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*In Vitro*Anthelminthic Efficacy of Ethanolic Root BarkExtract of *CalotropisProcera*

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Abstract
Background:*Calotropis Procera is a plant which is used in several traditionalmedicine and folklore system to cure various ailments.Many people around the world, especially in the developing countries, are increasingly relying on plant-derived traditional medicines. The world health organization defined medicinal plants as any plant that possess bioactive compounds in one or more of its organs that can be used for healing purposesThe ethanolic root bark extract of Calotropis Procera was evaluated for Phytochemical and anthelmintic activity using earthworms (Pheretimaposthuma). Alkaloids, Flavanoids, Cardiac Glycosides, Terpenoids, Carbohydrate, Phenols, Saponins, Quinones, Tannins, Steroids and Saponinswere confirmed in the plant extract while Oxalate, Sterols were not confirmed. Cardiac glycosides, Terpenoids and Carbohydrate were confirmed in high concentrations. The ethanolic extract exhibited dose-dependent inhibition of spontaneous movement (paralysis) and evoke response to shock. With higher dose of 30.0mg/ml of ethanolic root bark extractshowed paralysis at 10min:43sec and death at 19min:23 seconds against the earthworm (Pheretimaposthuma), the standard drug Albendazole at the concentration of 20.0 mg/ml showed paralysis at 17min:54sec and death in 26min:63sec. This indicates that ethanolic extract at 30.0 mg/ml dose showed highest activity when compared with Albendazole. The predominant effect of the extract on the worm is to cause a flaccid paralysis that result in the expulsion of the worm by peristalsis. The results showed that the root bark extract possesses wormicidal efficacy, therefore the root bark extract can be formulated and use as anthelmintic.*
Keywords*: Calotropis Procera, Anthelmintic, Phytochemical, Earthworm, Root Bark.*

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I. Introduction

Medicinal plants are used from the ancient time as the major sources of drugs. The fact is that presently available drugs can be obtain, either directly in the extract form or in the modified synthetic form [1.] Plant-based natural products, animal derived natural products and minerals have been the foundation of treatment of different human diseases [2.] Many people around the world, especially in the developing countries, are increasingly relying on plant-derived traditional medicines. The world health organization defined medicinal plants as any plant that possess bioactive compounds in one or more of its organs that can be used for healing purposes [3]. Modern anthelminthic medicines are very effective in curing worm infestation but also cause a number of side effects and sometimes may cause resistance. Herbal drugs are less effective in comparism to synthetic drugs but are relatively free from side effects. Intestinal parasites have been considered as a major public health problem throughout the world. Tropical countries have relatively much acute problem of worm infestation among adults and children due to various reasons. Modern anthelminthic medicines are very effective in curing worm infestation but also cause a number of side effects and sometimes may cause resistance. Species are already reported to possess anthelmintic properties such as Artemisia an-nua L. extracts [4]. Various plants have been scientifically validated for their anthelmintic property both *in vitro* and *in* vivo [5, 6]. *Calotropis Procera* is a plant which is used in several traditional medicine and folklore systems to cure various ailments as reported in the Hindu literature [7.]*Calotropis procera* belongs to the family of Asclepiadaceae and commonly known as Giant Swallow wort, milkweed is a small to medium-sized shrub. *Calotropis procera* (Ait) R. Br is a plant commonly distributed throughout the tropics of Asia, South America, Africa and the Middle East [8, 9, 9].This plant is popularly known because it produces large quantity of latex. *Calotropis procera* latex, roots, stems, leaves and flowers has different biological activities such as anti-inflammatory, antidote, anthelmintic, antipyretic, antidiarrhoeal, antibacterial and analgesic [10]. The plant, *Calotropis procera* is being used as herbal medicine by people living in desert areas [11].The plant, *Calotropis procera* is used in many African countries for the treatment of various ailment including helminth infestations. Various parts of the plant

