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Phytochemical and Antimicrobial Screening of the Ethanolic, N-Hexane and Aqueous Extracts of *Calotropis procera* (Sodom Apple) Flower on Selected Pathogens

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Abstract

Crude Ethanolic, N-hexane and Aqueous extract of *Calotropis procera* flower were extracted by cold maceration method, and investigated for the phytochemical contents. The flower contains various classes of bioactive secondary metabolites such as Alkaloids (++), Phenols (++), Tannins (+++), and Saponins (++) whereas Flavonoids (- -) were absent in all the three Solvent extracts. Antibacterial activity by agar well diffusion method against the various human pathogens were carried out. The microorganism used in this study include *S.aureus*, *S.typhi*, *P.aeruginosa*, *C.albicans*, and *E.coli* of concentration 50,100,200 and 400 mg/ml respectively. Zones of inhibition observed against the pathogenic strains were between 11-29 mm. All the three extracts of the flower showed broad spectrum of inhibition against the studied pathogens with exception of C. albicans, which showed no zone of inhibition (00). The Results revealed that the flower extracts of *C. procera* can be potentially used as an antibacterial agent against these pathogenic organisms. These results were sufficiently positive to encourage further investigations of the pharmaceutical applications of Sodom apple flower.

Keywords: Bactericidal, Ethanolic, Inhibitory, Pathogens, Phytochemicals, Tannins

Introduction

Plants are important in our everyday existence in maintaining the ecosystem, they provide our foods, produce oxygen we breathe and serve as raw materials for many industrial products such as clothes, foot wears and so many others [1]. The use of plants in traditional medical practice has a long drawn history, and remains the main stay of primary health care in most of the third world [2]. Herbal medicines exhibit a remarkable therapeutic diversity. *C. procera* is an Ayurvedic plant which is used in several traditional medicines to treat variety of diseases [3].

Traditional medicines are used by about 60% of the world population in both developing and developed countries where modern medicines are predominately used. Despite modern development in the treatment of diseases, herbal remedies have

been in continuous used universally as the cheapest and affordable in health care management [4].

Medicinal plant like *C. procera* are believed to be an important source of new chemical substances with potential therapeutic benefits which lie in the chemical substance present in the plant [1].

Researchers have explored and discovered the nature of secondary metabolites that are in various medical plants are of great importance in treating so many illnesses. Medical plant represents a precious and renewable source for new drugs [3]. Therefore, this study is undertaken to assess the phytochemical and antimicrobial screening of the ethanolic, n-hexane and aqueous extracts of *Calotropis procera* (Sodom apple) flower on selected pathogens towards the discovery of drugs to extend life expectancy.

Materials and Methods

Sample Collection

C. procera flowers were collected by hand picking using hand gloves from the garden at the National Animal Production Research Institute (NAPRI), Shika, Kaduna State, Nigeria, into a polyethylene bag and was transported for identification at the Taxonomy Division of the Medicinal Aromatic and Poisonous Plant Research Centre at Ahmadu Bello University, Zaria, Kaduna State, Nigeria in August, 2023. The specimen was assigned a voucher number ABU 0900219 at the Herbarium of the Department of Botany, Ahmadu Bello University, Zaria.

Preparation of flower powder and extraction

Fresh flower of *C. procera* were washed in clean water and placed on a polythene bag for drying at room temperature in the laboratory. After drying at room temperature for about 4-6 weeks, the flower was pulverized in a clean mortar and pestle, the powder was sieved and weighed (500 g) and kept for further purpose (Plate 1)

About 20 g of the powdered flower was weighed each time using an electronic weighing balance and made into packets using rezohdge filter paper and soaked into 200 ml of three solvents (Methanol, N-hexane and Aqueous) for extraction for 24hr, using cold water Maceration method of extraction (Plate:2)

The flower extract obtained from the use of each solvent was concentrated separately, by distillation and drying by evaporation in a water bath at 40°C (Plate 2)

PLATE 1: *C. Procera* plant and the pulverized flower

Phytochemical Analysis

Each extract was investigated for the presence of secondary metabolites like alkaloids, flavonoids, Saponins, phenol and tannins for the three different extractants, using qualitative standard method [8]

Test for Alkaloids

One gram of the dried powdered sample was heated with 10 mL of 10% HCl on a water bath for five minutes.

The extract was then filtered and allowed to cool. The pH was adjusted to about 6-7 by adding 10% ammonia and using litmus paper. The presence of turbidity or precipitation indicated presence of alkaloid [6]

Test For Flavonoids

One gram of the powdered sample was added to 10 mL of ethanol and 3 drops of $FeCl_3$ solution was added, dark green colour observed indicated the presence of flavonoid. One gram of powdered sample was added to distilled water and heated for 3 mins. It was allowed to cool and 2 mL of concentrated. H_2SO_4 was added, yellow coloration disappeared which shows the presence of flavonoid [14].

