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PHYTOCHEMISTRY AND *IN VITRO* TRYPANOCIDAL EFFICACY OF SELECTED MEDICINAL PLANTS OF SEMI-ARID NORTH-EASTERN NIGERIA

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ABSTRACT

Qualitative phytochemical and *In vitro* trypanocidal studies of aqueous extracts of the leaves of *Calotropis procera, Cassia sieberiana* and *Azadirachta indica* were conducted using standard procedures. Bioactive compounds of saponins terpenes, flavonoids, alkaloids, glycosides, tannins, pentoses, ketones, reducing sugars, and general carbohydrates were found in these plants. *In vitro* trypanocidal efficacy of graded concentrations of plant extracts in this study indicated a 100% *Trypanosoma brucei* mortality at 2.5mg/ml under one-hour incubation period. Thus, the parasite count was inversely proportional to the percentage inhibition as the extract concentration increases. Notably at lower extract concentrations of 0.875mg/ml, *Cassia sieberiana* was more effective with 94.6% mortality recorded compared with *C. procera* and *A. indica* that had 71.8% and 66.7% respectively ($p<0.05$). In conclusion, these extracts possess phytoactive properties and have trypanocidal activity on *T. brucei In vitro*.

Keywords: Phytochemistry, *In vitro* Trypanocidal Efficacy, *Cassia sieberiana, Calotropis procera, Azadirachta indica.*

INTRODUCTION

Calotropis procera (Asclepiadaceae) is native to tropical Africa, south and western Asia and Indo-China. In Nigeria, the whole plant or in combination with other herbs have been used to treat diseases such as, fever, cold, eczema, diarrhoea, asthma, cough, leprosy, rheumatism, jaundice, dysentery and elephantiasis (Rai *et al.*, 2000; Joshua, 2006; Jain *et al.*, 2007; Jan *et al.*, 2009; Kumar, 2009). Elsewhere it has been reported to treat headache, malaria and

convulsion (Prasad, 1985; Shah *et al.*, 2006), while its latex is used to neutralize scorpion and snake bites and to cure wounds (Smith *et al.*, 1995).

Cassia sieberiana is a savannah tree belonging to the family Caesalpiniaceae, and is distributed throughout West Africa (Gledhill, 1991). The whole plant or its leaves, roots and pods are widely used in traditional medicine as purgatives and in diabetic remedies. Decoctions are used to treat malaria and an infusion of the leaves sweetened with honey is taken against stomach ache, ulcers and diarrhoea, the twigs are used to treat sleeping sickness, while infusions of the root bark are used to treat haemorrhoids, bilharziasis, leprosy, dropsy and bloody dysentery, venereal diseases, sterility and dysmenorrhoea (Madusolumuo *et al.*, 1999).

Azadirachta indica of the family Meliaceae is a tree commonly found in the African Sahara which has been reported to remedy malaria, arthritis, rheumatism and skin conditions (Ganguli, 2002; Kausik *et al.*, 2002).

Problems have been associated with identifying plants to establish their claimed therapeutic actions, identifying and extracting their active principles, and inspite of their increasing demand in traditional remedies, only a small percentage have been scientifically evaluated in Nigeria (Gyang, 2001; Atawodi *et al.*, 2003). Hence, this study was done to evaluate the phytochemical constituents and *In vitro* trypanocidal activity of *Calotropis procera, Cassia sieberiana* and *Azadirachta indica* plant species commonly found in this study area.

MATERIALS AND METHODS

Plant Collection and Extraction: Fresh matured leaves of *Calotropis procera, Cassia sieberiana,* and *Azadirachta indica* were obtained from University of Maiduguri Campus, and were identified and authenticated by a botanist from the Department of Biological Sciences University of Maiduguri, Maiduguri, Nigeria. The leaves were rinsed in clean tap water to remove dirt, air dried under shade, and ground into coarse powder using wooden pestle and mortar, and sieved using a wire mesh to remove debris. About 76 grams of each plant fine powder was obtained and soaked in 100mls of distilled water, shaken for 1 hour and left on a desk top for 48 hours. The mixture was filtered and the filtrate concentrated using rotary evaporator, and the concentrate evaporated

to dryness in an oven at 60^{0}C overnight and stored at room temperature (± 27^{0}C) until used.

Phytochemical Screening: The aqueous extracts of the three plant species were subjected to qualitative phytochemistry to determine their bioactive components using standard procedures as described by Brain and Turner, (1975); Farnsworth, (1989); Wall *et al.*, (1992); Sofowara, (1993); Silva, *et al.*, (1998) and Trease and Evans, (2002).

