IN VITRO ANTI-TRYPANOSOMAL ACTIVITY OF *ANACARDIUM OCCIDENTALE* AND *CYMBOPOGON CITRATES* AGAINST TRYPANOSOMA BRUCEI IN MICE

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ABSTRACT

The anti-trypanosomal effect of ethanolic extracts of Anacardium occidentale and Cymbopogon citrates was examined in vitro for their trypanocidal activity using Trypanosoma brucei as the test trypanosome at effective concentrations of 2mg, 10mg, and 20mg/ml. Complete elimination of motility or reduction of motility of the parasites when compared to control was observed as indices of trypanocidal effects. Extract of Anacardium occidentale was slightly more active than that of Cymbopogon citrates. The result suggested that these two plant extracts possessed some trypanocidal activities, which may require further investigation. The presence of phytochemical constituents such as Alkaloid, Glycosides, Saponins, tannin, and steroids could account for the observed activities and potency of some of the active extracts against trypanosomes.

Keywords: Anti-trypanosomal, Anacardium occidentale, Cymbopogon citrates.

INTRODUCTION

Trypanosomosis is a haemo-parasitic disease in mammals caused by protozoan belonging to the genus Trypanosoma. These parasites can multiply in the bloodstream, lymphatic vessels, and tissues, including the cardiac muscle and central nervous system. They have *Glossina* as their common vector of all the livestock disease endemic to the African continent. Trypanosomosis has been seen as the single dominant disease which limits the number and productivities of cattle, sheep, and goat.The metacyclic form that develops in the vector salivary glands may be inoculated by the tsetse fly with its saliva into a mammalian host (Franco *et al*., 2014).

The economic importance of this disease calls for its control in Africa, which relies on vector control, chemotherapy, and chemoprophylaxis. This is related to the problem of the cost of the chemical and cumulative toxicity of chemical sprays on both plants and animals. Besides being expensive and toxic, they require lengthy parenteral administration. The disease evolves into the successive phases that characterize its clinical phases (Sternberg, 2004; Kennedy, 2013).

Both, human and animal trypanosomiases negatively affect the whole economy of Africa by weakening both human and animals health (John *et al*., 2012). The root bark extract of Terminalia superba inhibited the growth of trypanosomes in both rats and mice while the root bark extracts of Afzelia Africana and Khaya senegalensis resulted in parasite clearance in rats only (Antia *et al*., 2009).

In mammals, various species of Trypanosoma can cause disease including the following; *T. avium* found in birds, *Trypanosoma brucei (T. brucei),* which causes sleeping sickness in humans and nagana in cattle, *T. equinum* in horses, *T. lives* in rats and *T. melophagium* in sheep. The *Trypanosoma brucei* species comprise three subspecies*; T. b.*

brucei, T .b. gambiense and *T.b. rhodiesciense*, whereby the latter two cause diseases in humans

Therefore, there is need to seek new chemotherapeutic and chemoprophylactic agents for combating trypanosomes. Herbal remedies would provide cheaper alternatives to many kinds of expensive western drugs, but experimental trials must be carried out to ascertain their efficacy, safety, and economic risks, before widely promoting them.

Hence, this study is to determine the phytochemical compounds of *Anacardium occidentale* and *Cymbopogon citrates.* To determine the anti-trypanosomal activity of *Anacardium occidentale* and *Cymbopogon citrates* in vitro against *T. brucei and* to compare the efficacy of *Anacardium occidentale* and *Cymbopogon citrates*.

JUSTIFICATIONS FOR STUDIES

According to Tabuti *et al.* (2003) and others, studies on Ethnoveterinary medicine can be acceptable for three main reasons.

1. They can produce useful information that can be used to develop livestock healing practices and methods that are appropriate to the rural environment.
2. Ethnoveterinary medicine can contribute to biodiversity conservation.
3. EVM could be an input veterinary resource and could add useful new drugs to the pharmacopeia.

