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Phytochemical Constituents of Sap, Root and Leaf Extracts of *Calotropis Procera* from Select Regions in the Gambia

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Medicinal plants serve as potential cure for animal and human diseases as they contain phytochemicals for therapeutic values. Most of the modern drugs produced are isolated from herbal plants. This research reports on the phytochemical constituents of the methanolic and dichloromethane (DCM) extracts of the sap, root and leaf of *Calotropis procera* in three select regions in The Gambia namely the West Coast Region, North Bank Region, and Lower River Region. The sap was collected in an air-tight test tube; the root and leaf were dried at room temperature for three weeks and one month, respectively and further ground into powder. Soxhlet extraction of the sap was done using both DCM for 30 minutes at 40^{o}C to 50^{o}C and methanol for 3 h at 60^{o}C to 70^{o}C. The root and leaf were extracted using cold maceration for 72 h. The methanolic extracts showed the presence of alkaloids, quinones, saponins, flavonoids, terpenoids, steroids, and carbohydrates in the sap and leaf. Quinones were present in the DCM extract of the sap and

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root, but absent in the leaf. While flavonoids and terpenoids were present in the sap and leaf extracts, they were absent in the root. Alkaloids, saponins, and steroids were absent in the sap and root extracts, but found in the leaf. Tannins and phenols were absent in both the methanolic and DCM extracts. The results of each of the samples collected at the three different regions were similar in their phytochemical constituents for the extracting solvents; but varied with the different solvent. The findings of this research allude to the fact that the sap, root and leaf of *C. procera* are good sources of therapeutic compounds that are responsible for the use of this plant in herbal medicine in The Gambia.

Keywords: Calotropis procera; The Gambia; herbal plants.

1. INTRODUCTION

Medicinal plants have been sources of cure for animal and human diseases as they contain phytochemicals in different parts of the plant. Medicinal plants contain varying amounts of phytochemicals in different parts of the plant. Phytochemicals are chemicals produced by plants through primary or secondary metabolism. They generally have biological activity in the plant host and play a role in plant growth or defence against competitors, pathogens, or predators. These chemical compounds (secondary metabolites) help plants to resist fungi, bacteria and virus infections, consumption by insects and other animals, and have therapeutic values [1]. "*Calotropis"*, derived from the Greek terms "beautiful" and "keel of a boat," alludes to the scales on the flowers. The Latin name *procera*, which means "in favor of" and "wax," refer to the waxy aspect of the plant. *Asklepios* refers to the scientific name for the plant family. *Calotropis procera* is the scientific name for a flowering plant in the *Asclepediaceae* family which consists of 175–180 genera and 2200 species; also called Kisher and Usher in Arabic, giant milkweed, Rubber tree, *calotrope* cabbage and Sodom apple [2]. It is a xerophytic perennial shrub tree that grows in semi-arid and arid regions [3] and mostly located in tropical Africa and subtropical Africa, Asia, and Latin America [2]. In The Gambia, *Calotropis procera* is a ubiquitous weed plant and known by various local names such as *"Kunjunbor"* or "*Kikanpaw*" in Mandinka; "*Fufftaan*" in Wollof; "*Kupampaw*" in Fula and "*Pumba pumbeh*" in Jola (oral communications). Numerous investigations have indicated that *Calotropis procera* contains phytochemicals [4,5,6,7].

Phytochemical screening of *C. procera* showed the presence of alkaloids, tannins, and flavonoids, reducing sugars, triterpenoids, sterols, amino acids, and glycosides [8]. A variety of proteins, including N-acetyl-D-glucosaminidase and carbohydrate-binding proteins (lectins) are present in the latex fluid made up of uzarigenin and terpineol ester. The primary active ingredient is mudarine together with a bitter yellowish acid, resin, and three toxic glycosides called calotropin, uscharin, and calotoxin [5]. The stalk and leaves contain calotropin, calotropagenin, cardinolide, and proceragrin; while benzolylinesolone and benzolyisolelone are present in the bark. The alkaloids *calotropin, calotaxein*, and *uskerin* are stimulants to the heart [7]. *C. procera* traditionally has been used to treat certain diseases such as diarrhea, stomatic, sinus fistula, skin diseases, and jaundice [9]. In The Gambia, polio is treated with the bark of *C. procera*. Both the plant's bark and leaves were reported to be effective against both gram-positive and gram-negative bacteria due to their broad-spectrum antibacterial properties. The root bark contains oxypregnane oligoglycosides that can eradicate the U373 glioblastoma and PC-3 prostate cancer cell lines [10]. The root extract has potent cytotoxic impact on COLO 320 tumor cells; a derivative of cardenolide, which was isolated from the root bark of the plant, is cytotoxic on a number of human cancer lines [11]. A spray made from the leaf extract of *C. procera* can be utilized to treat stagnant water against the breeding of mosquitoes [6]. By immersing the affected limb for several hours over the course of three days, *C. procera* leaf and fruit extracts can be utilized to cure guinea worms. The burned dry leaves and pithy stem are administered to patients with paralysis, arthralgia, swelling, and sporadic fever either by inhaling the smoke or smoking it in pipes [12]. The flowers are reported to contain chlorophenyl acetate and several flavonoids [13].