including the inner bark, stem, leaf, sap, roots, fruits and bark of the root have been found useful in the management of syphilis, cold, fever, rheumatism, indigestion, eczema and diarrhea. The leaf, stem and root are utilized in traditional medicine for treatment of wounds, sores and skin diseases, diarrhea, sinus and fistula [12]. It strongly recommended in leprosy, hepatic and splenic enlargements, dropsy and worms. Due to the prevalence of parasitic infections and the developed resistance of some anthelmintic drugs is now an enclosing area in the field of research [13]. The world health organization (WHO) estimates that about 80% of the world population presently uses traditional medicine for some aspect of primary health care. The use of and search of drugs as dietary supplements derived from plants have accelerated in recent years. The bioactivities in the plants are generally ascribed to the presences of plant secondary metabolites which could have beneficial or adverse effects. (Rochfort and Panozzo, 2007).

Phytochemicals are natural bioactive compounds of secondary metabolite found in plants that work with nutrients and fiber's to act as a defense system against diseases. These phytochemicals are divided into two groups, which are primary and secondary constituents, according to their functions in plant metabolism. The primary constituents comprises common sugars, amino acids, proteins and chlorophyll while secondary constituents consist of alkaloids, terpernoids, phenolic compound, tannins (Krishnaiah*et al.,* 2009).The drugs which are currently used for the treatment of worms in modern medicine are far from satisfactory as they provide only symptomatic relief, produce several adverse effects and may lose effectiveness on continued used. The challenge of developing new effective, safe and long lasting anthelmintic drugs from natural products appear inevitable. Hence, the search for an effective, relatively safe, cost effective and broad spectrum anthelmintic drugs for which *Calotropis procera* root bark has been selected due to its reported anthelmintic activity in Nigerian fork lore medicine.

II. Materials And Methods

Sample Collection

Plant Material

Calotropis procera root bark was collected in June 2019 from Federal University Gashua, Yobe state, School Campus field. The plant material was identified at the field using standard keys and descriptions. The Root Bark was cleaned by washing with running water (tap water), followed by thorough washing with distilled water in other to remove the contaminants, the plant material was cut into pieces and shade dried at room temperature for 21 days. Thereafter it was grinded into coarse powder using mortar and pestle, the powdered sample was sieved and stored in an air tight container until required.

Worm Collection

Adult earth worms (*Phaeretimaposthuma*) were used to evaluate anthelmintic activity. The earth worms *Phaeretimaposthuma*(family-annelida) were collected from the local moist place, at Federal University Gashua field washed and kept in water. All earthworms were of approximately equal size of 5-6 cm length and 0.2-0.3 cm widths used for all the experimental protocol.

Reference Drug

Albendazole (Shalina Laboratories PVT. LTD; Batch No: J7015)was obtained as the standard drug for comparative study with ethanolic extract of *Calotropis procera* Root Bark.

Extraction and Preparation of Material for Phytochemical Screening

The solvent (ethanol) was used for the extraction of the Root Bark of *Calotropis procera* sample. The method adopted was the one described by [16, 17]. Exactly 120 g of the powdered sample was weighed and extracted by cold extraction in 800 ml of the solvent (ethanol). The mixture was agitated at 30 min interval for 3 h and then soaked for 72 h. Subsequently the resulting mixture was then filtered using muslin cloth and the filtrate was evaporated to dryness using a water bath. The resulting extracts was weighed and stored in a sterile bottle and refrigerated at 4°C prior to testing. The extract is screened for phytochemical investigation and for screening of anthelmintic activity [18]. The phytochemical screening to detect the presence of secondary metabolites in the crude ethanolic extract of *Calotropis procera* Root Bark were carried out using the methods used by [19, 20, 21].The ethanol extract was qualitatively tested for the presence of phytochemicals such as Flavonoids, Alkaloids, Terpenoids, Phenols, Cardiac glycosides (steroids), Saponins, Tannins, Sterols, Carbohydrate and Quinone.