MATERIALS AND METHOD

Plant collection and authentication***:*** the stem-bark Collection of *Anacardium occidentale* and *Cymbopogon citrates* leaves were obtained fresh from the wild in Akure, Akure South Local Government Area of Ondo State, and authenticated at the Forest Department of the Federal University of Technology Akure.

Extraction

The plants material was pulverized and grounded into powder form for easy extraction of the extract. The dry appearance of the herbs was measured and150g was saturated in a container containing 450ml of ethanol for 48hours before withdrawal was made. The muslin cloth was first used as a sieve to remove the coarse particles. The filtrate was moved over a rotary evaporator; the paste-like material was transfer into Petri-dishes for the evaporation of any residue and alcohol. The filtrate is then collected together and dried. Both dried filtrate and residue are collected into different tubes and kept in a refrigerator at freeze temperature at the Department of animal production and health laboratory of the Federal University of Technology Akure until needed for the experiment.

Phytochemical evaluation

The Phytochemical evaluation was carried out to determine the primary constituent in medicinal plant sample in the case of *Anacardium occidentale* and *Cymbopogon citrates.*

- Test for Alkaloids: 0.5g of each extract was dissolved with 5ml of one percent aqueous hydrochloric acid on a steam bath, 1ml of the filtrate was mixed with dragendorff's reagent by Merch Schuchardt OHG. Turbidity or precipitation with the reagent was seen as evidence for the alkaloid presence in the extracts.

- Test for saponins: 0.5g of each plant extract was stunned with distill-water in a clean test tube. Frothing which persists on warming was observed as evidence for the presence of saponins.
- Test for tannins: 0.5g of each plant extract was titrated with 10ml of distilled water, ferric chloride reagent added to the filtrate. A blue-black, blue-green or brownish precipitation was evident for the presence of Tannins (Trease and Evans, 1985).
- Test for phlorotannins: Deposition of red precipitate when 0.5g of an aqueous extract of plant sample was heated with 1% aqueous hydrochloric acid was shown as evidence for the presence of phlorotannins.
- Test for Anthraquinone: About 5g of each plant was shaken with 10ml benzene, filtered and 5ml of 10 percent ammonia solution added to the filtrate. The mixture was shaken, and the presence of a pink, red or violet color in the ammonical phase indicates the presence of anthraquinone.
- Keller – Killian Test: 0.5g of the extract was dissolved in 2ml of glacial acetic acid and containing one drop of ferric chloride solution. The result was then mixed with 1ml of 10% concentrated H_2SO_4. A brown ring obtained at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may be seen below the brown ring also in the ache and layer a greenish ring may form just above the brown loop and gradually spread throughout the coat.

Preparation of the crude plant extracts

The resultant concentrated crude extracts were measured into tube and 10mg was dissolved in 500ul (0.5ml) of 10% Dimethylsulfoxide (DMSO) in Phosphate Buffered Saline (PBS) to produce extract solutions of 20.0mg/ml (stock). Two other extract concentrations (10.0mg/ml and 2mg/ml) were prepared from the stock extract solution by appropriate dilution with PBS.

Test organism

The species of trypanosome that was inoculated was *Trypanosoma brucei brucei* which was collected from the National Institute of Trypanosomosis Research, VOM, Jos, Plateau State, Nigeria. This parasite was maintained in the mice house by continuous passaging of the parasite in mice and albino rats as a bank.

Experimental animals

Mice were the experimental animals used. The experiment lasted for two weeks; while diminazene aceturate Diminal® was the standard drug used.

Housing and feeding

Plastic cage with wire mesh was worn in the animal house. The pen was closed with wood chip, and the shaving was distorted weekly. Three different indicators of blue, red and black were taken in the identification of the mice being labeled as a test animal for each extract. The weight of each mouse was collected. Feeding was done once in a day using pelleted grower mash along with water usually in the morning.

Determination of parasitemia

Blood was collected from the passaged mice, and parasitemia levels were determined by examining the smear of blood taken via tail-cut under a microscope using the method of Murray *et al*., (1983). Briefly, the technique involves microscopic counting of parasites per field in uncontaminated blood or blood diluted with phosphate buffer saline (PBS) to give a log of 6.9m before it was injected intraperitoneally to the experimental animals.

