Phytochemical Screening and Antibacterial Activity of Whole Plant Crude Extracts of *Euphorbia hirta* Linn against Some Clinical Isolates

*Oloninefa, S. D.¹, Aisoni, J. E.², Fadayomi, V. K.³, Oghenemaro, O.⁴*

¹Department of Biological Sciences, Kogi State University, Kabba, Nigeria  
²Department of Microbiology, Bayero University, Kano, Nigeria  
³Department of Microbiology, Federal University, Lafia, Nigeria  
⁴Industrial Scientific Nutritional (ISN), Abuja, Nigeria

*Corresponding Author: sdonolinefa@ksukabba.edu.ng; Tel.: 08038893341*

**ABSTRACT**

The problem of bacteria resistance coupled with microorganisms (e.g. Bacteria, fungi, viruses and protozoa) been implicated as causative agents of many infectious diseases has led many researchers globally to search for substances (Phytochemicals) which can be used to stop the activity of these microorganisms and to reduce or eliminate their effect on the human population and to also curb the menace of bacteria resistance. This study aimed at carrying out phytochemical screening and antibacterial activity of whole plant crude extracts of *Euphorbia hirta* Linn against some clinical isolates. The selected clinical isolates (*Escherichia coli*, *Pseudomonas fluorescens*, *Klebsiella pneumoniae*, *Salmonella typhimurium*) were identified using biochemical tests. The extraction of whole plant of *Euphorbia hirta* with *n*-hexane, ethyl acetate, methanol and water was done using reflux extraction technique. The screening for phytochemicals was carried out using standard methods while the determination of antibacterial activity of the extracts against selected clinical isolates was carried out using agar well diffusion method. The results obtained show that phytochemicals such as: flavonoids, terpenoids, alkaloids, steroids, tannins, saponins, phenols, reducing sugars were present while cardiac glycosides was absent in all the extracts. The highest (21.33±0.33) antibacterial activity which is concentration-dependent was recorded against *Pseudomonas fluorescens* using ethyl acetate extract while *n*-hexane extract has no antibacterial activity against any of the clinical isolates which possibly may be due to insufficient bioactive agents in the *n*-hexane extract. The study revealed that *Euphorbia hirta* contained phytochemicals which have antibacterial activity and can be very useful in drug development, traditional and complementary medicine if utilized.

**Keywords:** Phytochemical screening, Antibacterial activity, *Euphorbia hirta*, Traditional medicine, Clinical isolates


**INTRODUCTION**

*Euphorbia hirta* Linn is a weed plant of the tropics and subtropics that belong to the family of *Euphorbiaceae*. It is commonly referred to as milkweed (Dudhy) and an asthma plant and known by different names in different parts of the world (Ghosh *et al*., 2018; Ghosh *et al*., 2019; Tripathi *et al*., 2021). It is found throughout tropical Africa, India, China, Japan, Taiwan and neighboring islands as well as in the islands of the southern Pacific. According to Pranabesh *et al.* (2019), it grows in continental tropical America and the Caribbean. It is an annual broad-leaved herb that has hairy stem, usually erect, slender stemmed; spreading up to 45 cm tall though sometimes can be seen lying down with many branches from the base to the top and the stem and leaves produce white or milky juice when cut as reported in the works of Tuhin *et al.* (2017) and Panzu *et al.* (2020). As reported by Asha *et al.* (2016)
and Gokulprasath et al. (2021), the usefulness of *E. hirta* in traditional and complementary medicine cannot be over emphasized. It is used to cure pimples, female disorders, respiratory ailments (cough, asthma, coryza and bronchitis), dysentery and tumors (Kausa et al., 2016). *E. hirta* contains phytochemicals such as: triterpenoids, sterols, alkaloids, glycosides, flavonoids, tannins, phenols, choline and shikimic acid while some of the reported activities include its use as an antispasmodic, antiasthmatic, expectorant, anticatarhal, antisyphilitic, antibacterial, antidiabetic, antivenom, wound healing, antimalarial, immunostimulatory, antithrombocytopenic, antioxidant, anticancer, anti-allergy, anti-inflammatory and diuretic (Gopi et al., 2016; Kanedi, 2017; Anjum et al., 2017; Kikete et al., 2018; Thabet et al., 2018; Aleksandrov et al., 2019; Rahman et al., 2019; Uzor, 2020; Mahabati et al., 2020; Tran et al., 2020; Gokulprasath et al., 2021). However, El-Mahmood (2009) reported that *E. hirta* is a very popular herb amongst practitioners of traditional medicine and some of its local names in Nigeria include “nonon furchiya” in Hausa, “tepel” in fulfulde, “Harvom” in Kaka and “Hammock sand mat” (Florida) while the exudate of the stem is used to treat eye and ear infections in Nigeria. This study focuses on phytochemical screening and antibacterial activity of whole plant crude extracts of *Euphorbia hirta*.

