British Journal of Pharmaceutical Research
4(6): 751-758, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

In vitro and *In vivo* Antitrypanosomal Effects of Petroleum Ether, Chloroform and Methanol Extracts of *Artemisia maritima* Linn

A. C. Ene[1*], S. E. Atawodi[2] and Y. E. O. Apeh[2]

[1]Department of Biochemistry, Federal University of Technology, Owerri, Nigeria.
[2]Department of Biochemistry, Ahmadu Bello University Zaria, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors ACE and SEA respectively designed the study. Author SEA managed the analyses of the study. Authors ACE and YEOA did all the laboratory work. Author ACE performed the statistical analysis, wrote the protocol, managed the literature searches and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Original Research Article

Received 30th October 2013
Accepted 18th January 2014
Published 1st February 2014

ABSTRACT

Petroleum ether, chloroform and methanol extracts of the whole plant of *Artemisia maritima* Linn were studied *in vitro* and *in vivo* for antitrypanosomal activity against *Trypanosoma brucei brucei* in Swiss albino mice. The extracts were also screened for phytochemicals/secondary metabolites. All the extracts showed trypanocidal activity against *T. brucei brucei in vitro* with the petroleum ether extract showing the highest activity. The *in vivo* study revealed that only the chloroform extract *A. maritima* exhibited antitrypanosomal activity. This extract at a dose of 100mg/kg body weight significantly ($p<0.05$) reduced the parasitemia in *T. brucei brucei* infected mice when compared with the other treatment groups. The chloroform extract of *A. maritima* at this dose reduced the level of parasitemia to 26%. This reduction in the level of parasitemia is statistically significant ($p<0.05$) compared to the other treatment groups and the untreated control group. The result of the phytochemical analysis revealed that the extracts contain secondary metabolites like flavonoids, terpenoids, steroids, anthraquinones and alkaloids. The presence of these secondary metabolites in this plant might be responsible for the antitrypanosomal activity exhibited by its extracts.

**Corresponding author: Email: chineduene@gmail.com;*

Keywords: Artemisia maritima, Antitrypanosomal effects, Trypanosoma brucei brucei, In vitro and in vivo studies.

1. INTRODUCTION

Trypanosomiasis is one of the major obstacles to livestock production in Africa. Trypanosomes which are the causative agents of trypanosomiasis are flagellated haemoparasites that are widely distributed in the animal kingdom. They are the causative agent of serious diseases of man and animal [1]. Trypanosomiasis has reemerged over the last few decades as a problem of human health and economic development [1].

Human African trypanosomiasis which is also known as sleeping sickness caused by *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense* are major causes of mortality and morbidity in Sub-Saharan Africa. African trypanosomiasis occurs in thirty six (36) African countries and about sixty (60) million people are affected. *Trypanosoma cruzi* is the causative agent of American trypanosomiasis, which is a major endemic disease in Latin America. The number of people affected are estimated to be from sixteen (16) million to eighteen (18) million, with a further one hundred (100) million considered to be at a risk [2]. Trypanosomiasis of domestic animals, called nagana cause the death of about three (3) million cattle a year, and surra is mainly a disease of the equines and camels. Trypanosomiasis has played an important role in the chronic lack of food proteins in tropical Africa. Indeed, the World Health Organization in listing major problems facing mankind, places trypanosomiasis high on the list with malaria, filariasis and leishmaniasis [2,3].

Trypanosomiasis eradication and control is principally based on chemotherapy and chemoprophylaxis. Problems faced are limited and expensive drugs, toxicity due to long period of treatment and drug resistance [1,3]. Several reports on the evaluation of different chemicals/drugs for trypanocical activity have appeared [4,5], just as are interesting reports on the antitrypanosomal effects of plant extracts and plant derivatives [6,7,8,9,10]. Some of these reports have indeed shown that, under *in vitro* conditions, many of these plants possess trypanocidal activity [6,7,8]. The result of Freiburghaus [7] has clearly indicated that different solvent extracts of the same plant may exhibit different trypanocidal activity just as extracts of different parts of the same plant.

Herbal preparations for the treatment of several diseases still hold a strong position in rural areas. In Northern Nigeria where trypanosomiasis is prevalent, traditional healers use medicinal plant either alone or in combination to treat both human and animal trypanosomiasis. Because of the limitations of the present drugs, searching for active substances of natural origin is necessary. More so, several semi-synthetic and synthetic drug derivatives were originally isolated from natural compounds [11,12].

