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Phytochemical Screening and Antibacterial Analysis of *Calotropis Procera* Flower Extracts

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ABSTRACT

Medicinal plants containing active chemical constituents with high antibacterial property play an important role in new drugs discovery. In this study we have identified the various chemical compounds present in the flower extract of *calotropis procera* found in Sokoto region and also assessed its antibacterial activity against some selected bacterial strains for the possibility of using them in the development of new antibiotics to reduce cost of health care and mortality rate due to bacterial resistance. Cool extraction method was adopted to extract the flower of *calotropis procera* using three different solvent (n-hexane, chloroform and ethanol) and also standard methods were used to assess the antibacterial activity of the extracts. The result obtained from solvent extraction of *calotropis procera* flower revealed that n-hexane, chloroform and ethanol had a mass of 2.19 g, 0.9 g, 3.2 g, with percentage yield of 4.30 %, 2.35 % and 11.98 % respectively. The result of the extraction revealed that *calotropis procera* flower consists of more polar compounds than non-polar ones. The phytochemical screening of the flower extracts of *calotropis procera* revealed the presence of alkaloids, steroids and tannins while flavonoids, antharaquinones, saponins and cardiac glycoside were not detected. The results of the antibacterial studies revealed that all the fractions has activity on the selected bacteria strains (*S. aureus, E. coli and Shagella*) with zone of inhibition that ranges between 2-8 mm. these results shows that the *calotropis procera* flower has a potential for the development of new antibiotic drugs.

Keywords: Phytochemical Screening, Antibacterial Analysis, Antibioltics, *Calotropis procera*, Drug resistance

INTRODUCTION

For thousands of years, plants have been utilized as medicines. Major constituents of more than 50% of all the drugs in clinical use today are natural products and their derivatives [1]. There has been a growing interest in the study of medicinal plants as natural products in diverse parts of the world. [1] Medicinal plants containing active chemical constituents with high antibacterial property play an important role in the prevention of various degenerative diseases. [1] Herbal medicines have become more popular in the treatment of many diseases due to popular belief that green medicine is safe, easily available and less side effects [2]. The World Health Organization (W.H.O.) reported that over 85% of the populations in Sub-Sahara Africa, including Nigeria still depend on herbal traditional medicine for their healthcare needs [2]. Natural products in general and medicinal plants in particular are believed to be an important source of new chemical substances with potential therapeutic effects [3]. Plant extracts as well their primary and secondary metabolites have important therapeutic role in the treatment of many human diseases [3]. Natural products mainly from plant kingdom offer a wide range of biologically active compounds that act as natural antibacterial agent with recognized potential in drug discovery and development [4]. This research project aim to screen the phytochemicals present in the *Calotropis procera* flower extract and identify it antibacterial potentials for the possibility of developing new drugs.

