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# PRELIMINARY PHYTOCHEMICAL AND *IN VITRO* ANTHELMINTIC PROPERTIES OF *CALOTROPIS PROCERA* ROOT EXTRACTS (*ASCLEPIADACEAE*)

**IBRAHIM T. BABALOLA1* AND UMAR M.UMAR1**
*Department of Chemistry, PMB 1144, Damaturu, Yobe State University, Damaturu Yobe State, Nigeria.*

## ABSTRACT.

*The medicinal value of plants in the treatment of internal parasites has been recognized dated back to 300 B.C by early physician- Theophrastus. Calotropis procera is a popular conspicuous weed-shrub in the North-Eastern Nigeria, belonging to the family Asclepiadaceae. Several works have been reported on the medicinal potential of the plant materials. The current study evaluates the phytochemicals and anthelmintic activity of Calotropis procera root extract. Methanol extract of the plant root was obtained through cold extraction method using Ultrasonicator. Phytochemical screening of the plant root extract reveals the presence of Alkaloids, Flavonoids, Cardiac glycosides, Saponins, Phenolics, Carbohydrates, but Steroids, Terpenoids, Anthraquinones and Oxalates were not detected. The crude methanol extract was partitioned into Petroleum-ether (PET) and Ethyl acetate (EtoAc) soluble fractions using solvent-solvent extraction. The anthelmintic activity of the crude methanol extract, PET soluble fraction, EtoAc soluble fraction and the residue were screened in vitro against Pheritimia pasthuma and Taenia solium at 50-25mg/ml in comparison with reference drug (Piperazine citrate, 10mg/ml) by measuring paralysis time (P) and time of death (D). The crude methanol extract exhibited most significant effect on Pheritimia pasthuma (P= 6.18 and D= 8.75) and against Taenia solium (P= 8.00 and D=15.05) at 50mg/ml when compared with other soluble fractions and the reference drug (P=34.38 and D= 43.17 for P. pasthuma); and for T. solium, (P=28.48 and D=39.5). This result provides support for the ethnomecinal use of Calotropis procera root as worm expellant.*

***Kerwords****: Calotropis procera; methanol root extract; solvent-sovent extraction; soluble fractions; phytochemicals; anthelimintic activity, Piperizine citrate.*

## Introduction.

*Calotropis procera* is a popular weed shrub belonging to the family *Asclepiadaceae.* It is indigenous to many parts of the world and commonly known as milk weed, Dead Sea apples, and Sodom apple in English, Pomme de Sodome in French, Tumfafiya in Hausa, Kayoci in Kanuri and Bomu bomu in Yoruba (Burkill, 1985). The plant is erect, tall, large, much branched and perennial shrub or small tree that grows on a height of 5-6m with milky latex throughout (Perwez and Mohammad, 2009; Kumar *et al.*, 2015).

Plants from this family have been used in African traditional ethnomedicine for several years for the treatment of various ailments. The powdered root mixed with goat milk is used in the treatment of epilepsy. The latex is used for treating ringworm, guinea worm blisters and scorpion stings. (Shivkar & Kumar, 2003; Verma *et al.*, 2010). *The stem* bark is used in extracting Guinea worms, and the leaves in treating cholera, eczema, leprosy, elephantiasis, asthma, cough, rheumatism and gout. Twigs are applied for the preparation of diuretics, stomarch tonic and anti-diarrhoetics. The juice of the stem and twigs were used for the purpose of infanticide and was sometimes given to women to induce abortion. The root is used exterminating intestinal worms (Orwa *et al.*, 2009; Amit, 2012; Shinde *et al*., 2013; Chaudhary *et al.*, 2017;). Several scientific reports have appeared in the literature on investigation into ethnomedicinal use of the plant (Meena *et al*., 2010; Chaudhary *et al*., 2011; Gupta *et al*., 2012; Khairnar *et al*., 2012; Quazi *et al.*, 2013; Gurung *et al.*,2016;). However, Investigation into anthelmintic activity of the plant is scanty in the literature.

In continuation of our studies on the search for active principles from phytomedicines, this paper reports on preliminary phytochemicals and anthelmintic activity of methanolic root extract of *Calotropis procera* hitherto unreported.

## Materials and Methods

### Plant Collection and Authentication

Root (1kg) of the plant *Calotropis procera* were collected from a farm land within Yobe State University Campus, along Gujba road, Damaturu. The plant

was authenticated by Botanist at the department of Biological Sciences, Yobe State University where the voucher specimen already exist.