Recently, Atawodi et al. [13] reported on plants claimed to be useful in the treatment of African trypanosomiasis in North-Central Nigeria. They screened for the *in vitro* antitrypanosomal effects of many indigenous plants in that area as reported[13]. As a follow up to that work, we present this review on the *in vitro* and *in vivo* assessment of petroleum ether, chloroform and methanol extracts of the whole plant of *Artemisia maritima* for their antitrypanosomal activity using *Trypanosoma brucei brucei*.

2. MATERIALS AND METHODS

2.1 Plant Collection and Sample Preparation

The plant *Artemisia maritima* was collected in Zaria and identified by a Taxanomist at the Herbarium Section of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. The Voucher number of this plant is 861. The whole plants of *Artemisia maritima* were air dried for two weeks in the laboratory and ground into powder form before being extracted with petroleum ether, chloroform and methanol in the order of increasing polarity.

2.2 Extraction Procedure

The powdered form of the whole plants of *Artemisia maritima* (20g) was weighed into a paper thimble and placed in a Soxhlet extractor. Extraction was carried out using 250 ml of each of the solvent in the order of increasing polarity. The extract was dried at room temperature ant last traces of solvent were removed by heating the extract in the water bath at 45ºC for an hour. The extracts were later stored in the refrigerator at 4ºC until required.

2.3 Test Organism

Trypanosoma brucei brucei used for this study was obtained from the Department of Parasitology and Entomolgy, Ahmadu Bello University, Zaria, Nigeria. The parasite was maintained in the laboratory by continuous passage in rats and mice until required. In passaging, approximately $1x10^3$ parasites were introduced intraperitoneally into rats or mice in 0.1 to 0.2 ml blood/Phosphate Buffered Saline (PBS) solution. For several passages, approximately 80% infected blood was obtained by cardiac puncture into 1 ml syringe containing 0.1 ml EDTA. This heparinized (because the blood cannot clot) *T. brucei brucei* infected blood was diluted with PBS and injected as described above into clean mice or rats acclimatized under laboratory conditions for one week.

2.4 Determination of Parasitemia

Parasitemia was monitored in the blood obtained from the tail of the mice. The number of parasites was determined microscopically at x400 magnification using the “Rapid Matching” method of Herbert and Lumsden [14]. The method involves putting drops of blood on a glass slide and covered with a cover slip, then viewed under the microscope at x400 to obtain the number of parasites per field. The number of trypanosomes per microscopic field was then compared with the table of logarithmic values. Logarithm values of these counts obtained was converted to antilog to provide absolute number of trypanosomes per ml of blood.

2.5 In vitro Test of Extracts for Trypanocidal Activity

Assessment of petroleum ether, chloroform and methanol extracts of *Artemisia maritima* for *in vitro* trypanocidal activity was performed in triplicates in a 96 well micro titre plate. The extracts were dissolved in 0.3% Tween 80 PBS solution. The extracts doses of 20.0 10.0 and 2.0 mg/ml were prepared by dissolving 20.0, 10.0 and 2.0 mg of the extracts respectively in 1 ml of PBS. Twenty micro litre (20µl) of infected blood was mixed with 5µl of extract solution of 20, 10 and 2 mg/ml to produce effective test concentrations of 4, 2 and 0.4 mg/ml respectively. A set of control which contained the parasites suspended in 0.3% Tween

80 only was included. For reference, tests were also performed with the same concentrations of diaminal which is a commercial trypanocidal drug.

The test mixtures above were incubated in a closed 96 well micro titre plate for 5min at 37ºC. About 2µl of the test mixtures were placed on separate microscope slides and covered with cover slips and observed every 5min for a total duration of 60min. Cessation or drop in motility of the trypanosomes was used as a measure of antitrypanosomal effect of the plant extracts and the standard drug under *in vitro* condition.

2.6 In vivo Evaluation of Petroleum Ether, Chloroform and Methanol Extracts of *Artemisia maritima* for Antitrypanosomal Activity

Thirty (30) male Swiss albino mice were used for this study. The mice were divided into five (5) groups. Six (6) mice were used for each of the test/treatment groups, diaminal standard control and untreated control groups. All the mice used for this experiment were infected with *T. brucei brucei* as earlier described. Group 1 animals which served as the untreated negative control were administered 0.3% Tween 80 PBS solution. Group 2 animals served as the standard control were treated with 3.5mg/kg of diminazine aceturate. All the animals in groups 3, 4 and 5 were treated with 100mg/kg each of the three extracts. The dose level of 100mg/kg of the extracts was selected from a pilot study carried out in mice and earlier studies [15,16]. The extracts were dissolved to the suitable dose level in solution and suspension, the later requiring total dissolution in 0.3% Tween 80 PBS solution. Treatments were performed daily for seven consecutive days starting 48hr after infection, with each treatment group receiving a total of seven intraperitoneal doses. The parasitemia was monitored in all the groups for 14 days as earlier described [14].