Test for Tannin

The Braemer's test: One gram of the powdered sample was decocted with 10 mL of distilled water by boiling for 10 minutes and filtered while hot and allowed to cool. A 0.1% ferric chloride reagent was added to the filtrate and observed. A blue-black, green or blue green precipitate indicated presence of tannins [6].

Test for Saponins

***Frothing*:** One gram of the powdered sample was transferred into a test tube containing 10 mL of distilled water and then boiled for five minutes and then filtered. The filtrate was mixed vigorously and observed. The presence of froths indicated the presence of Saponins. The mixture was shaken vigorously and kept for 3- min after which it was

observed. A honey comb like froth formed indicated the presence of Saponins [14].

Test For Phenols

One gram of powdered of sample was added to 10 mL of ethanol and three drops of phenol solution added. A dark green colour observed indicated the presence of phenol. For steroids, two ml of acetic anhydride were added to 0.5 g of ethanol extract of each sample with 2 mL H_2SO_4. The colour change from violet to blue or green in some samples indicated the presence of steroids [5].

Antibacterial Analysis

Antibacterial activity of the extracts was carried out by using overnight cultures of the tested bacteria. An already solidified sensitivity test medium on petri dishes were seeded with the test organisms using sterile swab stick (spread plate method) after which four wells were made using cork-borer (6mm).

The extracts were reconstituted by adding 0.2 mL of ethanol to 0.0001g of the paste like form of *C. procera* after proper mixing, 1mL of distilled water was then added. Various concentrations of 50, 100, 200 and 400 mm/mL were obtained. The plates were incubated at 37 ^oC for 24 hours and the zones of inhibition were measured in millimetre using transparent meter rule.

Antibiotics sensitivity disc was used as positive control, the test organisms were also prepared using spread plate method, while CPX and FLU serves as control. Each antibacterial assay was carried out in triplicate.

Determination of Minimum Inhibitory Concentration (MIC)

A serial dilution ranges of the extracts to obtain concentrations (50,100,200 and 400 mg/ml) were made to determine the MIC. The bacterial strains were cultured in Nutrient broth in the micro plate (96- well U-Bottom (50063), Gaithersburg). 1.0 mL of test concentrations of each was added to 1.0 mL suspension of microorganism and incubated at 37^oC for 24 hrs [5].

Determination of minimum bactericidal concentration (MBC)

Suspensions from the MIC studies were used for the MBC determination to a solid media a bacterial streaking of equal streaks of the suspension from the MIC was made and the procedures were repeated all through the required numbers of the corresponding isolates. The concentrations were incubated at 37^oC for 24 hrs. And the plates were observed.

Results and Discussion

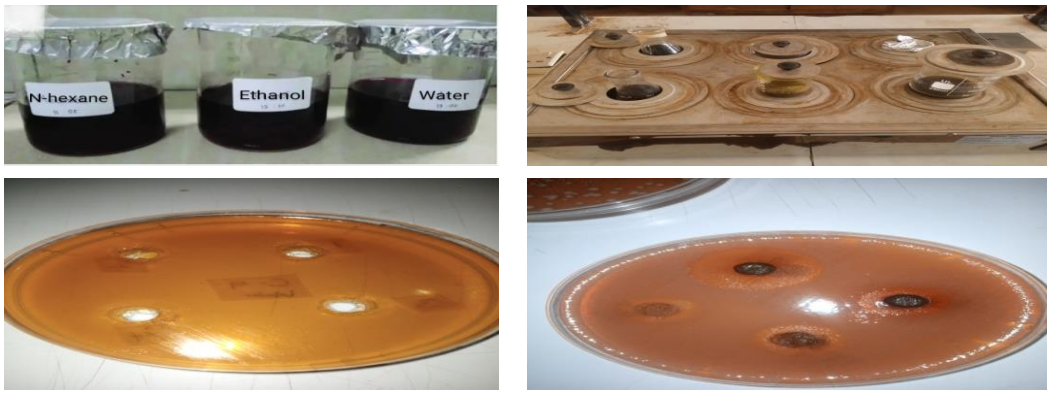

PLATE 2: Different extracts, water bath and zone of inhibitions

TABLE 1: Phytochemical analysis result for the three solvent used

Phytochemicals	Solvent		
	Ethanol	N-hexane	Aqueous
Alkaloides	+ +	+ +	+ + +
Phenols	+ +	+ +	+ + +
Flavonoides	–	–	–
Tannins	+ + +	+ +	+ + +
Saponins	+ +	+ +	+

Key: +++ = Highly Present, ++ = Moderately present, + = Low presence, **-** = Absent