**Plant Extraction**

The plant material (445.81g) was immersed in redistilled Methanol by maceration at room temperature (320C) for 96hrs. After solvent recovery, the percentage yield was determined and the plant extract were stored in sterile sample bottle until when required for analysis. 4.0g of crude methanol extract was partitioned into Petroleum ether and Ethylacetate respectively. The resultant PET soluble fraction, EtoAc soluble fraction and the residue were concentrated in vacuo using rotarvap and the percentage yield determined

**Phytochemical Analysis**

Qualitative analysis of the methanolic extract was carried out using standard procedure as decribed Sabri et al., 2012).

**Worm Collection and Authentication**

*Pheritimia pasthuma (earth worm,* mean weight 0.006-0.08g) and *Taenia solium(tapeworm,* 2.2-2.5g) were obtaine from freshly slaughtered cows in Dikumari Abattoir, Damaturu, with the help of Veterinary Health Technicians attached to the Abattoir. The earth worm were collected from water logged area inside vegetable market (‘Kaswan gwari’) along Portiskum road, Damaturu. The worms were authenticated at the Parasitology Unit, YSUTH.

**Anthelmintic Assay.**

Two worms (same type) were placed in 10.5cm Petri dishes in solution of crude extract/soluble fractions in two different concentrations (25 and 50 mg/ml in distilled water), respectively. This was done in duplicate for all the worms.
Mean times for paralysis (P, in minutes) were taken when no movement of any sort could be observed, except when the worms were shaken vigorously. Times of death (D, in minutes) were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50oC). Distilled water was included as control. This method is similar to previous method by Ajaiyeoba & Okogun, 1996).

**Results & Discussion.**

The methanol extraction of root of *Calotropis procera* afforded 8.15g of a creamy colored extract with percentage yield of (1.8%). 4.0g of crude methanol extracts pertitioned between Petroleum ether and Ethylacetate afforded 0.35g and 0.37g soluble fractions ( percentage yield of 8.83% and 10.38%) respectively.

Phytochemical screening of the methanol extract using standard methods confirmed the presence of Alkaloids, Flavanoids, Cardiac glycosides, Saponins, Phenolics, Sterols, Resins, Terpenoids, Anthraquinones, Oxalates and Carbohydrates as previously reported( Shamim et al., 2017; Najam, 2018).

Review of literature has shown that different species of worms have been used to evaluate the anthelmintic compounds from plant sources due to similarity in morphology (Shivkar & Kumar, 2003).

In this study, the crude methanol extract displayed intrinsic anthelmintic properties. The extract showed dose dependent anthelmintic activities with the two worms used in this study with 50mg/l giving a shortest time of paralysis (P) and death (D) for the two worm types.

The results from Table 1 showed that the crude methanol extract exhibited a more pronounced effect on *Pheritimia pasthuma.* The worms were more sensitive to crude methanol extract than PET and EtoAc soluble fractions. It produced paralysis at 6.18mins and death time of 8.75mins for the *Pheritimia pasthuma*, while paralysis and death for the reference drug were 34.38 and 43.17mins respectively. In the case of *Taenia solium*, 8min was obtained as time taken to paralyze the organism and death time of 15mins under same condition. 28.48mins and 39.5 mins were time taken for paralysis and death respectively for the reference drug. The Petroleum ether soluble fraction exhibited noteworthy anthelmintic properties on *Taenia solium*, paralyses and death time at 11mins and 20mins respectively. This indicates that *Taenia solium* are more susceptible than *Pheritimia pasthuma* (P = 16min, D = 25min). The ethylacetate soluble fraction showed that the worms reacted in the most none-relative pattern. *Taenia solium* were more fragile and so they die in the shortest time possible (22mins) but took 17mins to paralyse in the case *Pheritimia pasthuma*, the organism took longer time to die in ethylacetate soluble fraction (30mins) while they paralyze in the shortest time possible (i.e 16mins), the ethylacetate soluble fraction was more effective in causing the death of the

*Pheritimia pasthuma* rather than the paralysis when compared with reference drug. The residue is less active against both worms. In both cases, P and D values were more than 70% of the duration taken by the reference drug. The resultant P and D time for *P. pasthuma* and the *T. solium* by the residue showed that *Pheritimia pasthum* paralyses in 20mins while *Taenia solium* 19mins. Death time was found to be 40mins and 32mins for *P. pasthuma* and *T. solium* respectively. Generally, *Pheritimia pasthuma* (earthworm) are most susceptible to the extracts compared to reference drug- Piperazine citrate (10mg/ml). It is established that the role of worm expellers like Piperazine citrate is to cause paralysis of worms which then enables them to be expelled through man and animal faeces.

The crude methanol extract of *C.procera* root has not only demonstrated this property, they also cause the death of the worms at a shortest time contact. It is logical to conclude here that ethnomedicinal use of this plant in treating intestinal worm infestation (Dalziel, 1937; Burkhill, 1995) is justified.

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