***Trypanosoma brucei* Stock**: *T. brucei* was obtained from stabilates (donor rats) maintained at the Nigerian Institute for Trypanosomiasis Research (NITR), Vom, Plateau State, Nigeria. The parasites were regulated through continuous passage in albino rats maintained at the Parasitology Laboratory of the Faculty of Veterinary Medicine, University of Maiduguri, Nigeria until when required. Passage was done at parasitaemia levels of 16-32 parasites/ field obtained at 3-5 days post inoculation. For each passage 1ml containing 4x10^6 parasites diluted with 0.2ml phosphate buffered saline solution was inoculated intraperitoneally into albino rats.

Parasitaemia levels were determined by sterilizing (with methylated spirit) the tail of infected albino rats and collecting blood which was examined (using wet mounts) microscopically at x400 of the Olympus light microscope and the rapid matching technique of Herbert and Lumsden (1976) was used to obtain the absolute number of trypanosomes per millilitre of blood.

***In vitro* Testing**: A serial dilution of the three extracts into graded concentration of 0.875, 1.75 and 2.5mg/ml in phosphate buffered saline solution was prepared into test tubes replicated 8 times for each concentration of each extract tested. Two drops of infected blood and glucose D® (Glaxo Nigeria Ltd) was added to each test tube which were then incubated at 37^{0}C in water bath for a period of 1 hour. For reference tests, 2 control groups comprising of a negative control with test tubes inoculated with 2 drops of infected blood in 1ml of phosphate buffered saline (PBS) solution and a positive control group (3 test tubes) with 2 drops of infected blood in 1ml of PBS and added glucose D® and treated with 0.875, 1.75 and 2.5mg/ml of diminazene aceturate (3.5mg/kg), a commercially prepared trypanocidal drug.

All tests were allowed to stand for a period of 1 hour in a water bath at 37^0C after which the improved Neubauers chamber was used to count the number of *T. brucei* per field under the Olympus light microscope at x400.

Trypanocidal activity was evaluated by observing cessation of motility of the parasite (Atawodi *et al.*, 2003). It is to be noted that under *In vitro* tests, *T. brucei* in the negative control could survive for up to 4 hours. Percentage mortality/inhibition of motility was calculated from:

$$100 \times \frac{Parasite\ Count\ of\ \ Count - Parasite\ Count\ of\ Treated}{Parasite\ Count\ of\ the\ Control}$$

Statistical Analysis: Data were expressed as mean ± standard deviation (SD) and subjected to a paired "t" test with a p values equal to or less than 0.05 regarded as statistically significant (GraphPad, 2003).

RESULTS

Table 1 shows the phytoactive components of the aqueous extracts of *C. procera, Cs. sieberiana* and *A. indica* leaves. Saponins had high scores (+++) for *C. procera* and *A. indica.* Alkaloids, tannins, glycosides, flavonoids, reducing sugars and pentoses had low to moderate concentrations in all the 3 extracts, while terpenes and general carbohydrates were of high concentrations in *Cs. sieberiana,* in which ketones were also present only.

Table 2 shows the comparative efficacy of graded concentrations of the 3 extracts. In all there was a 100% mortality of *T. brucei* at concentrations of 2.5mg/ml. However, the mean percentage inhibition of *T. brucei* was positively correlated to an increase in the concentration of all the 3 extracts treated.

Table 1: Phytochemical Components of Aqueous Extracts of *Calotropis procera, Cassia sieberiana* and *Azadirachta indica* Leaves

Active Components	Tests	Scoring		
		C. procera	*Cs. Sieberiana*	*A. indica*
Alkaloids	Dragendorff's	+	+	+
Saponins	Frothing	+++	–	+++
Tannins	Ferric chloride	+	+	++
Glycosides	Salkowski's	+	+	++
Combined anthraquinones	Borntrager's + sulphuric acid	–	–	–
Anthraquinone derivatives	Borntrager's	–	–	–
Terpenes	Lieberman-Buchard	++	+++	+
Flavonoids	Pew's	++	++	+
Reducing sugars	Fehling's	+	++	+
Ketones	Standard	–	++	–
Pentoses	Standard	+	+	+
Monosaccharides	Barfoed's	+	–	–
General carbohydrates	Molisch's	++	+++	+

Key:

- = Absent
+ = Low Concentration
++ = Moderate Concentration
+++ = High Concentration

Table 2: Comparative Efficacy of Graded Concentrations of Aqueous Extracts of *Calotropis procera, Cassia sieberiana* and *Azadirachta indica* Leaves Against *T. brucei* following One Hour Incubation Period

Concentration of Extracts (mg/ml)	Parasite Count (x10^6/mm^3)	% Inhibition
***Calotropis procera**:*		
2.5	00 ± 00	100
1.75	00 ± 00	100
0.875	0.46 ± 0.36 (0.25-0.87)	77.9 ± 16.4 (57-98)
Control	2.15 ± 0.07 (2.05-2.25)	00 ± 00
***Cassia sieberiana**:*		
2.5	00 ± 00	100
1.75	00 ± 00	100
0.875	0.61 ± 0.75 (0.3-1.65)	94.6 ± 20.4 (95-98)
Control	1.9 ± 0.07 (1.75-1.95)	00 ± 00
***Azadirachta indica**:*		
2.5	00 ± 00	100
1.75	0.6 ± 0.37 (0.3-1.1)	84.4 ± 24.9 (83-86)
0.875	1.24 ± 0.36 (0.75-1.75)	66.7 ± 17.8 (67-72)
Control	2.3 ± 0.07 (2.05-2.25)	00 ± 00

DISCUSSION

The phytochemistry for active bio-components in this study has revealed the presence of saponins, terpenes, flavonoids, alkaloids, tannins and glycosides in the 3 plants except saponins which was absent in *Cassia sieberiana.* It has been

reported that phytochemicals confer pharmacological relevance on plants, and that scientific evaluation of medicinal plants is important to the discovery of novel drugs and helps to assess toxicity risks associated with the use of either herbal preparations or conventional drugs of plant origin (Toma *et al.*, 2009; Ullah *et al.*, 2011).

Flavonoids have been referred to as "nature's biological response modifiers" due to their inherent ability to modify the body's reaction to pathogens, allergens and carcinogens (Onyeama *et al.*, 2012).

Tannins are diverse organic compounds producing physiological astringent properties that hasten wound healing, ameliorates inflamed mucus membrane and has haemostatic properties and alkaloids are reported to have analgesic, anti-inflammatory and adaptogenic activities which help to alleviate pain, develop resistance against disease and endurance against stress, and have anti-hypertensive action, glycosides provide physiological action, and cardiac glycosides are choice drugs for congestive heart failure, and they have laxative, diuretic and antiseptic properties, while saponins are active immune boosters and terpenes have shown activity against several protozoal diseases (Atawodi, 2005; Ojo *et al.*, 2012; Sandabe *et al.*, 2006).

The *In vitro* trypanocidal efficacy trial has shown that the concentration of 2.5mg/ml of aqueous extracts of the leaves of *Calotropis procera, Cassia sieberiana* and *Azadirachta indica* demonstrated 100% mortality against *T. brucei,* and there was a positive correlation between parasite counts and all the extract concentration levels (i.e. 0.875; 1.75 and 2.5mg/ml). This finding agrees with the reports of Atawodi *et al.*, (2003) that plants of different families could possess potent trypanocidal activity and that extracts showing potent trypanocidal activity have been reported to contain alkaloids, flavonoids, phenolics and/or terpenes. Also several medicinal plants of Nigerian, Tanzanian and Ugandan origin have shown trypanocidal activity (Freiburghaus *et al.*, 1998; Atawodi *et al.*, 2002; 2003; Wurochekke and Nok, 2004; Alli *et al.*, 2011).

Plants bioactive products interfere with the redox balance of haemoprotozoans, while others impede glucose catabolism and polymerase synthesis, and inhibit ornithine decarboxylase, or through interactions with the kinetoplast DNA of trypanosomes (Al-Bayati and Al-Mola, 2008; Ogunleye and Ibitoye, 2003; Musa *et al.*, 2008; Alli *et al.*, 2011).

In conclusion, this study has revealed bioactive components and trypanocidal efficacy of *Calotropis procera, Cassia sieberiana* and *Azadirachta indica* and it has been reported that among the advantages of phyto-therapeutics that currently justify their use are the synergistic effects of its compounds, the combination of mechanisms for substances acting on different molecular targets, the lower risk of side effects and lower costs in research (Paulo *et al.*, 2001; Yunes *et al.*, 2001; Politi *et al.*, 2012).

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