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Phytochemical Analysis and *In-vitro* Antitrypanosomal Activity of Selected Medicinal Plants in Niger State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors FMM, AYK and AM designed the study, wrote the protocol and supervised the work. Authors FMM, JNA and AOA carried out all laboratories work and performed the statistical analysis. Authors FMM and JNA managed the analyses of the study. Author FMM wrote the first draft of the manuscript. Authors AYK, AM and AA managed the literature searches and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Trypanosomiasis is a disease caused by protozoan parasite belonging to the genus *Trypanosoma.* The disease affects both humans and animals. In this study, phytochemical analysis and *in-vitro* antitrypanosomal screening of crude methanol extracts of the leaves of *Waltheria indica, Vernonia amygdalina, Albizia ferruginea, Camellia sinensis, Chamaecrista mimosoides* and *Hyptis suaveolens* were carried out using standard methods. Highly parasitized blood from infected donor rats was diluted with glucose phosphate buffered saline solution and incubated with varying concentrations (1 mg/ml, 2 mg/ml and 4 mg/ml) of the extracts in Eppendorf tubes for 60 minutes. Aliquots from the mixtures were removed and observed under microscope for parasite motility at 5 minutes interval. Phytochemical screening revealed the presence of tannins, alkaloids, flavonoids,

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phenols and glycosides in all the plants' extracts. Saponins were absent in *H. suaveolens* while steroids and phlobatannins were absence in *C. mimosoides* and *H. suaveolens.* All the plants' extracts showed significant cessation of parasite motility with increase in incubation time and concentration of the extract. Complete cessation of the parasite motility was observed for all the extracts within 60 minutes of the study. The most active extract was *W. indica* at 4mg/ml which caused complete cessation of the parasite motility within 5 minutes whereas the least active plants *C. mimosoides* and *H. suaveolens.* Both caused complete cessation of the parasite motility within *25* minutes. Berenil, the standard drug, however, caused cessation of trypanosomal motility within 5 minutes even at 1 mg/ml. These results showed that methanol l extracts of the plants leaves screened especially *Waltheria indica* and *Vernonia amygdalina* have significant *in vitro* antitrypanosomal activity and may be potential source for the *in vivo* treatment of trypanosomiasis.

Keywords: In-vitro; antitrypanosomal activity; Trypanosoma brucei brucei; Waltheria indica; Vernonia amygdalina; Albizia ferruginea; Camellia sinensis; Chamaecrista mimosoides and Hyptis suaveolens.

1. INTRODUCTION

Human African trypanosomiasis, which is commonly known as sleeping sickness, is a parasitic vector borne disease. The parasites involved are protozoans belonging to the genus *Trypanosoma*. They are transmitted to humans by tsetse fly (*Glossina genus*) bites which have acquired their infection from human beings or animals harbouring the human pathogenic parasites [1]. The disease affects mostly poor populations living in the remote rural areas of Africa. If untreated, it is usually fatal. Travellers also risk being infected if they venture through regions where the insect is prevalent. Generally, the disease is not found in urban areas, although some cases have been reported in the suburban areas of big cities in some disease endemic countries [2]. Sleeping sickness occurs only in 36 sub-Saharan Africa countries where there are tsetse flies that can transmit the disease. In 1995, WHO Expert Committee estimated that 60 million people were at risk with an estimated 300 000 new cases per year in Africa, with fewer than 30,000 cases diagnosed and treated [3]. Two species of *trypanosome brucei* infect human, these subspecies include *T. brucei gambiense* and *T. brucei rhodesiense*. *T. brucei gambiense* account for over 90% of reported cases. It causes chronic condition that can remain in passive stage for weeks, months or years before symptoms begin and the disease can last about 3 years before death results. *T. brucei rhodesiense* on the other hand result in acute form of infection and mortality can result within weeks and it's more virulent and rapid developing than *T. b gambiense* [4]. Animal's African trypanosomiasis (AAT), also results from infection from other subspecies of trypanosome such as *T. brucei brucei*, *T. congolense, T. vivax, T. cruzi*, and *T. equiperdum*. These diseases are called by different names like Nagana, Dourine, Surra all of which have negative impact on the development of agriculture in Africa. Those affecting cattle are apparently the major economic important since they are major cause of reduce meat and milk production [5]. *T. congolense* is the most important causative agent of animals' trypanosomasis in Africa though a rinder-pest pandemic of the 1890 removes many host animals resulting in the near eradication of most tsetse species. Further suppression was achieved through spraying with dichlorodiphenyl trichloroethane (DDT) [6].