Calotropis procera (giant milkweed) is a perennial, greyish-green, woody shrub with broad fleshy flower that grows wild in the tropics and in warm temperate regions [4]. The plant is found in most parts of Nigeria but most abundant in the northern part of the country [5]. It is primarily harvested because of its distinctive medicinal properties [5], It is used in the treatment of pain as well as inflammation and to aid digestion. The latex of the plant is used as an antidysenteric, antirheumatic, a diaphoretic, an expectorant and for the treatment of bronchial asthma and skin conditions [6]. In African and Asian countries, the latex of *C. procera* is utilized as an arrow poison, molluscide, a fungicide, an anti-syphilitic, an anti-inflammatory, a purgative, as well as in the treatment of leprosy and bronchial asthma. The dried latex and root are used as an antidote for snake poisoning. It is also used as an abortifacient and for the treatment of piles and intestinal worms [6]. The previous pharmacological studies include reports of anticancer, antifungal and insecticidal activities of *C. procera*. The flowers and latex of the plant exhibited hepatoprotective, anti-inflammatory, antipyretic, analgesic, antimicrobial and larvicidal activity .[6] The latex of the plant is also reported to possess analgesic and wound healing activity, as well as anti-inflammatory and antimicrobial activities while the roots are reported with anti-fertility and anti-ulcer effects [7]. Though some studies have been reported on the antidiabetic potentials of *C. procera* but there is dearth of information on the mechanism of antihyperglycemic action of this plant. The plant is an erect, tall, large, much branched and perennial shrubs or small trees that grow to a height of 5.4 m., with milky latex throughout. Bark is soft and corky. Branches stout, terete with fine appressed cottony pubescence (especially on young) [7], Flower sub-sessile, opposite, decussate, broadly ovate-oblong, elliptic or obovate, acute, thick, glaucous, green, covered with fine cottony pubescent hair on young but glabrous later and base cordate. Flowers in umbellate-cymes and tomentose on young, Calyx glabrous, ovate and acute. Corolla glabrous, lobes erect, ovate, acute, coronal scales 5 - 6, latterly compressed and equally of exceeding the staminal column. Follicles are sub-globose or ellipsoid or ovoid. Seeds broadly ovate, acute, flattened, minutely tomentose, brown coloured and silky coma is 3.2 cm long [7].

Plate 1.1: *Calotropis procera* flower

Calotropis procera Linn have been widely used in the Sudanese, Unani, Arabic and Indian traditional medicinal system for the treatment of various diseases namely leprosy, ulcers, piles and diseases of the spleen, liver and abdomen. The latex is used as an abortifacient, spasmogenic and carminative properties, antidysentric, antisyphilitic, antirheumatic, antifungal, mullusccide, diaphoretic and for the treatment of leprosy, bronchial asthma and skin affiction [8]. Different parts of the plant have been reported to possess a number of biological activities such as proteolytic, antimicrobial, larvicidal, nematocidal, anticancer, antiinflammatory. Its flowers possess digestive and tonic properties. On the contrary, the powdered root bark has been reported to give relief in diarrhoea and dysentery. The root of the plant is used as a carminative in the treatment of dyspepsia. The root bark and flower of *Calotropis procera* are used by various tribes of central India as a curative agent for jaundice [8]. This study seeks to identify the various chemical compounds present in the flower extract of *calotropis procera* found in Sokoto region and also to evaluate its antibacterial active agent (compound) for the possibility of using them in the development of new antibiotics to reduce cost of health care and mortality rate due to resistance of bacteria to already developed drugs.

MATERIALS AND METHOD

All solvents and chemicals used were of analytical grade and were obtained from sigma aldrich and BDH Chemicals England respectively. Nutrient Agar and Nutrient Broth were obtained from Titan Biotech L.T.D India.

Sample collection

The *calotropis procera* flower was collected in January, 2022 from Mabera Salame Area in Sokoto state Nigeria.

Sample treatment

The flower was first washed thoroughly with distilled water to remove dirty and other impurities and was allowed to dry at room temperature for 5 days. The dried flower was ground to fine powder using mortar and pestle. The powdered samples were stored in an air tight water free polyethene bag and used for the analysis.

Determination of moisture content

The flower was taken in a pre-weighed empty dish (W_0) and their resulting weight was denoted by (W_1). The dish containing the flower sample was transferred into an oven at 100 ^{0}C for 24 hours then removed. The

weight of the dish containing the dried sample was determined and denoted by (W_2). The percentage moisture content of the flower was calculated using equation below.

$$\% \text{ moisture} = \frac{W_1 - W_2}{W_1 - W_0} \times 100 \quad \text{——————————} \quad (2.1)$$

Where W_0 = weight of the empty dish

W_1 = weight of empty dish + sample

W_2 = weight of the empty dish + dry sample

Extraction of flower

The flower powder (50 g) was extracted by maceration in a 1000 cm^3 glass stoppered conical flask. N-hexane (150 cm^3) was first used to defat (extract) the flower to remove non polar compound. The marc was allowed to dry and then extracted with chloroform to remove moderately polar compound and then extract with ethanol to remove polar compound. The extraction was done according to the order of increasing polarity of the solvents. In each case the flower was left in contact with the solvent for 24 hours with intermittent stirring to ensure maximum extraction. After 24 hours, the extracts were filtered using Whitman filter No.1 and the filtrates were transferred to evaporation dishes and concentrated using air circulated oven at temperature between 80-100 ^{0}C.

