

Original Research Article

Antibacterial Activities and Phytochemical Screening of Crude Extracts of *Calotropis gigantea* (Giant Milk Weed)

ABSTRACT

The root and leaf of were screened for its antimicrobial and phytochemical activities. The solvents used for the roots and leaves extraction were ethanol and water. The extracts were tested against infectious disease causing bacterial such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus*, *Klebsiella aerogenes* and α -haemolysin using the well diffusion method. The aqueous extracts of root of *Calotropis gigantea* against all the test bacteria ranged from 6.0 mm to 20.0 mm diameter zones of inhibition. The ethanolic extract of root of *Calotropis gigantea* inhibition against some the test microbe ranges from 6 mm to 14.0 mm diameter inhibitory zone. The ethanolic leaf extract of *C. gigantea* also showed an inhibition of 8mm to 20.0 mm. In present study, bacterial extract showed a varying zone of inhibition of the growth of tested organism than ethanol. Phytochemical properties of root and leaves of *Calotropis gigantea* obtain from ethanol and aqueous extracts were investigated. The results confirmed that presence of antibacterial activity and phytochemical in the shade dried extract of *Calotropis gigantea* against the human pathogenic organisms.

Keywords: Antibacterial, *Calotropis gigantea*, Crude extracts, Phytochemical

1. INTRODUCTION

The Africa continent is rich in medicinal plants and is one of the richest continents in terms of genetic diversity of medicinal plants after the Asia continent. It exhibits a wide range in topography and climate. Plants have been a rich source of medicines because they produce wide array of bioactive molecules, most of which probably evolved as a chemical defense against predation or infection. *Calotropis* is one such genus of flowering plants which contains many phytochemicals with potential pharmacological activity. *Calotropis* belongs to *Asclepiadaceae* or Milkweed or *Aak* family which includes 280 genera and 2,000 species of world-wide distribution but most abundant in the sub-tropics and tropics, and rare in cold countries. They are commonly known as milkweeds because of the latex they produce. *Calotropis* species are considered common weeds in some parts of the world [1]

Studies have shown that medicinal plants are good materials for the formation of new drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy [2]. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity [3]. As a result, a number of medicinal plants used in

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Commented [A3]: From 7 – 12 mm (in Table 1)

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indigenous medicine have been tested and found to possess bactericidal properties. Phytochemical screening is very important methods of identifying bioactive compound that useful in creating new drugs. These simple, cheap, sensitive, selective and rapid chemical tests to determine the presence of certain groups of compounds is an initial step to select plants for further phytochemical studies [4].

Calotropis gigantea which is generally referred to as giant milk weed, Sodom apple, crown flower and shallow wort is a perennial, greyish-green, woody shrub with broad obovate fleshy leaves that grows wild in the tropics and in warm temperate regions [5]. The plant is found in almost all parts of Nigeria but more abundant in the northern part of the country [6]. In northern Nigeria, it is referred to as tumfafia, in Yoruba Parlance; the plant is generally referred to as “*Ewe bomu bomu*”.

All the parts of the plant including root, stem, leaf and flowers of *C. gigantea* are in common use in indigenous system of medicine. *Calotropis* is also a reputed Homoeopathic drug .The plant shows anticancer, antifungal and insecticidal activities. The roots are reported to have antifertility and anti-ulcer effects. The latex of the plant is reported to possess analgesic and wound healing activity, as well as anti-inflammatory, antimicrobial activity and also exhibited local anesthetic activity. The protein fraction derived from the whole latex of *C. gigantea* possesses antinociceptive activity, which is independent of the opioid system .Leaf extract shows antimicrobial activity. The flowers of the plant exhibit hepatoprotective activity, anti-inflammatory, antipyretic, analgesic, and antimicrobial effects and larvicidal activity [1].

Over the years *Calotropis gigantea* had invited attention of the researchers worldwide for its pharmacological activities such as anti-diabetic, antitoxin, antihepatotoxin, antioxidant and wound healing activity. Latex contains the cardiac glycosides, calotropin, uscharin, calotoxin, calactin and uscharidin; gigantinin. All parts of the tree are considered to possess medicinal properties and used in the treatment of syphilis, boils, inflammation, epilepsy, hysteria, fever, muscular spasm, warts, leprosy, gout, snakebites, and cancer [7]. Therefore, the objectives of this study are to determine the phytochemical constituents in the leaves of *Calotropis gigantea*, and to examine the efficacy and potency of the crude aqueous and ethanolic extracts of *C. gigantea* leaves and roots against some clinical pathogens.

