacterial and fungal growth which can be attributed to the presence of some essential secondary metabolites present. Thus, the result of this study provides support for the traditional use of *Jatropha curcas* as an antibacterial and antifungal agent.

REFERENCES


**Cite this article as:** J. H. Wakirwa, P. Ibrahim, S. J. Madu. Phytochemical screening and in vitro antimicrobial analysis of the ethanol stem bark extract of *Jatropha curcas* Linn. (Euphorbiaceae). *Int. Res. J. Pharm.* 2013; 4(3):97-100

Source of support: Nil, Conflict of interest: None Declared