The percentage yield of the extracts was calculated as follows:

$$\% \text{ extract yield} = \frac{\text{Weight of extract}}{\text{Initial weight of sample}} \times 100 \quad \text{——————————} \quad (2.2)$$

Qualitative phytochemical screening of extract

The tests for flavonoids, tannins, saponnins, steroids/triterpenoids, alkaloids, cardiac glycosides and anthraquinones were carried according to the methods described by [12-17].

Antibacterial Studies

Test Organisms

The test organisms were obtained from the Department of microbiology, Faculty of Sciences, Sokoto State University, Sokoto, Nigeria. The microorganisms are standard laboratory strains of *Staphylococcus aureus (*gram +ve)*, Shigella* (gram -ve)*, Escherichia coli (*gram –ve).

Susceptibility Test

The antibacterial test was conducted using the petri dish plate method described by [16]. The Nutrient Agar plates prepared according to manufacturer's instruction were allowed to solidify for 15 minutes at room temperature and incubated without inoculum for 24 hours at 37 ^{0}C to ensure the sterility of the medium. The Nutrient Agar plates were flooded with 1 ml of the inoculum and the excess was removed using Pasteur pipette. Five wells (cups) of about 6 mm in diameter were cut on each Nutrient Agar plate using a sterile cork borer and the agar plugs were removed using sterile ampoule file. The extract solution (0.1 mL) was placed in each of the wells and were allowed to settle for two hours at room temperature and then incubated for 24 hours at 37 ^{0}C. The inhibition zone was observed and then recorded in millimeters using a transparent ruler. Standard antibiotic was used.

Minimum Inhibitory Concentration (MIC)

This was carried out as described by [16]. Minimum inhibitory concentration (MIC) was defined as the lowest concentration where no visible turbidity would be observed in the test tubes. The MIC was determined for the micro-organisms that showed reasonable sensitivity to the test extracts. In this test, the micro-organisms were prepared using the broth dilution technique. The stock solution (20 mg/mL) of the leaves extract was prepared by dissolving 0.2 g of the leaves extract in 10 mL Dmso. Four concentration were prepared (0.25mg/mL, 0.5 mg/mL, 1.0 mg/mL and 2.0 mg/mL), from the stock solution using serial

dilution technique and later inoculated with 0.2 mL suspension of the test organisms. After 24 hours incubation at 37 °C, the tubes were observed for turbidity. The lowest concentration where no turbidity was observed was determine and noted as the Minimum Inhibitory Concentration (MIC).

Minimum bactericidal concentration (MBC)

The minimal bactericidal concentration was determined from broth dilution test resulting from the MIC tubes as described previously by inoculating the content of each test tube on a nutrient agar plate. The plates were then incubated at 37 °C for 24 hours. The lowest concentration of the extract that showed no growth was noted and recorded as the minimum bactericidal concentration.

RESULTS AND DISCUSSION

Results

Table 1 and 2 give the details of the percentage moisture content, percentage yield and mass of the fractions obtained from *calotropis procera* flower extract respectively. The flower had a percentage moisture content of 62.64 %. The mass and the percentage yield of the three fractions obtained from n-hexane, chloroform and ethanol were 4.19 g, 8.38%, 2.90 g, 7.59%, and 1.20 g, 4.49% respectively. N-hexane fraction contains more of the extract.