2. MATERIAL AND METHODS

2.1 Plants Collection and Preparation

The plant parts were collected from Ado-Ekiti, Ekiti State, Nigeria. The plant parts (leaf and roots) were adequately washed with clean water and air dried at room temperature ($25\pm 2^{\circ}\text{C}$) for about 20 days. They were then pulverized (crushed) separately with grinding machine to obtain smooth powder.

2.2 Preparation of Crude Extracts

One hundred grams (100g) of the ground powder of each of the plant parts were soaked separately in 500 ml sterile distilled water for 72 hours at room temperature. Ethanol was used in this study. One hundred grams of the ground powder of each of the plant parts (leaf and root) were soaked separately with the solvents in 750 ml capacity flask for 72 hours. They were then filtered through using a sterile no.1 filter paper. The extract was stored at 4°C .

2.3 Reactivation and Identification of Bacterial Culture

Colonies were picked with a flamed inoculated loop and cultured in test tube of McConkey broth, incubated in an incubator at 37°C for 18 hours. Subsequently, a loop full of the suspension was streaked on an overdried McConkey agar and incubated at 37°C for 24 hours.

The pure bacterial isolates were identified on the basis of their morphological and biochemical tests such as pigmentation, shape, elevation, consistency, margin, Gram staining, Catalase test, Fermentation of sugars, Indole production and sensitivity tests [8]. The test bacteria were identified as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Bacillus subtilis* and *Alpha haemolysin*.

2.4 Antibacterial Susceptibility Testing

The method employed by Aiyegoro *et al.* [9] was adopted. Different concentrations (0.5g, 1.0g, 1.5g, 2.0g, and 2.5g) of each extract were weighed and dissolved separately in 2 mL of ethanol and distilled water. These extracts were incorporated into sterilized paper disks made from Whatman No. 1 filter paper. The medium (nutrient agar) was prepared according to the manufacturer specifications; the broth culture of each organism was serially diluted to 10⁻³. A loopful of 10⁻³ dilution was spread onto the prepared agar plates. The disks with the different concentrations were placed on the inoculated plates, incubated at 37°C for 18-24 hr and observed for growth and the diameter of the zones of inhibition was measured in millimeter using a metre-rule.

2.5 Antibiotic Susceptibility Testing

The antibiotics susceptibility of the bacteria pathogens were determined by disc diffusion method on Mueller-Hinton agar according to CLSI [10]. The antibiotic discs were aseptically, carefully and firmly placed on the inoculated plates using sterile forceps. The plates were then inverted and incubated for 24 hours at temperature of 37 °C. After incubation, the plates were examined for growth and the diameters of zone of inhibition were measured and the results were interpreted with reference to CLSI [10]. The test bacteria were screened for resistance to gram-negative and gram-positive antibiotic discs which comprise Chloramphenicol (CH 30µg), Ciprofloxacin (CPX 10µg), Amoxicillin (AM 30µg), Gentamycin (CN 10µg), Streptomycin (S 30µg), Erythromycin (ERY 15µg), Ampiclox (APX 10µg), Levofloxacin (LEV 5µg), and Rifampicin (RD 5µg).

2.6 Phytochemical Screening the Plant Extracts

Test for Alkaloids: Five grams of each plant extract was mixed with 5 ml of 1% (v/v) aqueous hydrochloric acid on a steam bath, 1ml of the filtrate was treated with few drops of Dragendoff's reagent. Blue-black turbidity serves as preliminary evidence of alkaloids presence.

Test for Saponins: Five grams of each plant extracts was shaken with distilled water (5 ml) in a test tube. Frothing which persisted on warming was taken as preliminary evidence of the presence of saponins.

Test for Tannins: Five grams of each plant extract was added to 100 ml distilled water, stirred and filtered through Whatman No 1 filter paper. Ferric chloride reagent was added to the filtrate. A blue-black or blue green precipitate determined the presence of tannins.

Test for Flavonoids: Presence of flavonoids in the plant extracts was tested using FeCl₃ and lead ethanoate solutions. A green-blue or violet coloration on addition of FeCl₃ solution

and appearance of buffcoloured precipitate on addition of lead ethanoate solution indicated the presence of flavonoids in the extract.