2.7 Statistical Analysis

All the results generated were analyzed using Analysis of Variance (ANOVA).

3. RESULTS

The three extracts of *Artemisia maritima* (ie petroleum, chloroform and methanol) exhibited *in vitro* antitrypanosomal activity, with the petroleum ether extract exhibiting the highest activity (Table 1). Indices of antitrypanosomal effect were described by the complete elimination of motility or reduction in motility of *T. brucei brucei* parasites. The petroleum ether extract of *A. maritima* caused complete cessation of *T. brucei brucei* motility in 2 mg/ml drug concentration in 20 min compared to the complete cessation of motility of the parasites by the chloroform and methanol extracts in 25 and 50 min respectively at the same drug concentration (Table 1).

Diaminal eliminated trypanosomal motility within 25 min even at the lowest concentration tested (0.4 mg/ml). The effect was such that after 10 – 20 min incubation, no motility was visible with drug/extract concentrations of 4 and 2 mg/ml (Table 1). The trypanosomal motility persisted in the untreated control even after 60min (Table 1).

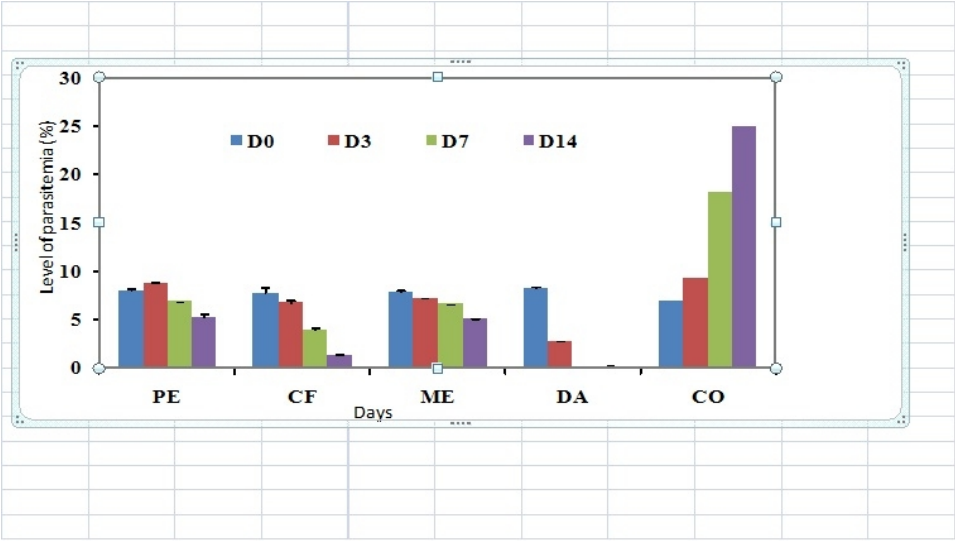
The *in vivo* studies showed that the chloroform extract of *A. maritima* exhibited the highest antitrypanosomal activity compared to the petroleum ether and methanol extracts of the same plant and the untreated control. Though, the parasitemia was not completely cleared, it was drastically reduced in the chloroform extract treated group (Fig. 1). Parasitemia was

reduced from 7.70±0.12 in day 0 to 6.80±0.17 in day 3, and from this to 3.90±0.00 in day 7, and finally reduced to 1.30±0.00 in day 14. A statistically significant difference ($p<0.05$) was observed when the level of parasitemia of *T. brucei brucei* infected mice treated with the chloroform extract was compared with the level of parasitemia of infected mice treated with the petroleum ether and methanolic extracts of *A. maritima* and the infected mice not treated (Fig. 1). However, diaminal at the standard dose of 3.5mg/kg cleared the parasitemia in the *T. brucei brucei* infected mice after 3 days of drug administration (Fig. 1). The parasitemia in this standard treatment group was reduced from 8.25±0.10 in day 0 to 2.75±0.20 in day 3 and finally cleared in day 4. There was no recrudescence in days 7 and 14 (Fig. 1). On the other hand, all the infected mice not treated died within 8 days of infection due to increased parasitemia (Fig. 1).