Percentage moisture content of *calotropis procera* flower extract

The percentage moisture content obtained for *calotropis procera* flower extract is presented in Table 1

Table 1: Percentage moisture content of *calotropis procera* flower (%)

S/N	Weight of empty dish (W0)	Weight of empty dish + sample (W1)	Weight of empty dish + dry sample	Moisture content (%)
1	2.93	5.50	3.89	62.64%

Extraction of *calotropis procera* flower

The mass of the fractions obtained and the yield are presented in Table 3.2

Table 2: Mass of fractions (g) and percentage yield of *calotropis procera* flower extracts (%).

Extract	Original mass (g)	Mass (g) of Extract	Percentage Yield (%)
N-hexane	50	4.19	8.38
Chloroform	38.2	2.90	7.59
Ethanol	26.70	1.20	4.49

Qualitative phytochemical screening of *calotropis Procera* flower extract

Table 3 show the results of the qualitative phytochemical screening of the fractions obtained. Alkaloids, tannins, and steroids, were detected in all the three fraction of *calotropis procera* flower while antharaquinones, flavonoids, saponins and cardiac glycoside were not detected in all the three fractions.

Table 3 Qualitative phytochemical screening of *calotropis procera* flower extract

Phytochemicals		N-hexane	Chloroform	Ethanol
Alkaloids				
(a)	Mayer's test	+	+	+
(b)	Wagner's test	+	+	+
(c)	Dragendorff's test	+	+	+
Flavonoids				
(a)	Sodium hydroxide	-	-	-
(b)	Ethyl acetate test	-	-	-
(c)	Shinoda's test	-	-	-
Steroids				
(a)	Salkowski's test	+	+	+
(b)	Lieberman-Buchard's test	+	+	+
Saponins				
(a)	Foam test	-	-	-
Tannins				
(a)	Iron (III) chloride test	+	+	+
(b)	Lead acetate	+	+	+
Antharaquinones				
(a)	Borntrager's test	-	-	-
Cardiac glycoside				
(a)	Keller-killiani's test	-	-	-

Keys:(+): detected (-): not detected

Antibacterial activity of *calotropis procera* flower

Table 4(a), 4(b), 4(c), 4(d), 4(e), 4(f) and 34(g) show the details of the results obtained from the antibacterial activity test of the n-hexane, chloroform and ethanol fractions of *calotropis procera* flower. The results deduced that all the three fractions, has activity on bacterial strain with zone of inhibition ranging between 2-8 mm.

Table 4(a) Antibacterial activity of n-hexane fraction of *calotropis procera* flower

Concentration (mg/ml)	Zone of Inhibition		
	S-aureus	E-coli	Shigella
0.25	0.00	2.00	6.00
0.50	2.00	2.00	7.00
1.00	3.00	3.00	7.00
2.00	4.00	4.00	8.00

Table 4(b) Antibacterial activity of chloroform fraction of *calotropis procera* flower

Concentration (mg/ml)	**Zone of Inhibition**		
	S-aureus	**E-coli**	**Shigella**
0.25	6.00	6.00	2.00
0.50	5.00	5.00	2.00
1.00	4.00	4.00	2.00
2.00	3.00	3.00	0.00

Table 4(c) Antibacterial activity of ethanol fraction of *calotropis procera* flower

Concentration (mg/ml)	**Zone of Inhibition**		
	S-aureus	**E-coli**	**Shigella**
0.25	8.00	0.00	0.00
0.50	6.00	2.00	7.00
1.00	5.00	2.00	6.00
2.00	4.00	4.00	5.00

Table 4(d) Antibacterial activity of standard antibiotic (Amoxicillin)

Antibiotic concentration	**Zone of Inhibition (mm)**		
	S-aureus	**E-coli**	**Shigella**
Amoxicillin (125mg/mL)	12.00	10.00	10.00

Table 4(e) Minimum inhibitory concentration (mic) and minimum bactericidal concentration (mbc) of n-hexane fraction of *calotropis procera* flower