The fight against the disease has relied mainly on vector control system and chemotherapies; their effectiveness and safety remains a source of concern due to the side effect of the drugs and resistance shown by the parasite to the drugs [7]. These dilemma call for the need for continued effort to identify new chemical compounds for the treatment of Human African tryponosomiasis (HAT). And this work is designed to screen some selected medicinal plants in Niger state, Nigeria. The plants include *Waltheria indica*, *Hyptis suaveolens, Albizia ferruginea, Vernonia amygdalina, Camellia sinensis* and *Chamaecrista mimosoides.* These plants have been used traditionally by the traditional medicine practitioners for the treatment of various diseases in Nigeria and many African countries. Their uses vary from one country to another.

Durawhite, for example, an extract of sleepy morning (*Waltheria indica*), is used in a commercial cosmetic for its ability to inhibit melanin synthesis and whiten the skin [8]. The plant contains steroid derivatives and alkaloids of the adouetine group that perhaps make it

physiologically active. Various extracts are used as standard febrifugal, purgative, emollient, tonic, analgesic, and astringent herbal medicines in Africa [9]. In Hawaii, the root is chewed to relieve sore throat [10]. A decoction of the leaves of *A. ferruginea* is administered as a purgative, as an analgesic and against inflammation. In Central and West Africa, this plant is used for the treatment of skin diseases, bronchitis, tapeworm, headaches and sinusitis. The extracts of *A. ferruginea* were also reported to have significant antimicrobial activity on selected microorganisms [10] and antiplasmodial activity. Lipophilic extracts of *A. gummifera* (another member of the genus *Albizia*) revealed very promising antitrypanosomal activity. Many herbalists and naturopathic doctors have recommended the aqueous extracts of *V. amygdalina* for their patients as treatment for emesis, nausea, diabetes, loss of appetite-induced ambrosia, dysentery and other gastrointestinal tract problem [11].

Theophylline in *Camellia sinensis* is used to prevent respiratory diseases like wheezing, shortness of breath, and difficulty in breathing caused by asthma, chronic bronchitis, emphysema, and other lung diseases. It relaxes and opens air passages in the lungs, making it easier to breathe [12]. *Camellia sinensis* is used as an age-old home remedy for burns, wounds and swelling.

Therefore, this work is aimed at screening plants with antitrypanosomal activity used by the inhabitants of Niger State to treat trypanosomiasis to ascertain their activity. *In vitro* antitrypanasomal studies were carried out using blood from an trypanosoma brucei brucei infected mice by checking the parasite motility.

2. MATERIALS AND METHODS

2.1 Plant Collections and Identification

Plants were collected from different bushes around Minna, Niger State, Nigeria, between the months of February and March, 2015. They were taken to the Department of Biological Sciences, Federal University of Technology, Minna, Nigeria for the authentication.

2.2 Sample Preparation and Extraction

The plants were destalked; the leaves washed with running tap water and dried under laboratory condition. The dried plant samples were milled into powdered form using electric blender. Exactly 50 g of each powdered samples were weighed and extracted with 300 ml of 70% methanol by reflux extraction for 2 hours. The extracts were dried in hot water bath at 65ºC and stored in sample bottles in the refrigerator at 4ºC until required [13].

2.3 Phytochemical Screening

Each of the plant extracts was screened qualitatively for the presence of tannins, saponins, flavonoids, steroids, terpenoids, cardiac glycosides, alkaloids, phenols and phlobatannins as described by Sofowora [14].

2.4 Test Organism

T. brucei brucei was gotten from stabilates maintained at the Nigerian Institute of Trypanosomiasis Research (NITR), Kaduna, Kaduna State, Nigeria; in the month of August, 2015. The parasites were maintained in the laboratory by serial passage in rats until required for use. Passage was carried out when parasitaemia was in the range of 16 - 32 parasites per field (usually 3 - 5 days post infection). In passaging, 1× 103 parasites in 0.1 - 0.2 ml blood/PBS were injected intraperitoneally into normal rats acclimatized under laboratory condition for two weeks.

2.5 Determination of Parasitaemia

Parasitaemia was monitored in blood gotten from the tail, pre-sterilized with methylated spirit. The number of parasites was determined microscopically at ×400 magnification using the ''Rapid Matching'' method of Herbert and Lumsden [15]. Briefly, the method involves microscopic counting of parasites per field in pure blood or blood appropriately diluted with phosphate buffered saline (PBS, pH 7.2). Logarithmic values of these counts obtained by matching with the table of Herbert and Lumsden [15] is converted to antilog to provide absolute number of trypanosomes per ml of blood.