Test for the presence of Cardiac glycosides (Keller-killiani test): Five grams of each plant extracts was mixed in 2 ml glacial acetic acid and a drop of ferric chloride solution were added. This was underlaid with 1ml of absolute H₂SO₄. Development of a brown ring at the interface indicates the presence of a deoxy-sugar characteristic of cardenolides. A violet ring might appear below the brown ring, while in the acetic acid layer, a green ring might form which would gradually spread throughout the acetic acid layer.

Test for the Presence of Terpenoids: Five milliliter of each plant extracts was mixed in 2 ml of chloroform 100% (v/v), and absolute H₂SO₄ (3 ml) was carefully added to form a layer. Formation of a reddish brown layer at the interface showed the presence of terpenoids.

Test for steroids: One gram of the plant extracts was mixed in a beaker with 5 ml of concentrated acetic acid. It was gently warmed and cooled. One drop of concentrated sulphuric acid was added along the sides of the test tube. Appearance of green colour indicates the presence of steroids.

2.7 Statistical Analysis

Analysis of variance was computed using Statistical Package for the Social Sciences (SPSS) 15 software for each attribute and the Duncan multiple range test was used to separate the means where significant difference existed. Statistical significance was considered at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Results

The aqueous leaf extract of *C. gigantea* inhibit high antibacterial activity against selected pathogens compared to root extract shown in table 1. So that the leaf extract was highly effective, maximum range (8.0 mm to 20.0 mm). Aqueous root extract of *C. gigantea* inhibit antibacterial activity against *Bacillus* spp. compared to other pathogenic test organisms.

The result also showed that the ethanolic extracts of both the leaf and root of *C. gigantea* have antibacterial activities on *E. coli* and *Bacillus* spp. but with no activity in root against *S. aureus*, *Klebsiella* spp. and *Pseudomonas* spp. while leave extract shows no activity against *E. coli* at all concentrations. However, the antibacterial effect was more pronounced against *Bacillus* spp., and *Pseudomonas* spp. in leaf which was seen to be more sensitive to the both the leaf and root ethanolic extracts at a concentration of 5.00g/mL with zones of inhibition of 16.0 mm and 12.0 mm (Table 2). In the case of *E. coli*, it was only inhibited by the root ethanolic extract with a highest recorded zone of inhibition (10.0 mm) at 5.00 g/mL while no activity was observed at all concentrations in leaf ethanolic extract.

Commented [A6]: No activity in leaf against *E. coli*

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Table 3 shows the antibiotics sensitivity of test bacteria pathogens; *E. coli* recorded the highest sensitivity to all the antibiotics, followed by *Pseudomonas* spp. which also had no resistance to any of the antibiotics. *Bacillus* spp. was sensitive to all the antibiotics also *Staphylococcus aureus* was sensitive to all the antibiotics except (CN, RD, E, CH, and APX). While *Klebsiella* spp. and *α-haemolysin* had the highest resistance to all the antibiotics used because there was no growth in the immediate area around the disc used.

C. gigantea possess alkaloids, cardiac glycosides, tannins, flavonoids, terpenoids, sterols, proteinases and non-protein amino acid as major phytochemical groups. A series of bioactive components have been reported from the different parts of *C. gigantea*, are reported in table 4.

UNDER PEER REVIEW

Table 1. Antibacterial activity of aqueous extracts of *Calotropis gigantea* leaves and roots against bacterial pathogens

Test organisms	Diameter of zones of inhibition (mm)									
	Concentrations (mg/mL)									
	0.50		1.00		1.50		2.00		2.50	
	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root
<i>S. aureus</i>	8.0±0.01 ^a	0.0±0.00 ^b	10.0±0.01 ^a	0.0±0.00 ^b	12.0±0.01 ^a	0.0±0.00 ^b	16.0±0.01 ^a	0.0±0.00 ^b	20.0±0.01 ^a	0.0±0.00 ^b
<i>B. cereus</i>	7.0±0.02 ^a	0.0±0.00 ^b	9.0±0.02 ^a	0.0±0.00 ^b	10. ±0.01 ^c	7.0±0.01 ^c	12.0±0.01 ^c	10.0±0.01 ^c	14.0±0.02 ^a	12.0±0.01 ^c
α-haemolysin	0.0±0.00 ^a	0.0±0.00 ^a	6.0±0.00 ^a	0.0±0.00 ^a	9.0±0.02 ^b	0.0±0.00 ^a	11.0±0.02 ^b	0.0±0.00 ^a	14.0±0.01 ^c	0.0±0.00 ^a
<i>E. coli</i>	6.0±0.02 ^a	0.0±0.00 ^b	10.0±0.01 ^c	0.0±0.00 ^b	12.0±0.01 ^c	0.0±0.00 ^b	14.0±0.01 ^c	0.0±0.00 ^b	17.0±0.02 ^a	0.0±0.00 ^b
<i>P. aeruginosa</i>	0.0±0.00 ^a	0.0±0.00 ^a	0.0±0.00 ^a	0.0±0.00 ^a	0.0±0.00 ^a	0.0±0.00 ^a	0.0±0.00 ^a	0.0±0.00 ^a	0.0±0.00 ^a	0.0±0.00 ^a
<i>K. aerogenes</i>	7.0±0.01 ^a	0.0±0.00 ^b	8.0±0.02 ^c	0.0±0.00 ^b	10.0±0.01 ^a	0.0±0.00 ^b	13.0±0.01 ^a	0.0±0.00 ^b	14.0±0.02 ^c	0.0±0.00 ^b