**MATERIALS AND METHODS**

**Collection of Samples**

**Plant material**

Fresh whole plant of *Euphorbia hirta* were collected around house-holds in Sauka-Kahuta area of Minna, Niger State, Nigeria and taken to the Department of Biological Sciences in Federal University of Technology, Minna for botanical identification. The identified plants were authenticated by Department of National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja. Nigeria with the voucher number, NIPRD/H/6864 which was then deposited in the same Herbarium Department of the Institute.

**Clinical Isolates**

The *Escherichia coli*, *Pseudomonas fluorescens*, *Klebsiella pneumoniae*, *Salmonella typhimurium* used were collected from the Microbiology Department of General Hospital, Minna.

**Identification of the Clinical Isolates**

The identification of the clinical isolates were carried out by Gram reaction and biochemical tests (such as catalase, citrate utilization, methyl red, indole, urease and triple sugar iron) as outlined in Cheesbrough (2010) and then sub cultured into Nutrient Agar slants, Eosin Methylene Blue Agar slant and Deoxycholate Agar slant and kept in a refrigerator until they were ready for further use.

**Preparation of Extracts**

The identified *E. hirta* was thoroughly washed with water to remove any extraneous substances and dirt and then dried at room temperature away from sunlight and dust. The air-dried whole plant were grounded to powder using Panasonic electric blender model MX-J210PN. The extraction was done using reflux extraction method as described in the work of Oloninefa et al. (2018).

**Phytochemical Screening of the Crude Extracts**

The crude extracts were subjected to phytochemical analysis to screen for the presence of bioactive components such as alkaloids, reducing sugar, saponins, flavonoids, cardiac glycosides, terpenoids, steroids and tannins. The phytochemical screening was carried out using standard procedure as described in the study by Bandiola (2018).

**Antibacterial Activity of the Crude Extracts**

**Standardization of clinical isolates**

The population of the clinical isolates were determined from the 0.5 McFarland Turbidity Standard prepared (Murray et al., 2007).

**Preparation of extract concentration**

The extract concentration was prepared as described in the work of Oloninefa et al. (2018). Two hundred milligram (200 mg) of the *n*-hexane, ethyl acetate, methanol and aqueous crude extract were weighed in 5 ml each of 20% Dimethyl sulfoxide (DMSO) (20 ml DMSO was made up to 100 ml with distilled water) to give 40 mg/ml concentrations respectively. The concentrations of 60 mg/ml; 80 mg/ml and 100 mg/ml were prepared following the same procedure.

**Determination of the antibacterial activity of the crude extracts**

The antibacterial activity of the crude extract was carried out using Agar Well Diffusion method outlined in CLSI (2015). Mueller Hinton Agar (MHA) was prepared and sterilized according to manufacturer’s instruction. Petri dishes containing about 20 ml MHA were streaked with standardized 24 h culture of the clinical isolates using sterilized wire inoculating loop. Wells were cut with a 6
mm sterile cork borer and then sealed at the bottom with a drop of molten agar so as to prevent the extract from sipping beneath the agar. Four holes were made on each plate and adequately spaced out. About 100 µl of each of the crude extracts (40, 60, 80 and 100 mg/ml) (namely aqueous, methanol, n-hexane and ethyl acetate extracts) was delivered into each well and 40 mg/ml of the standard drug (Ciprofloxacin) were used for positive control while dimethylsulfoxide (DMSO) served as the negative control. One-hour pre-diffusion time was allowed after which the plates were incubated at 37°C for 24 h. The zones of inhibition were measured by direct linear measurement using a meter scale rule. The above method was carried out in triplicates and the mean of the triplicate result were taken.