Evaluation of Antihelminthic Activity of Calotropis Procera Root Bark

The anthelmintic assay was carried out as per the method of [22] with minor modifications. Here the anthelmintic activity was assessed using earthworms because of their anatomical and physiological resemblance with that of the intestinal roundworm parasites of human being [23]. They are widely used as effective tools for

anthelmintic study because of their easy availability [24]. Normal saline water was used to wash all the worms and to remove all fecal matters. Extracts were weighed and dissolved in 10 ml of distilled water to obtain the concentrations of 10, 20, and 30 mg/ml. Earthworms were divided into five groups (each containing four worms) in petri dish. The extract was applied to the petri dishes and the time of paralysis and death was determined. Albendazole (10, 20, and 30mg/ml) was used as reference standard and normal saline (0.9% NaCl) as control. All the test solutions and standard drug solution were prepared freshly before starting the experiment. When no movement of any sort could be observed except when the worms were shaken vigorously, was considered as paralysis time. Time for death of worms (minutes) was recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50°C) followed by fading away of their body colors. The test results were compared with reference drug Albendazole treated samples.
The percentage yield of the Root Bark extract of *Calotropis procera* was calculated as follows;

$$\text{Percentage (\%) yield of extract} = \frac{weight\ of\ the\ extraxt}{weigt\ of\ the\ sample} \times 100$$

Statistical Analysis
The comparison of the experimental data was done by using One-Way analysis of variance (ANOVA). The results were presented as Mean ± SEM. Differences between means of treatment and control group were accepted significant at $p<0.05$.

III. Results

The result of Phytochemical screening of ethanolic Root Bark extract of *Calotropis procera* in Table 1revealed the presence of Alkaloids, Flavonoids, Cardiac Glycosides, Terpenoids, Steroids, Saponins, Tannins, Phenols, Quinone's and Carbohydrates, Oxalate and Sterols were not confirmed in the analysis.

Table no 1: Phytochemical Constituents of *Calotropis procera* Root Bark

Constituents	Calotropis Procera (Root Bark)
Alkaloid	+
Flavonoids	+
Saponins	+
Cardiac glycosides	++
Oxalate	-
Quinone	+
Terpenoids	++
Tannins	+
Sterols	-
Phenols	+
Carbohydrates	++

Key: + = present; - = absent; ++ = strongly present

Table 2: Shows the result of anthelminthic activity of *CalotropisProcera*root bark, the result revealed that different concentration of *Calotropis procera* Root Bark extract caused paralysis followed by death of the worms at all tested dose levels, the wormicidal effect increases with increase in concentration of the extract. The perusal of the data shown revealed that the ethanolic extractat the concentration of 30mg/ml showed paralysis at 10min:43sec and death at 19min:23secondsagainst the earthworm (P*heretimaposthuma*), the standard drug Albendazole at the concentration of 20.0 mg/ml showed paralysis at 17min:54sec and death in 26min:63sec. This indicates that ethanolic extract at 30.0 mg/ml dose showed highest activity when compared with Albendazole. The predominant effect of Albendazole on the worm is to cause a flaccid paralysis that result in the expulsion of the worm by peristalsis.

Table no2:Anthelmintic Activity of *Calotropis Procera* Root Bark Extract in Comparison with Reference Drug Albendazole

Groups	Concentration (mg/ml)	Time of Paralysis (m)	Time of Death (m)
Standard Albendazole	-	-	-
	10	21:19±0.009*	31:34±0.009*
	20	17:54±0.292**	26:63±0.405**
	30	14	
Calotropis Procera (Root Bark)	10	20:49±0.006*	29:79±0.344**
	20	15:50±0.006*	25:46±0.009*

30	10:43±0.012*	19:23±0.017*

Values were expressed as Mean±SEM (N=3) of four worms in each group.Values were find out by using one way ANOVA followed by Barlett's test. Values with (*) are not significantly different at $p<0.05$, while those with (**) are significantly different at $p>0.05$.