Values with the same superscripts in the same row are not significantly different ($p < 0.05$).

Table 2. Antibacterial activity of ethanolic extracts of *Calotropis gigantea* leaves and roots against bacterial pathogens

Test organisms	Diameter of zones of inhibition (mm)									
	Concentrations (mg/mL)									
	0.50		1.00		1.50		2.00		2.50	
	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root
<i>S. aureus</i>	0.0±0.00 ^a	0.0±0.00 ^a	0.0±0.00 ^a	0.0±0.00 ^a	6.0±0.02 ^b	0.0±0.00 ^a	8.0±0.02 ^b	0.0±0.00 ^a	10.0±0.03 ^c	0.0±0.00 ^a
<i>B. cereus</i>	7.0±0.02 ^a	0.0±0.00 ^b	8.0±0.03 ^c	7.0±0.03 ^c	10.0±0.02 ^a	8.0±0.02 ^a	14.0±0.03 ^c	9.0±0.02 ^a	16.0±0.02 ^a	12.0±0.03 ^c
α-haemolysin	0.0±0.00 ^a	0.0±0.00 ^a	10.0±0.02 ^b	0.0±0.00 ^a	10.0±0.01 ^c	0.0±0.00 ^a	12.0±0.01 ^c	0.0±0.00 ^a	14.0±0.03 ^d	0.0±0.00 ^a
<i>E. coli</i>	0.0±0.00 ^a	0.0±0.00 ^a	0.0±0.00 ^a	6.0±0.02 ^b	0.0±0.00 ^a	7.0±0.01 ^b	0.0±0.00 ^a	8.0±0.01 ^b	0.0±0.00 ^a	10.0±0.01 ^b
<i>P. aeruginosa</i>	8.0±0.02 ^a	0.0±0.00 ^b	12.0±0.02 ^a	0.0±0.00 ^b	12.0±0.03 ^c	0.0±0.00 ^b	14.0±0.01 ^d	0.0±0.00 ^b	16.0±0.02 ^a	0.0±0.00 ^b
<i>K. aerogenes</i>	6.0±0.03 ^a	0.0±0.00 ^b	10.0±0.01 ^c	0.0±0.00 ^b	10.0±0.02 ^d	0.0±0.00 ^b	12.0±0.02 ^d	0.0±0.00 ^b	14.0±0.01 ^c	0.0±0.00 ^b

Values with the same superscripts in the same row are not significantly different ($p < 0.05$).

Table 3: Antibiotic sensitivity of test bacteria

Test Organisms	Concentrations (gm/mL)								
	Diameter of zones of inhibition (mm)								
	CPX	CN	AM	S	RD	ERY	CH	APX	LEV
<i>S. aureus</i>	16.0	8.0	14.0	15.0	0.0	0.0	0.0	0.0	14.0
<i>B. cereus</i>	23.0	31.0	31.0	32.0	32.0	32.0	25.0	32.0	34.0
α -haemolysin	0.0	0.0	14.0	14.0	0.0	0.0	0.0	0.0	0.0
<i>E. coli</i>	25.0	28.0	26.0	38.0	27.0	25.0	8.0	30.0	31.0
<i>P. aeruginosa</i>	11.0	24.0	25.0	10.0	24.0	10.0	20.0	6.0	34.0
<i>K. aerogenes</i>	14.0	0.0	0.0	15.0	13.0	0.0	0.0	0.0	14.0