TABLE 2: Minimum Inhibition for the test organisms

Test Bacteria	**Concentration Of Extract (in mg/ml) And zone (in mm)**												**Test Bacteria**	
	Ethanol				N-Hexane				Aqueous				CPX (10mg)	FLU (10mg)
	400	200	100	50	400	200	100	50	400	200	100	50		
S. aureus	19	14	11	9	21	18	14	00	26	19	17	10		
E. Coli	20	18	14	12	22	20	17	13	26	16	15	13		
P.aeruginosa	23	20	18	16	23	21	18	16	27	20	18	14		
S.thyphi	29	27	21	17	22	20	18	13	22	17	14	11		
C.albicans	00	00	00	00	00	00	00	00	00	00	00	00		

Table 3: Minimum Inhibition for the test organisms

Test Bacteria	**Concentration Of Extract (in mg/ml) And zone (in mm)**											
	Ethanol				N-Hexane				Aqueous			
	400	200	100	50	400	200	100	50	400	200	100	50
S. aureus	-	MIC	+	+	-	-	MIC	+	-	-	MIC	+
E. Coli	-	-	-	MIC	-	-	-	MIC	-	-	-	MIC
P.aeruginosa	-	-	-	MIC	-	-	-	MIC	-	-	-	MIC
S.thyphi	-	-	-	MIC	-	-	-	MIC	-	-	MIC	+
C.albicans	Not Determined				Not Determined				Not Determined			

Discussion

Table 1 presents the result of phytochemical analysis carried out on the Ethanolic, N-hexane and Aqueous extract of *C. procera*. The phytochemicals tested where Alkaloids, Phenols, Flavonoids, Tannins and Saponins. The result indicates the presence of the bioactive components of interest such as alkaloids, flavonoids, Saponins, Phenols and tannins. The bioactive components seen in this work are similar to the findings of Al-Sulaibi *et al.* [2] who tested for the presence of similar bioactive components in *C. procera* extracts

Phytochemicals are known to show medical as well as antimicrobial activities. Flavonoids were found to be absent in all the three extracts. This implies that the plants flower may not be effective in inhibiting or killing microorganisms known to be sensitive to these phytochemicals.

Al-Sulaibi [2] reported that flavonoids which are widely distributed in plants have many functions including protection from microbial and insect's attacks. Al-Rowaily *et al.* [3] also reported that flavonoids are responsible for the colour, fragrance, and flavour characteristics. In plants, flavonoids perform many functions like regulating

cell growth, attracting pollinator's insects, and protecting against biotic and abiotic stresses The test carried out on the aqueous extract indicated that only Alkaloids, phenol and tannins were present in trace and sufficient quantities, whereas Saponins were found present but at a low concentration and flavonoids were found absent.

The phytochemical analysis carried out on the N-hexane extract shows that Alkaloids, phenols, tannins and Saponins were present in the extract of the flower plant in moderate quantities. The same phytochemicals were also found to be present in the Ethanolic extract of the flowers plant but here tannins were found to be highly present. The result shows that the flower plant when extracted with organic solvent (especially ethanol) would be more effective than when it is extracted with water as an antimicrobial agent. This result agreed with the work reported by [14].The phytochemical analysis indicate the presence of tannins, this could be useful in the leather industries for the production of tannins for leather processing.

Antimicrobial activity

Antimicrobial activity shows that ethanolic extract of *C. procera* flower, was effective on *S. aurous* and *S. typhi* but in effective for *E. coli, P. aeruginosa*, and was not susceptible for all the extract of *C. procera* flower on *C. albicans.* The results of this work generally shows that *C. Procera* can be utilized as a source of both traditional and modern medicine as world health organisation (WHO) has advocated traditional medicine from plants as safe remedies for ailment of both microbial and non-microbial origin [8]

The result also confirms that the choice and use of *C. Procera* flowers alone or combined with other herbs traditionally in Nigeria and other countries for the treatment of common diseases such as fever, rheumatism, indigestion, cold, eczema and diarrhoea [12]

The result of this work further confirms that the work could be used as an anticancer agent treatment as it has been found to reduce the viable count in water [8].

Conclusion

The result of the phytochemical analysis of the Ethanolic, N-hexane and aqueous extract of *C. Procera*, in this work indicated that the flower plant contains some of the selected secondary metabolites (phytochemicals) such as Tannins, Saponins, phenols, and Alkaloids which are essential parts of the flower plant, whereas Flavonoids were found absent. Therefore this implies that the flower of *C. Procera* can be utilised as a source of both traditional and modern medicine in the treatment of different pathogenic organisms.

The world health organisation has estimated more than 80% of the world's population in developing countries depend primarily on herbal medicines for their basic healthcare needs in recent years, ethno-botanical and traditional uses of natural

compounds, especially those of plants origin, have received much attention as they are well known for their efficacy and are generally believed to be safe for human use. It is best to use the classical approach in the search for new molecules to manage a variety of diseases. Batool *et al* [12] Review and published a literature on *C. procera* shows that it is a popular remedy in a variety of ethnic groups, as well as Ayurveda and traditional practitioners for the treatment of a range of ailments. Researchers are exploring the therapeutic potential of this plant as it is likely to have more therapeutic properties than are currently known.

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