In Senegal the milky latex is used locally to cure skin conditions like ringworm, syphilitic sores, and leprosy. Nigerian natives have historically used *C. procera* alone or in combination with other plants to treat ailments like fevers, rheumatism, indigestion, colds, dermatitis, and

diarrhea. The milky latex of *Calotropis procera* is used by traditional folk healers to treat a variety of illnesses. When applied to a new wound, leaf latex rapidly stops the bleeding. In combination with honey, the milky latex has been used to make anti-rabies and toothache remedy [14]. In India the plant's sap is utilized as a purgative, while the flowers are used as a digestive aid, stomach tonic, and asthma preventative. The plant is infamous for its poisonous chemicals including iridocylitis and dermatis, which operate as toxins. Asclepin and mudarin are the main active pharmaceuticals that give the plant its digitalic and emeto-carthartic characteristics [15]. The latex is a potent antimycotic agent dermatomycosis *in vitro* (Aliyu *et al.*, 2011) and contains high amount of the poisonous *cardenolides* (cardiac glycosides) [16]. A considerable dose-dependent analgesic effect is produced by a single oral dose of dry latex starting at 165 mg/kg and 830 mg/kg in the treatment of acetic acid-induced writhing. In comparison to oral dose of 100 mg/kg the effect of dry latex at 415 mg/kg is stronger [17]. To cure skin conditions, swelling of the abdominal viscera, intestinal worms, and ascites, the root bark is used as medication [18]. Mashed root paste of *C. procera* when combined with rice vinegar is used to treat the scrotum and legs of an elephant. Hay from *C. procera* is high in digestible crude protein content, but is cardiotoxic and hepatotoxic [19].

Medicinal plants have varieties of curative effects but under-utilized in The Gambia as research on herbal plants, including *Calotropis procera*, is scarce. The importance of such curative effects can be incorporated into The Gambia herbal medicine and health care system. In this research, investigation of phytochemicals in *C. procera* in three select regions in The Gambia was conducted and reported.

2. MATERIALS AND METHODS

2.1 Identification and Collection of Samples

Calotropis procera (Plate 1) was identified by a Taxonomist at the National Agricultural Research Institute (NARI) at Yundum, The Gambia. The sap, leaves, and roots of *C. procera* were collected from three different regions of The Gambia namely Essau (located in Lower Niumi District in the North Bank Region), Pakalinding (located in Jarra West, Lower River Region), and Kainilai (located in Foni Bondali District in the West Coast Region of southern Gambia near the border with Senegal).

Plate 1. The *Calotropis procera* plant

A clean, dry test tube with a rubber cork was used to collect 19 cm^3 of the milky latex from the sap of *C. procera*.

2.2 Treatment of Samples

The collected latex was safely stored in a 50 cm^3 test tube, corked, and kept in the chemistry laboratory of The UTG (University of The Gambia) at room temperature for further analysis. The cut leaves were rinsed in running water to remove dust and dirt and dried under the shade for 1 month. The roots of *C. procera* were washed with running water to remove sand and dirt and dried under shade for 3 weeks. The dried roots were cut into pieces and ground using a wooden pestle and mortar. The dried leaves were grinded using a wooden pestle and mortar to reduce their surface size and further pulverized with a grinder into fine powder.

2.3 Extraction of Samples

Into the thimble of the Soxhlet extractor was poured 18 cm^3 of the latex of *C. procera*. Both methanol and dichloromethane (DCM) were the extracting solvents. The extraction process for DCM was for 30 minutes at 40^oC to 50^oC. The methanol extraction lasted for 3 h at 60^oC to 70^oC. The extract was evaporated on a water bath and carefully stored for further analysis.

The powdered leaves (100 g) were macerated in 75cm^3 DCM for 72 h and filtered. The filtrate was evaporated to dryness. The concentrate was kept in a test tube with rubber-stopped cork for qualitative analysis. The same procedure was repeated using methanol.

The root sample (155 g) of *C. procera* was macerated in 75 cm^3 of DCM for 72 h and

filtered. The extract was evaporated to dryness. The concentrate was kept in a test tube with rubber cork for qualitative analysis. The same procedure was repeated using methanol.

2.4 Phytochemical Screening

The methods previously described by [1] and [20] with minor modifications were used to carry out the phytochemical screening for alkaloids, quinones, phenols, saponins, flavonoids, terpenoids, steroids, tannins, and carbohydrates.

3. RESULTS AND DISCUSSION

Table 1 presents results of the phytochemical constituents of methanolic extracts of the sap, root and leaf of *Caltropis procera* in three regions West Coast Region (WCR), North Bank Region (NBR), and Lower River Region (LRR) of The Gambia and showed similar distributions of the phytochemicals. Tannins and phenols were absent in the sap, root and leaf extracts. On the other hand, alkaloids quinones, saponins, flavonoids, terpenoids, and steroids were present in the methanolic extracts of the sap and leaf, but absent in the root extracts. Tannins are known for their antioxidant and antimicrobial properties as well as for soothing relieve, skin repair, anti-inflammatory and diuresis, and interact with protein to give the astringent effects which helps in the treatment of ulcer [21].