***In vitro* test for trypanocidal activity**

The assessment of *in vitro* activity was performed in triplicate in ninety-six (96) well plate or microtitre plates. 40µl of blood containing log 8.4 parasites per field obtained as described under "Parasitemia determination" was mixed with 10µl of extract solution of 10mg/ml and 20mg/ml respectively, 10% DMSO was used as phosphate buffer solution (PBS) to ensure thorough solubility of the extract and a standard drug Diminal® was also used as control. These were monitored continuously at 5 minutes interval.

After 5 minutes of incubation in closed tubes maintained at 37^0C, about 2µl of test mixture were positioned on separate microscope slides covered with slide cover, and the parasites were monitored/observed every 5 minutes for a total duration of 60 minutes. The blood sample containing parasite when mixed thoroughly with prepared extract and PBS was observed for motility of the parasites, the parasite develops into dead (non-motile) after 30 minutes for *Anacardium occidentale* and after 45 minutes with *Cymbopogon citrates.*

RESULTS

Phytochemical

The phytochemical studies carried out on the plants extract, the result for the *Anacardium occidentale* showed (Table 1) the presence of tannin, phlorotannins, glycoside, and flavonoid but saponin was absent.

While *Cymbopogon citrates* showed the presence of tannin, phlorotannins, glycoside, saponin, and flavonoid.

In vitro anti-trypanosomal activity of Anacardium occidentale.

The *in vitro* activity of *Anacardium occidentale* extract as an anti-trypanosomal agent on *T. brucei* showed (Figure 1) that at 20mg/ml of the concentration of the extract there was 9% motility effect on the parasites at 5 minutes. 66.3% of parasites ceased moving at 25 minutes. A total cessation of the parasite motility was seen at 30 minutes of the experimental time.

Moreover, at 10mg/ml of the concentration motility of the parasite was observed to extend a few minute beyond that experimental at 20mg/ml concentration. 2.2% inhibitory effect of the extract on motility of the parasite at 5 minutes while at 20 minutes, 88.8% motility effect on the organism as observed under a microscope and gradually the motility of the parasites decreased. Finally, the parasites ceased moving at 40 minutes.

The standard drug used (Diminal^R) on *T. brucei* showed that at 7.87mg/ml concentration drastically eliminate the entire parasite at 10 minutes. The unincubated parasites remained 100% motile even at 40 minutes of the test and motility reduced by 3% up to 60 minutes of the experimental time.

In vitro antitrypanosomal activity of Cymbopogon citrates

The *in vitro* activity of *Cymbopogon citrates* showed (Figure 2) that at 20mg/ml concentration there was a 3% drop in motility at 10 minutes, which increased to 12% at 25 minutes. About 30% decline in motility at 35 minutes and the 45 minutes, there was zero percent motility.

However, at 10mg/ml there was no significant effect on the motility until about 20minutes when there was 1.5% decline in motility and gradually increased to 15% inhibitory effect on motility at 45 minutes there was a total cessation in parasite motility at 55 minutes interval.

The standard drug (Diminal®) at the concentration of 7.87mg/ml achieved 100% cessation in parasites motility in 10 minutes. The unincubated showed no significant reduction in motility ($p>0.5$) gradually declined to 1-2% reduction in motility at 55 minutes.

TEST	*Anacardium occidentale*	*Cymbopogon citrates*
Tannin	+	+
Phlorotannins	+	+
Glycosides	+	+
Saponin	_	+
Flanovoid	+	+

+ = present, - = absent

Table 1: Plants constituent of *Anacardium occidentale* and *Cymbopogon citrates*