Data Analysis

The analysis of variance (ANOVA) of the values obtained for the antibacterial susceptibility was carried out using IBM SPSS Statistics Version 23. All data were expressed as mean ± standard error of the mean. The values with different superscripts along the same column were significantly different (P < 0.05).

RESULTS

Gram Reaction and Biochemical Characteristics of the Clinical Isolates

The findings from the clinical isolates tested revealed the presence of E. coli, S. typhimurium, K. pneumoniae and P. fluorescens from Table 1).

Phytochemical Components in Whole Plant Crude Extracts of Euphorbia hirta

The phytochemical screening of the whole plant extract shows some bioactive components. The results showed that flavonoids, terpenoids, alkaloids, steroids, tannins and phenols were present in n-hexane crude extract (NHE) whereas cardiac glycosides, saponins and reducing sugars were absent. On the other hand, alkaloids, flavonoids, phenols, tannins, terpenoids, reducing sugars and steroids were present in ethyl acetate crude extract (EAE) but saponins and cardiac glycosides were absent. Meanwhile, saponins, flavonoids, terpenoids, tannins, phenols, alkaloids, steroids and reducing sugars were present in methanol extract (ME) whereas cardiac glycosides was absent. The aqueous crude extract (AQE) had saponins, flavonoids, terpenoids, tannins, phenols and alkaloids while cardiac glycosides and steroids were absent (Table 2).

Antibacterial Activity of Whole Plant Crude Extracts

The n-Hexane Extract (NHE) had no antibacterial activity against all the isolates. The antibacterial activity results of Ethyl Acetate Extract (EAE) against the isolates were as follows: S. typhimurium (11.67-15.67); K. pneumoniae (10.33-12.00) and P. fluorescens (19.67-21.33) while methanol extract (ME) had antibacterial activity against P. fluorescens (16.00-17.33). More so, the antibacterial activity results of aqueous extract (AQE) were as follows: E. coli (11.67); S. typhimurium (9.00-13.00); K. pneumoniae (9.67-12.67) and P. fluorescens (10.67). However, Ciprofloxacin had antibacterial activity against the isolates as follows: E. coli (11.67); S. typhimurium (11.67-13.00); K. pneumoniae (9.67-12.67) and P. fluorescens (10.67). The DMSO had no antibacterial activity against all the isolates. The results are shown in Tables 3 and 4.

Table 1: Gram Reaction and Biochemical Characteristics of the Clinical Isolates

<table>
<thead>
<tr>
<th>Tests</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram Reactions</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TSI</td>
<td>A/A, G (+), H₂S (-)</td>
<td>K/A, G (+), H₂S (+)</td>
<td>A/A, G (+), H₂S (-)</td>
<td>K/K, G (-), H₂S (-)</td>
</tr>
<tr>
<td>Methyl Red</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Indole</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Citrate Utilisation</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Urease</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Identity</td>
<td>E. coli</td>
<td>S. typhimurium</td>
<td>K. pneumoniae</td>
<td>P. fluorescens</td>
</tr>
</tbody>
</table>

KEY: + = Positive; - = Negative; TSI= Triple Sugar Iron; K= Alkaline; A= Acid; G= Gas; H₂S= Hydrogen Sulphide
Table 2: Phytochemicals Components in Whole Plant Crude Extracts of *Euphorbia hirta*

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Saponins</th>
<th>Flavonoids</th>
<th>Terpenoids</th>
<th>Cardiac Glycosides</th>
<th>Tannins</th>
<th>Phenols</th>
<th>Alkaloids</th>
<th>Steroids</th>
<th>Reducing Sugars</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>n</em>-hexane</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Methanol</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Aqueous</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

KEY: +: Present; -: Absent

Table 3: Antibacterial Activity of Whole Plant Crude Extracts of *Euphorbia hirta* (mm)

<table>
<thead>
<tr>
<th>Extracts/Control</th>
<th>Escherichia coli 40 mg/ml</th>
<th>Salmonella typhimurium 40 mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>n</em>-Hexane</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Ethyl Acetate</td>
<td>0.00±0.00</td>
<td>11.67±0.33</td>
</tr>
<tr>
<td>Methanol</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Aqueous</td>
<td>11.67±0.33</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>10.33±0.67</td>
<td>44.33±0.67</td>
</tr>
<tr>
<td>DMSO</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
</tbody>
</table>

Results represent mean ± standard error of mean of triplicate determination. Values with the same superscript in the same column are not significantly different at p<0.05