While the results of the methanolic extract of the sap corroborate the findings of [22], [5], and [23], they differ with the findings of Morsy et al. [24].

Saponins are expectorants, cough depressants and administered for hemolytic activities [25]. Saponins exhibit cytotoxic effects and growth inhibition against a variety of micro-organism thereby, making them to have anti-inflammatory and anticancer properties [26]. The frothy effects of saponins in the plant may be responsible for the lowering of blood cholesterol levels and the risk of cancer. [18] reported that the root bark was used to cure skin conditions. This property of *C. procera* can be attributed to the presence of saponins in the root extract of the plant [27].

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Flavonoids are powerful water-soluble antioxidants and free radical scavengers. The presence of flavonoids in could be the reason for the reported use of *C. procera* for the treatment of abdominal tumors [28]. Flavonoids are responsible for various colourations in the barks, leaves, flowers, fruits and seeds of plants, and are known for their antibacterial, antiviral, anti-inflammatory, anti-allergic, antineoplastic, antiangioic anti-mutagenic, cytostatic, analgesic, and antioxidant activity for the protection of the body against degenerative diseases such as cancer [29, 30, 25, 23].

Table 1. Phytochemical constituents of methanolic extracts of *Caltropis procera* from West Coast Region, North Bank Region and Lower River Region

		WCR			NBR			LRR		
S/N	**Phytochemicals**	***S***	**R**	**L**	**S**	**R**	**L**	***S***	**R**	**L**
1.	Alkaloids	+	-	+	+	-	+	+	-	+
2.	Quinones	+	+	+	+	+	+	+	+	+
3.	Phenols	-	-	-	-	-	-	-	-	-
4.	Saponins	+	+	+	+	+	+	+	+	+
5.	Flavonoids	+	+	+	+	+	+	+	+	+
6.	Terpenoids	+	-	+	+	-	+	+	-	+
7.	Steriods	+	-	+	+	-	+	+	-	+
8.	Tannins	-	-	-	-	-	-	-	-	-
9.	Carbohydrates	+	+	-	+	+	-	+	+	-

WCR = West Coast Region' NBR = North Bank Region, LRR = Lower River Region, S = sap, R = root, L = leaf, + means present, – means absent

Table 2. Phytochemical constituents of DCM extracts of *Caltropis procera* from West Coast Region, North Bank Region and Lower River Region

		WCR			NBR			LRR		
S/N	**Phytochemicals**	***S***	**R**	***L***		**R**	**L**		**R**	***L***
1.	Alkaloids	-	-	+	-	-	+	-	-	+
2.	Quinones	+	+	-	+	+	-		+	-
3.	Phenols	-	-	-	-	-	-	-	-	-
4.	Saponins	-	-	+	-	-	+	-	-	+
5.	Flavonoids	+	-	+	+	-	+	+	-	+
6.	Terpenoids	+	-	+	+	-	+	+	-	+
7.	Steriods	-	-	+	-	-	+	-	-	+
8.	Tannins	-	-	-	-	-	-	-	-	-
9.	Carbohydrates	+	+	-		+	_		+	-

WCR = West Coast Region' NBR = North Bank Region, LRR = Lower River Region, S = sap, R = root, L = leaf, + means present, – means absent

Terpenoids have been reported to have insecticidal properties. The presence of terpenoids in the methanolic extract of the sap and leaf of *C. procera* may be responsible for its use in the treatment of stagnant water against the breeding of mosquitoes as reported by [6]. Quinones were present in the DCM extracts of the sap and root, but absent in the leaf (Table 2). While flavonoids and terpenoids were present in the sap and leaf extracts, they were absent in the root. Alkaloids, phenols, saponins, steroids, and tannins were absent in the sap and root extracts, but present in the leaf. For the same region, phytochemical constituent were present within the same plant part for a particular solvent but absent in the same plant part using a different solvent.

This could be due to the varying solubility of the phytochemicals and solvent polarity; methanol being more polar, and therefore, the more extractable solvent than DCM. All the three regions showed similar results for the sap, root and leaf possibly due to their related geography and topography. The presence of the phytochemicals in the sap, root, and leaf of *C. procera* explains the potentials of the plant in herbal medicine.

4. CONCLUSION

This study investigates the phytochemical constituents of *C. procera* from three different regions of The Gambia using two different solvents methanol and dichloromethane (DCM). The different solvents varied in their extractabilities of the phytochemicals. The methanolic extracts showed that tannins and phenols were absent in the sap, root, and leaf extracts; but alkaloids, quinones, saponins, flavonoids, terpenoids, steroids, and carbohydrates are present in the sap and leaf. Quinones are present in the DCM extracts of the sap and root; but flavonoids and terpenoids are contained the sap and leaf extracts. Alkaloids, phenols, saponins, and steroids were absent in the sap and root extracts, but present in the leaf. The isolation, quantification, and purification of these phytochemicals in this traditional herb can lead to the development of new medicine in conjunction with conventional medicine.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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