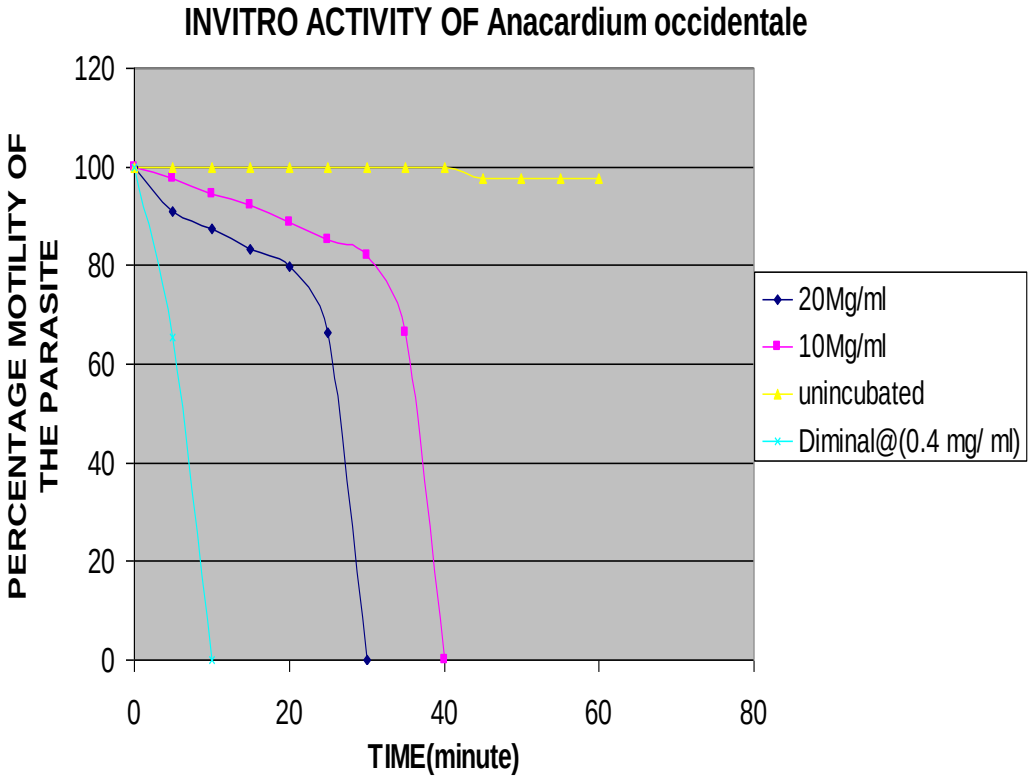

Figure 1: *In vitro* anti-trypanosomal activity of *Anacardium occidentale*

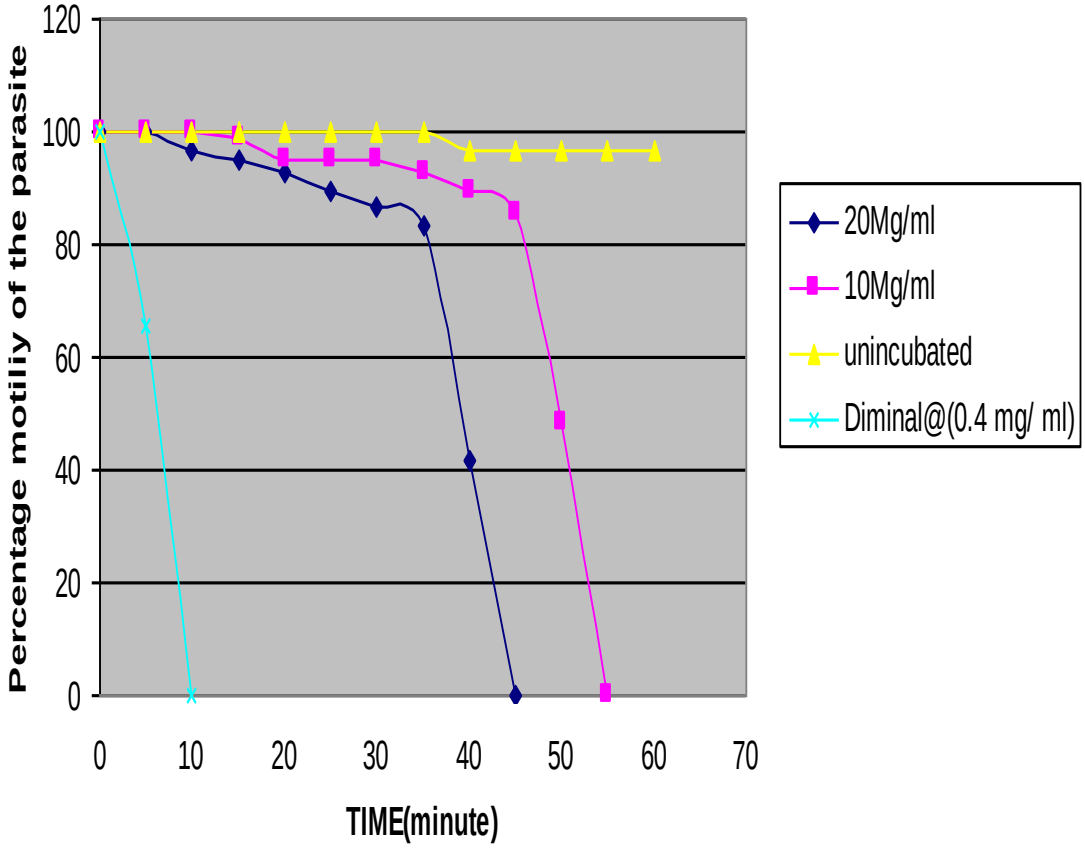

Figure 2: *In vitro* trypanosomal activity of *Cymbopogon citrates*

DISCUSSION

Recent effort on ethnopharmacology search revealed extracts showing potent trypanocidal activity to contain tannin, phlorotannins, glycoside, flavonoid, and saponin (Hoest et al., 2004). Hoest *et al.*, (2004) reported that plant extracts phytochemical constituents like tannins, saponin, and phenols show potent trypanocidal activity *in vitro* for Trypanosome brucei bloodstream forms.

However, it is not possible to compare many of our results with those of earlier reports because most plants investigated here were not previously studied for trypanocidal activity, although the use of some of the plant in the traditional management of trypanosomosis has recently been observed (Atawodi *et al.*, 2002).

Quantitative differences in activity may be due to known variation in chemical composition arising from differences in geographical location and time/season of collection. That most plants showed differential activity between extracts and between parts confirmed our earlier assertion (Atawodi *et al.*, 2003) that any statement on a plant's trypanocidal movement should be in use within the context of the plant part and the solvent extract tested.

The obtained result on *Anacardium occidentale* and *Cymbopogon citrates* revealed that they are potential anti-trypanosomal agents against *T. brucei* infection in mice in vitro.

The two concentration of test showed the inhibitory effect of the extract on the motility of the parasites compared with the infected not treated with plant extracts.