Table 1. *In vitro* effect of petroleum ether, chloroform and methanol extracts of Artemisia *maritima* on motility of *Trypanosoma brucei brucei*

S/N	Treatment	Latency for ceasing/reducing motility of *Trypanosoma brucei brucei* (Sec)		
		4 mg/ml	2 mg/ml	0.4 mg/ml
1	PE extract of Artemisia maritima	10	20	30
2	*Chloroform extract of Artemisia maritima*	15	25	40
3	Methanol extract of *Artemisia maritima*	35	50	>60
4	Diaminazine Aceturate	10	20	25
5	Control (0.3% Tween 80)	>60	>60	>60

Latency for ceasing / reducing motility of Trypanosoma brucei brucei by different effective concentrations (4, 2, 0.4 mg/ml) of Artemisia martima extracted in different solvents, diaminal standard and untreated controls.



Key: PE= Petroleum ether extract, CF= Chloroform extract, ME= Methanol extract, DA= Diaminal standard, CO= Control

Fig. 1. *In vivo* antitrypanosomal screening of petroleum ether, chloroform and methanol extracts of *Artemisia maritima* on *Trypanosoma brucei brucei*

4. DISCUSSION

The results of the present study showed that the petroleum ether, chloroform and methanol extracts of the whole plant of *Artemisia maritima* had *in vitro* activity against *T. brucei brucei*. The *in vitro* trypanocial activity exhibited by these extracts can be supported by similar studies conducted by other workers [6,7,10,17,18]. They showed conclusively that extracts of different plants exhibit *in vitro* trypanocidal activity. They equally indicated clearly that plants of different families could possess potent trypanocidal activity. In fact, natural products with trypanocidal activity and belonging to a variety of phytochemicals have been identified [9,19].

The results of the *in vivo* study showed that the chloroform extract of *A. maritima* reduced the level of parasitemia significantly in the *T. brucei brucei* infected mice compared to the other extract treatment groups and the untreated control. The antitrypanosomal activity exhibited by this extract can be attributed to the presence of some secondary metabolites/phytochemicals in it. This is because many phytocemicals have been shown to have antitrypanosomal activity [20].

Bizimana et al. [21], in a similar study evaluated some medicinal plants from Mali for *in vitro* and *in vivo* trypanocidal activity. They established that out of 165 extracts of the plants screened for *in vitro* trypanocidal activity, only 24 were active. They stated that the aqueous extracts of the leaves of *Terminalia avicennoides* (Combretaceae) and the stem bark of *Ceiba pentandra* (Bombacaceae) were able to reduce the parasitemia in *T. brucei brucei* infected mice treated at a dose of 100mg/kg intraperitoneally. This is in agreement with result of the *in vivo* evaluation of the chloroform extract of *A. maritima* in this study.

It is difficult to speculate the mechanism by which these extracts exhibit their trypanocidal action. However, accumulated evidence [9] 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 cellular defenses against oxidative stress. 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 [9]. It is also known that some agents act by binding with the kinetoplast DNA of the parasite [10].

The result of Freiburghaus [7] has clearly indicated that different solvent extracts of the same plant may exhibit different trypanocidal activity just as extracts of different parts of the same plant. Therefore, the statement that a plant is trypanocidal or not should be taken within the context of the solvent used and the parts investigated. Moreover, a plant with high *in vitro* trypanocidal activity may have no *in vivo* activity and vice-versa, because of the peculiarities in the metabolic deposition of the plant's chemical constituents[7]. This is in agreement with the *in vitro* and *in vivo* trypanocidal activity of petroleum ether extract of *A. maritima* in this study. This extract showed the highest trypanocidal activity *in vitro*, but has little or no activity *in vivo*. Therefore, plants found to be active *in vitro* must be tested *in vivo* before a definite statement can be made on their trypanocidal potentials.

5. CONCLUSION

The *in vitro* antitrypanosomal activity of the petroleum ether, chloroform and methanol extracts of *Artemisia maritima* has been demonstrated. It is equally shown that only the

chloroform extract of this plant has *in vivo* antitrypanosomal activity. These plant extracts are therefore currently being fractionated by modern chromatographic and spectroscopic technique in order to isolate the pure active components which make them exhibit this antitrypanosomal activity.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee".