Test Organism	**MIC (mg/ml)**	**MBC(mg/ml)**
S.aureus	2.00	2.00
E.colli	0.50	0.50
Shagella	0.50	0.50

Table 4(f) Minimum inhibitory concentration (mic) and minimum bactericidal concentration (mbc) of chaloroform fraction of *calotropis procera* flower

Test Organism	**MIC(mg/ml)**	**MBC(mg/ml)**
S.aureus	0.25	0.50
E.colli	2.00	1.00
Shagella	0.50	0.50

Table 4(g): Minimum inhibitory concentration (mic) and minimum bactericidal concentration (mbc) ethanol fraction of *calotropis procera* flower

Test Organism	**MIC(mg/ml)**	**MBC(mg/ml)**
S.aureus	1.00	1.00
E.colli	0.25	0.25
Shigella	0.25	1.00

DISCUSSION

The result obtained from moisture content analysis of *calotropis procera* flower revealed that the flower had a percentage moisture content of 62.64% (Table 1). The mass and percentage yield of the fraction (n-hexane, chloroform and ethanol) obtained are 4.19 g, 2.90 g, 1.20 g, and a percentage yield of 8.38% 7.59%, and 4.49% respectively (Table 2). Polar compounds can only be extracted by polar solvent, example ethanol [18]. On the other hand non-polar compounds can only be extracted by non-polar solvent in which n-hexane is an example [18]. The results of the extraction revealed that *calotropis procera* flower consist of more polar compounds than non-polar ones. The phytochemical screening of the flower extracts of *calotropis procera* revealed the presence of alkaloids, tannins, steroids while flavonoids, saponins, anthraquinones, cardiac glycoside were not detected (Table 3).These classes of secondary metabolites are known to be responsible for the antibacterial activities demonstrated by plant extracts [19]. Alkaloids and tannins have been reported to exhibit antimicrobial properties [19]. The chloroform extract exhibited good antibacterial activity on all the test organisms with zones of inhibition ranging between 2-8 mm (Table 4b). The n-hexane fraction showed a high level of activity on gram positive bacteria than gram negative bacteria, but all the organisms were found to be susceptible. The demonstrated activity shown by the n-hexane fraction may be due to the presence of tannins and alkaloids (Table 3) that are known to have some antibacterial activity as reported by [20]. The results when compared with Amoxicillin (Standard Antibiotic -Table 4(d), the zone of inhibition produced by the antibiotic against the test organisms was found to be appreciable in relation to those activities produced by the organism under study. However, according to [21] diameter of zones of inhibition ≥ 10 mm are considered active. From these results, it can be deduced that the Chloroform and ethanol fraction is bacteriostatic than the fractions and N-hexane fraction is bactericidal on all the test organisms at 0.2 mg/ml. In view of the above results, the extract showed considerable activity against *S. aureus*, a gram positive bacterium known to play a significant role in invasive skin diseases [22]. The antibacterial activities of the extracts are related to the plant secondary metabolites detected. In line with these findings, [23] reported that tannins had been widely used as an application to sprains, bruises and superficial wounds. According to [24], cytotoxicity is the common biological property of alkaloids and it has been associated with medicinal uses for centuries. The analgesic, antispasmodic and antibacterial properties of alkaloids have also been reported by several workers. Plant extracts contain steroids which are very important compounds because it has a relationship to body compounds such as sex hormones [25].

CONCLUSION

Based on the percentage yield of the extract obtained from the flower of *calotropis procera*, it could be concluded that ethanol extracts produced more of the phytochemicals than chloroform and n-hexane extracts. Similarly, the plants contain tannins, alkaloids and steriod while saponins, cardiac glycosides, flavonoids and anthraquinones were not detected. This justifies the result obtained from the antibacterial analysis which shows that the flower extracts were capable of inhibiting the growth of the bacterial strain with zone of inhibition between 2-8 mm which is appreciable when compared with the standard antibiotic drug.

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