2.6 *In vitro* Trypanocidal Activity

Assessment of *in vitro* trypanocidal activity was performed in triplicate in 96 well micro-titre plates [16]. Exactly 0.5 g of each extract was dissolved in 25 ml distilled water to obtain 20 mg/ml stock solution. Concentrations of 10 and 5 mg/ml were

also prepared giving three different concentrations for each extract. The 20, 10 and 5mg/ml concentration of each plant extract were prepared in triplicates [16,17].

Twenty microlitre (20 µl) of blood containing about 20-25 parasite/field obtained as described above, were mixed with 5µl of each extract to produce effective test concentrations of 4 mg/ml, 2 mg/ml and 1mg/ml respectively. To ensure that the effect monitored was that of the extract alone, two sets of control were set up. First Berenil (Diminazene aceturate- a standard drug) was used as positive control and blood suspended in glucose PBS was used as a second (negative) control. Berenil was prepared in the same concentration as the extracts (445 mg Diminazene aceturate + 555 mg Antipyrine, Eagle Chemical Company Ltd, Ikeja, Nigeria).

Each of the test mixtures was incubated for 5minutes in closed Eppendorf tubes maintained at 37ºC. 2 µl of test mixture was placed on separate microscope slides and covered with a cover slip and the parasites observed every 5 minutes for a total duration of 60 minutes. Cessation or drop in motility of the parasites in extract-treated blood compared to that of the parasite-loaded control blood suspended in glucose phosphate buffered saline without extract was taken as a measure of trypanocidal activity [16,17].

3. RESULTS

3.1 Extract Yield

The percentage yield of the various plants extract is shown in Table 1. *Chamaecrista mimosoides had the* highest extract yield (37.95%), followed by *Vernonia amygdalina (21%), Camellia sinensis* (18.40%), *Albizia ferruginea (16.18%), Waltheria indica* (13.68%) while *Hyptis suaveolens* had the least extract yield (13.24%).

3.2 Phytochemical Composition

Table 2 shows the phytochemical composition of crude methanol extract of the plants'leaves. The table revealed presence of tannins alkaloids flavonoids, phenols and glycosides in all the six (6) plant extracts. Saponins were absent only in *Hyptis suaveolens* while Steroids and phlobatannins were absent in *Chamaecrista mimosoides* and *Hyptis suaveolens*.

3.3 *In vitro* Antitrypanosomal Activity

Table 3 presents the effects of different concentrations of the methanol leaf extracts of the selected medicinal plants on motility of *T. brucei brucei.* All the six plants evaluated in

Table 1. The percentage (%) yield of the plants extract

Plants	Leaf powder (g)	Methanol extract (g)	Extract yield (%)
Waltheria indica	50	7	14
Vernonia amygdalina	50	11	21
Albizia ferruginea	50	8	16
Camellia sinensis	50	9	18
Chamaecrista mimosoides	50	19	38
Hyptis suaveolens	50	7	13

Table 2. Phytochemical compositions of crude methanol leaf extract of the plants extract

Phytochemicals	*Waltheria indica*	*Vernonia amygdalina*	*Albezia ferruginea*	*Camellia sinensis*	*Chamaecrista mimosoides*	*Hyptis suaveolens*
Alkaloids	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Saponins	+	+	+	+	+	-
Steroids	+	+	+	+	-	-
Phenols	+	+	+	+	+	+
Terpenoids	+	+	-	+	+	+
Tannins	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+
Phlobatannins	+	-	+	+	-	-

Note: -= absent, += present

Table 3. Effect of different concentrations of the methanol leaf extracts of the selected medicinal plants leaves on motility cessation of *T. brucei brucei*

Plants	Family	Part used	Time (Min)		
			1 mg/ml	2 mg/ml	4 mg/ml
Waltheria indica	Sterculiaceae	Leaf	50	20	5
Vernonia amygdalina	Asteraceae	Leaf	45	30	10
Albizia ferruginea	Fabaceae	Leaf	35	40	15
Camellia sinensis	Theaceae	Leaf	35	25	15
Chamaecrista mimosoides	Fabaceae	Leaf	45	30	25
Hyptis suaveolens	Lamiaceae	Leaf	45	35	25
Diminazene aceturate	Standard		5	5	5

this study at concentrations of 1- 4 mg/ml exhibited complete cessation of parasite motility within 60 minutes.