ACKNOWLEDGEMENT

We wish to express our gratitude to Mr. Bala of the Department of Parasitology and Entomology, Ahmadu Bello University, Zaria, Nigeria for supplying us the *Trypanosoma brucei brucei* used in this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Gutterridge WE. Existing chemotherapy and its limitations. British Medical Bulletin. 1985;41:162-168.
2. World Health Organisation (WHO). Control and Surveillance of African Trypanosomiasis. Report of a WHO Expert Committee, World Health Organisation Technical Report Series, Geneva. 1998;1-6, 1-114.
3. World Health Organization A new form of human trypanosomiasis in India. Description of the first human case in the world caused by *Trypanosoma evansi*. Weekly Epidemiology Record. 2005;80(7):62-3.
4. Bodley AL, Wani MC, Wall ME, Shapiro TA. Antitrypanosomal activity of complothecin analogs- structure activity correlations. Biochemical Pharmacology. 1995;50: 937-942.
5. Bodley AL, Shapiro TA. Molecular and cytotoxic effects of camplothecin, a lopoisomerase 1 inhibitor on Trypanosomes and Leshmania. Proc. Nati. Academy of Science. 1995;92:3272-3730.
6. Ene AC, Atawodi SE, Ameh DA, Nnamani CN, Apeh YEO. Antitrypanosomal effects of petroleum ether, chloroform and methanol extracts of *Artemisia maciverae* Linn. Indian Journal of Experimental Biology. 2009;47:981-986.
7. Freiburghaus F, Kaminsky R, Nkuna MHN, Brun R. Evaluation of African medicinal plants for their *in vitro* trypanocidal activity. Journal of Ethnopharmacology. 1996;55:1-11.
8. Freiburghaus F, Steck A, Pfander H, Brun R. Bioassay-guided isolation of a diastereoisomer of Kolavenol from *Entada absyssinica* active on *Trypanosoma brucei rhodesiense*. Journal of Ethnopharmacology. 1998;61:179-183.

9. Sepulveda-Boza S, Cassels BK. Plant metabolites active against *Trypanosoma cruzi.* Planta Medica. 1996;62:98-105.
10. Nok AJ, Esievo KAN, Lingdet I, Arowosafe S, Onyenekwe PC, Gimba CE, Kagbu JA. *In vitro* activity of leaf extracts against *Trypanosoma brucei brucei*. Journal of Clinical Biochemistry and Nutrition. 1993;15:113-116.
11. Cragg GM, Newman DJ, Snader KM. Natural products in drug discovery and development. Journal of Natural Products. 1997;60:52-60.
12. Soejarto DD. Biodiversity, and benefit sharing: Perspective from field. Journal of Ethnopharmacology. 1996;51:1-15.
13. Atawodi SE, Ameh DA, Ibrahim S, Nok AJ, Nzelibe HC, Onyike E, Anigo KM, Abu EA, James DB, Njoku GC, Sallau AB. Indigenous knowledge system for treatment of trypanosomiasis in Kaduna state of Nigeria. Journal of Ethnopharmacology. 2002;79:279-282.
14. Herbert WJ, Lumsden WHR. *Trypanosoma brucei*: A rapid "matching" method for estimating the host's parasitemia. Experimental Parasitology. 1976;40:427-431.
15. Teferi G, Heinz J. Treatment of malaria in Ethiopian folk medicine. Tropical Doctor. 2002;32:206-209.
16. Ene AC, Ameh DA, Kanashie HO, Agomo PU, Atawodi S.E. Preliminary *in vivo* antimalaria screening of petroleum ether, chloroform and methanol extracts of fifteen plants grown in Nigeria. Journal of Pharmacology and Toxicology. 2008;3(4):254–260.
17. Atawodi SE, Bulus T, Ibrahim S, Ameh DA, Nok AJ, Mamman M, Galadima M. *In vitro* trypanocidal effect of methanolic extract of some Nigerian Savannah plants. African Journal of Biotechnology. 2003;2(9):317-321.
18. Asuzu IU, Chineme CN. Effects of *Morinda lucida* leaf extracts on *Trypanosoma brucei brucei* infection in mice. Journal of Ethnopharmacology. 1990;30:307-313.
19. Hoop KH, Cunningham LV, Bromel MC, Schemester LJ, Wahba KSK. *In vitro* antitrypanosomal activity of certain alkaloids against *Trypanosoma lewisi.* Lloydia. 1976;39:375-377.
20. Hoet S, Opperdoes F, Brun R, Quetin-Leclercq C. Natural products active against African trypanosomes: a step towards new drugs. Natural Products Rep. 2004;21:353-364.
21. Bizimana N, Tietjen U, Zessin K, Diallo D, Djibril C, Melzig MF, Clausen P. Evaluation of medicinal plants from Mali for their *in vitro* and *in vivo* trypanocidal activity. Journal of Ethnopharmacology. 2006;103(3):350-356.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=407&id=14&aid=3507