The phytochemistry and the toxicology of these extracts are also been examined with a view to establishing the possibility of developing these plant extracts into new generation of useful and safe trypanocidal agents for combating trypanosomosis, a disease that has continued to be of immense economic and health importance in many tropical countries of the world, especially in Africa (Welburn *et al*., 2001).

The phytochemical screening of *Anacardium Occidentale* and *Cymbopogon citrates* showed energetic anti-trypanosomal activity *in vitro* against *T. brucei* , which could be due to the presence of tannin and saponin (Hoest *et al*.,(2004). It has also been observed that saponin suppresses ruminal protozoa by complexing with cholesterol in their cell membrane (cheek, 2000).

CONCLUSION

The studies explain the potency of plant extracts compared with standard drugs. It is necessary to appreciate the value of plant materials as they can be made for the treatment of animal's diseases. Further advanced purification suggests that there will be an increase in the potency of the extract as compared with the standard drug to fight against definite ailment in animals. Advancement in the technology on the method of extract will make plants extract highly efficacious also the certainty of reducing the cost of production.

RECOMMENDATION

Since *Anacardium occidentale* and *Cymbopogon citrates* have shown anti-trypanosomal activity through its phytochemical screening *in vitro*, these plants should be used in the experimental treatment of trypanosome in cattle, sheep, and other animals. Further research should be done to isolate and identify the fraction that is responsible for the observed activity. The extraction method should be standardized to improve its overall effectiveness.

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