4. DISCUSSION

Plants used in treatment of diseases are said to contain active compounds called phytochemicals some of which are responsible for the characteristic odours, purgensies and colour of plants while others give a particular plant its culinary medicinal or poisonous virtues [18]. There is considerable interest by phytochemists to identify the therapeutic agent contained in these plants in order to establish the basis for their uses in traditional medical practice. This study revealed the presence of various important medicinal phytochemicals in methanol extracts of *Waltheria indica, Vernonia amygdalina, Albizia ferruginea, Camellia sinensis, Chamaecrista mimosoides* and *Hyptis suaveolens* leaves*.* Flavonoids are the most diversified groups of phenolic compound found in plant. It biological activities include antibacterial, anti-inflammatory, anti-allergic, anti-ulcers, viruses and antitumor effect [19]. Alkaloids are the most efficient therapeutically significant plant substances. Pure isolated alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic, antispasmodic and antibacterial effects [20], Alkaloids has been found to have microbiocidal effect and the major anti-diarrheal effect is probably due to their effects on small intestine and also exhibited antihypertensive antifungal, antiinflammatory, antifibrogenic effect [21]. Tannins can be toxic to filamentous fungi, yeast and bacterial [22].

Saponins have been reported to have anti-inflammatory, cardiac depressant and hyper-cholesterolemic effects [23]. Saponins also appear to kill or inhibit cancer cells without killing the normal cells in the process [24]. Steroids are also of importance and interest in pharmacy due to their relationship with sex hormones [25] and promote immune function in the skin and also reduce inflammation [26]. The presence of all these important phytochemicals in methanol extracts of these plants is an indication that these plants if properly screened could yield drugs of pharmaceutical significance. However the absence of steroids and phlobatannins in *Chamaecrista mimosoides* and *Hyptis suaveolens* agree with earlier studies which also found that not all phytochemicals are present in all plant and those that present differs according to the solvent used in the extraction process [26].

Cessation of parasite motility is a reliable index for determining anti-trypanosomal activity of crude plant extracts *in vitro*. Following extensive review, Bashir et al. [27] documented, 18 West African plants that inhibit *T. brucei brucei* motililty in less than 30 minute at 4 mg/ml. Twenty three (23) West African plants inhibit the parasite motility within 31-60 minutes at 4 mg/ml [27].

In the present study, the highest activity of the plants was observed at 4 mg/ml where complete cessation of the parasite motility was observed in 5 minutes *(Waltheria indica)* which confirms the findings of Bala et al. [16]. 10 minutes *(Vernonia amygdalina),* 15 minutes *(Albizia ferruginea* and *Camellia sinensis*) and 25 minutes (*Chamaecrista mimosoides* and *Hyptis suaveolens*). The *in-vitro* antitrypanosomal effect demonstrated by the plants investigated in this study, especially *Waltheria indica* and *Vernonia amygdalina is* comparably higher than activities reported for most of other African plants [27]. The results also showed that after 5 minutes incubation in Eppendorf tubes maintained at 37ºC, the parasites survived for about 2 hours without extract which is in conformity with the observations of [17].

Moreover, a plant with high *in vitro* trypanocidal activity may have no *in vivo* activity and vice versa. This is because there are peculiarities in the metabolic disposition of the chemical

constituents of plants [16]. Therefore plants found to be active in this study must be tested *in vivo* before a definite statement can be made on their trypanocidal potentials [28].

The *in vitro* trypanocidal effects demonstrated by the plant extracts in this study, could be credited to their Phytoconstituents such as flavonoids, alkaloids, terpenes, quinones, polyphenols, Triterpenoids and sterols which are the most frequently implicated phytochemicals in the plants with trypanosome activities [27].

Furthermore, it is difficult to speculate the mechanism by which the plants extracts' exhibit their trypanocidal action. However, previous study suggest that many natural products exhibit their trypanocidal activity by virtue of their interference with the redox balance of the parasites acting either on the respiratory chain or on the cellular defences against oxidative stress [28]. This is because natural products possess structures capable of generating radicals that may cause peroxidative damage to trypanothione reductase that is very sensitive to alterations in redox balance [16]. It is also proposed that iron chelation is an effective way of killing trypanosomes and the prime target is the enzyme ribonucleotide reductase whose activity is central to DNA synthesis prior to cell division as depicted in trypanosomiasis infection [27].

5. CONCLUSION

From the research work, it could be inferred that the screened methanol leaf plants extract possessed trypanocidal activities, These results showed that methanol leaf extracts of the plants leaves screened especially *Waltheria indica* and *Vernonia amygdalina* have significant *in vitro* antitrypanosomal activity and may serves as a potential source for the *in vivo* treatment of trypanosomiasis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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