> 19.0mm – susceptible < 13.0mm – resistant

CPX – Ciprofloxacin (10 μ g); CN – Gentamycin (10 μ g); AM – Amoxicillin (30 μ g); S – Streptomycin (30 μ g); RD – Rifampicin (5 μ g); ERY – Erythromycin (15 μ g); CH – Chloramphenicol (30 μ g); APX – Ampiclox (10 μ g); LEV – Levofloxacin (5 μ g)

Table 4: Phytochemical screening results of crude extracts of *Calotropis gigantea* leaves and root

Parameters	Root	Leaves
Alkaloids	+	+
Cardiac Glycoside	+	+
Tannins	+	+
Saponin	+	+
Flavonoids	+	+
Terpenoids	+	+
Sterols	+	+

+ positive

3.2 Discussion

From the results it is evident that the ethanolic extracts showed better and broader spectrum of activity compared to other extracts. Ethanolic extract was active against one Gram negative bacteria. Aqueous extract exhibited moderate activity against one Gram negative bacteria and one Gram positive bacteria. The solvent blank and aqueous extract did not show any activity against tested bacteria. The rapid emergence of resistance to antibiotics amongst pathogens generates visions of the 'potential post-antibiotic era threatening present and future medical advances'. In view of the crossover of resistance across related compounds the future can see sharply depleting antibiotic resources. Laboratories around the world have literally screened thousands of phytochemicals having in vitro inhibitory effect against a wide spectrum of microbes [11].

In most of the screened plant extracts, the most active fraction is known but individual active compounds are not characterized. An interesting observation is that majority of the active crude extracts and their fractions are almost equally active both against drug resistant and sensitive bacterial strains. Multi target based approaches of screening of medicinal plant extracts and herbal drugs are expected to yield novel activities. The prepared extracts of *Calotropis gigantea* showed activity comparable with standard drug Ciprofloxacin inhibiting the growth of most of the above tested bacterial strains. The demonstration of antibacterial activity of *C. gigantea* extract against both Gram positive and Gram negative bacteria may

be an indicative of the presence of broad spectrum antibacterial components [12]. This indicates that the plant may be a useful source for the development of novel antibiotics against pathogenic bacteria.

The results of phytochemical screening of aqueous and ethanolic root and leaf extracts of *C. gigantea* revealed the presence of alkaloids, flavonoids, saponins, tannin, cardiac glycoside, steroid, and terpenoids. The concentrations of the various classes of secondary metabolite vary amongst the extracts evaluated. The concentrations of the constituents are in order of water > ethanol. The presence of these components in this species is an indication that it may have some medicinal potential. This is due to the fact that each of the components identified has one therapeutic usage or another. For instance, plants rich in saponins have immune boosting and anti-inflammatory properties [13]. Similarly, tannins have been reported to have antibacterial potential due to their basic character that allows them to react with proteins to form stable water soluble compounds thereby killing bacteria by directly damaging its cell membrane. The antibacterial activities of alkaloids and flavonoids have been reported by a number of authors [14, 15].

This could be due to the presence of the dissolved phytochemicals at low concentrations in the aqueous extracts; hence the phytochemicals were more dissolved in the ethanol and thus responsible for the antibacterial activity exhibited in this study. The apparent resistance of the test bacteria against these extracts at almost all concentrations may be a result of transfer of resistance plasmids or indiscriminate and sub-therapeutic use of the extracts [16]. Previous studies have shown that tannins bind the cell wall of bacteria, preventing growth and protease activity and can also be toxic to filamentous fungi, yeasts and ruminal bacteria [17]. Cardiac glycosides, which have been reported to have antimicrobial properties, were found in all the extracts. Saponins were detected in all the extracts. They are effective in the treatment of syphilis and certain skin diseases [18]. Flavonoids are known for their anti-allergic effect as well as a wide variety of activity against Gram-positive and Gram-negative bacteria, fungi and viruses [19].

4. CONCLUSION

The results showed that extracts obtained from different parts of the plant *Calotropis gigantea* using various solvents are rich sources of potent phytochemicals especially the roots and leaf extract and has inhibitory effects on the experimental microbes. From previous studies and the current work it is clear that the plant is rich source of alkaloids, glycoside, tannins, saponins, flavonoids, terpenoids etc. Since other parts exhibits multidimensional pharmacological activities, in future, the study on leaves and root extracts can reveal some of these properties and would be useful to mankind. The observed antibacterial activities of leaves extract in different solvents justify the traditional use of this plant against several antibacterial infections.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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