Table 4: Antibacterial Activity of Whole Plant Crude Extracts of *Euphorbia hirta* (mm)

<table>
<thead>
<tr>
<th>Extracts/Control</th>
<th>Klebsiella pneumoniae 40 mg/ml</th>
<th>Pseudomonas fluorescens 40 mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>n</em>-Hexane</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Ethyl Acetate</td>
<td>10.33±0.67</td>
<td>11.33±0.88</td>
</tr>
<tr>
<td>Methanol</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Aqueous</td>
<td>9.67±0.88</td>
<td>52.00±0.58</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>50.00±0.00</td>
<td>53.00±0.33</td>
</tr>
<tr>
<td>DMSO</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
</tbody>
</table>

Results represent mean ± standard error of mean of triplicate determination. Values with the same superscript in the same column are not significantly different at p<0.05
DISCUSSION

The presence of phytochemicals such as saponins, flavonoids, terpenoids, tannins, phenols, alkaloids, steroids and reducing sugars in the whole plant crude extracts of *Euphorbia hirta* were in agreement with the previous studies by Abalaka et al. (2016); Ewansiha et al. (2016); Mekam et al. (2019); Haleshappa et al. (2020); Nirmal et al. (2020) and Vadalia et al. (2020) but the results disagreed with work of Awomukwu et al. (2014) because terpenoids and steroids were absent in the study. The difference in results may possibly be due to the type of solvents used for the extraction and the difference in the geographical location where the plants were collected (Ughachukwu et al., 2014). Abalaka et al. (2016) and Oloninefa et al. (2018) opined in their previous studies that phytochemicals have antimicrobial effects and as such they can be used for the treatment of diseases. In addition, Mann et al. (2014) Haleshappa et al. (2020) and Nirmal et al. (2020) revealed in their studies that the presence of the phytochemical components in the crude extracts are responsible for their effectiveness against many microbes and also enable the plant parts to function as herbs or drugs by producing biological activity in animals and in humans.

Furthermore, the results of antibacterial activity of the whole plant crude extracts of *E. hirta* showed that n-hexane extract (NHE) had no antibacterial activity when tested against all the clinical isolates. This may possibly be due to insufficient bioactive components in the NHE to effect antibacterial activity against the clinical isolates. Similarly, the four crude extracts had no antibacterial activity against *E. coli* at 40 mg/ml. This result is not in agreement with the previous studies carried out by Nikunj and Kaushik (2014); Jakhar and Dahiya (2017) and Nazeer (2017) that recorded antibacterial activity with all the crude extracts used in their studies. The variation in results obtained in this study may possibly be due to the solvent used for extraction, geographical location of the plant and the characteristics of the soil as opined by Ughachukwu et al. (2014).

The methanol extract (ME) had no antibacterial activity when tested against *E. coli*, *S. typhimurium* and *K. pneumoniae*. This result did not agree with the work of Nazeer (2017). This may likely be due to the clinical isolates used and insufficient bioactive components in the ME to cause antibacterial activity.

In addition, it was observed that ethyl acetate extract (EAE) had the highest zones of inhibitions with *P. fluorescens* compared with other crude extracts. This result did not agree with the work of Jakhar and Dahiya (2017) that recorded highest activity with *Klebsiella pneumoniae*. The use of different crude extracts and the isolates may likely be responsible for this result. More so, the values obtained for antibacterial activity of the crude extracts against the clinical isolates showed a significant difference at p<0.05. However, there was increment in the zone of inhibitions as the concentration of Ciprofloxacin (positive control) increases from 40-100 mg/ml while the DMSO (negative control) does not contain antibacterial agent hence the results obtained were expected.

CONCLUSION

The study revealed that the whole plant crude extracts of *Euphorbia hirta* contained phytochemicals which can be very useful in traditional and complementary medicine. The antibacterial activities of the crude extracts against the selected clinical isolates which results from the study revealed to be concentration-depended will also go a long way to significantly reduce even though does not eradicate microorganisms which have been implicated as causative agents of many infectious diseases. The results from this study show that crude extracts obtained from *Euphorbia hirta* Linn are very promising and can be used for drug development in pharmaceutical industries in the nearest future.

CONFLICT OF INTEREST

There is no conflict of interest among the authors.

REFERENCES