Table 3:Shows the Percentage Yield Determination and Physical Characteristics of Crude Extract of *Calotropis procera* Root bark. Ethanolcrude extracts of*Calotropis procera* root bark (20.04g) was obtained from theextraction of 120.00 g powders of the root bark using ethanol as the solvent as shown in Table 3 below.
As reported by [24], most anthelmintic agents that have been identified from plants are soluble in organic solvents and this reveals the better efficiency of ethanol as extracting solvent than water.

Table no3: Physical Characteristics and Percentage (%) Yield of the Extract obtained from *Calotropis procera* Root Bark

Solvent (800ml)	Dried sample	Output (extract)	% Recovery	Colour	Odour	Texture
Ethanol	120g	20.04g	16.70	Brown	Pleasant	Sticky

IV. Discussion

The presence of these phytochemical components in the root bark extract is an indication that it may have some medicinal potential [25]. They are the secondary metabolites that combine with nutrients, combat free radicals, and thus provide protective effect against dreadful diseases and early ageing. For instance, plants rich in Saponins have immune boosting and anti-inflammatory properties [26]. Similarly, it is possible that tannins contained in the ethanolic extract of *Calotropis procera* Root Bark can bind to free protein in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and cause death [27]. Flavonoids are the most important of all bioactive compounds in plants and good sources of natural antioxidants [28, 29]. They are also reported to show inhibitory activity against peroxidation of lecithin [30, 31]. Tannins contribute to plants wound healing activity. Tannins also are known to cause anti-inflammatory effect due to decrease in vascular permeability [32]. Phenols and phenolic compounds have been extensively used in disinfections and remain the standard with which other bactericides are compared [33]. Alkaloids are basic natural products occurring primary in plants. They occur as one or more heterocyclic nitrogen atoms and are generally found in the form of salts with organic acids. Alkaloids are the most efficient therapeutically significant plant substances [33].In a study by [34], aqueous, n-butanol, n-hexane, chloroform and methanolic extracts of *Calotropis procera* leaves was evaluated for anthelminthic activity. They found that the leaf extract of *Calotropis procera* not only demonstrated paralysis, but also caused death of worms especially at higher concentration of 20 mg/ml in shorter time as compared to reference drug Piperazine citrate. The perusal of the data reveals that the 70 % hydroethanolic
extract at the concentration of 12.5 mg/ml showed both paralysis and death in 18min: 58sec and 29min: 05sec respectively. Similarly n-butanol and chloroform extract at the concentration of 12.5mg/ml showed both paralysis and death at 21min: 03sec and48min: 26sec and 26min: 53 sec and 51min: 25secrespectively. The effect increased with concentration. It was observed that 70% hydro ethanol extract shown better anthelmintic activity as compared to n-butanol and chloroform extract of *Calotropis Procera* extract and the reference piperazine citrate.From the observations made, higher concentration of extract produced paralytic effect much earlier and the time to death was shorter for all worms. Ethanolic extract showed anthelmintic activity in dose-dependent manner giving shortest time of paralysis (P) and death (D) with 30 mg/ml concentration, for the worms. It is possible that the parasite paralysis and/or death observed may have been attributed to secondary metabolites [26]like tannins, alkaloids and saponins. [32] acknowledged that plant metabolites action may be additive, synergistic or antagonistic in manner acting at single or at multiple target sites. It is therefore likely that a number of compounds could have contributed to the anthelmintic activity observed in the Root Bark extract.

V. Conclusion

From the results shown, it is concluded that *Calotropis procera* Root Bark used by tribals traditionally to treat intestinal worm infections, showed significant anthelmintic activity. The experimental evidence obtained in the laboratory model could provide a rationale for the traditional use of this plant as anthelmintic. This study has indicated the potential usefulness of *Calotropis procera* Root Bark against earthworm infections. It will be beneficial to identify and characterize the active component responsible for the anthelmintic activity and to study its pharmacological actions *in vivo*.

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