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Archives of Applied Science Research, 2011, 3 (6):1-5
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-508X
CODEN (USA) AASRC9

Antileishmaniasis and Phytotoxicity of three Nigerian *Acalypha* species

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ABSTRACT

Antileishmaniasis and phytotoxicity of the methanolic extracts of the stem and leaves of three Nigerian medicinal Acalypha species namely: Acalypha hispida, Acalypha torta and Acalypha wilkesiana was evaluated in vitro, as part of the screening of ethno - medically useful plants from the Nigerian flora as source for bioactive compounds. Leishmaniasis has received increasing attention in developed countries because of the growing number of cases seen in AIDS patients. Prevalent drugs not only have several adverse effects but drug resistance and treatment failures are becoming increasingly common especially in immuno-compromised patients who often fail to respond or relapse. There is thus, still need for development of new drugs. It has also been observed that bioactive and natural antitumor compounds can inhibit the growth of Lemna minor. The antileishmanial activity was assessed using promastigote culture of Pakistani leishmanial strain (L. major) in 96 well micro titer plate bioassay and phytotoxicity using the Lemna bioassay. The leaf extract of A. torta exhibited dose dependent phytotoxicity with significant phytotoxicity at the highest dose investigated. The leaf extract of A. hispida and the stem extract of A. wilkesiana also gave significant phytotoxicity at a high concenteration. The leaf extract of A. wilkesiana and the stem extract of A.torta on the other hand, displayed only moderate phytotoxicity at the highest dose studied. Only the leaf extract of A. hispida was found to be leishmanicidal with an IC_{50} of 71.75 $\mu g\ ml^{-1}$. All the other extracts tested were not leishmanicidal. These results are indicative of the presence of bioactive compounds in these plants and could be considered a valuable support of the ethno medicinal uses of these plants.

Keywords: *Acalypha species*,Euphorbiaceae, methanolic extracts, antileishmaniasis, phytotoxicity.

INTRODUCTION

Leishmaniasis, one of the parasitic diseases caused by trypanosomated protozoan *Leishmania* (transmitted by the female phlebotomus sand fly), is one of the major health problems of tropical, subtropical and Mediterranean regions. Human infection is caused by about 21 of 30

species of the genus *Leishmania* that infect mammals. 12 million new cases of the most common forms, cutaneous (causes skin sores) and visceral (affects internal organs of the body such as spleen, liver, bone marrow) leishmaniasis has been estimated to occur every year. Many novel compounds isolated from various medicinal plants have been reported for their leishmanicidal activities in the last few years. Till date chemotherapy remains the only means of controlling the disease. Amphotericin B and its new lipid formulations are used as second line of treatment. However these are severely limited due to prolonged length of therapy and adverse reactions Thus, there is still need for development of new drugs [1-4].

hispida Burm.f, , *A. torta* Muell and *A. wilkesiana* Mull.-Arg belong to the family Euphorbiaceae, a family of dicotyledonous plants that include herbs, shrubs and trees. They are primarily found in the tropical region of Africa. The herbs, shrubs or trees are fleshy and cactus like. Plants belonging to Euphorbiaceae family have been reported to contain cyanogenic alkaloids, saponins, flavonoids, alkaloids, tannins, resins, and carbohydrates. Different parts of *A. hispida* are used for the treatment of leprosy, gonorrhea, asthma, pulmonary problems, kidney ailments and as a diuretic. *A. torta* on the other hand, is useful traditionally in the treatment of neonatal jaundice, diarrhea and skin disease while *A.wilkesiana* is known for its antimycotic, antibacterial, anti-inflammatory, hemostatic, anthelmintic and analgesic activities [5-7].

To the best of our knowledge, there is no previous ethno - medical report on the leishmanicidal and phytotoxicity activities of *A. hispida*, *A. torta* and *A. wilkesiana* methanolic stem and leaf extracts. Our decision was based on the observation of their use in the treatment of chronic skin ailments, as well as their anti-inflammatory property. In continuation of our studies of the biological activity of ethno medically useful plants and sources for bioactive compounds from the Nigerian flora [8-10], as source for development of new drugs, the antileishmaniasis and phytotoxicity of the methanolic extracts of the stem and leaves of three Nigerian medicinal *Acalypha* species: *Acalypha hispida*, *Acalypha torta* and *Acalypha wilkesiana* is hereby presented.

MATERIALS AND METHODS

Collection, Authentication and extraction of Plant material

Stem and leaves of *A. hispida, A. torta* and *A. wilkesiana* were collected from the flower kingdom, Ibadan. Voucher specimens (FHI107319, FHI107324 and FHI107322, respectively) were identified by Mr. Felix Usang of Forest Research Institute of Nigeria (FRIN) where they were deposited. The air dried stem (1,2kg, 1kg, 1.1kg, respectively) and leaves (900g, 800g, 850g, respectively) of the three plants were each extracted with methanol for 48 hours and the resulting methanolic plant extracts of the stem (51g, 16.3g, 19.6g, respectively) and leaves (33.7g, 38.1g, 37g, respectively) were stored in the refrigerator prior to use.

Parasite culture

The promastigote culture of Pakistani leishmanial strain (*L. major*) were maintained in blood agar based modified NNN diphasic medium supplemented with RMPI-1640 (Sigma R-7388), with 20mM HEPES and L-glutamine without $NaHCO_3$ at 25^oC [11]

Leishmanicidal assay

Leishmanial promastigotes were grown in bulk early in modified NNN diphasic medium using normal saline. Parasites at log phase were centrifuged at 2000 rpm for 10 mins, washed three times with saline at same speed and time. Parasites were diluted with fresh culture medium to a final density of 10^6 cell/ml. Subsequently 100 µl of culture was added in all wells except first

column which received 180 µl. The last two rolls were left for negative and positive controls. Negative control received medium with solvents while the positive control contained varying concentrations of the standard antileishmanial compound Amphotericin B.

Serial dilutions of *A. hispida, A. torta* and *A. wilkesiana* methanolic extracts were performed in 96 well micro titer plates in triplicates. 20 µl of solubilized extracts were added into the first wells and mixed well by micropipette. 100 µl of sample was removed and added into the next well, mixed well and repeated till the 8th well was reached. The remaining 100 µl was discarded. By doing this, the first well received a final concentration of 100 µg/ml while the last had 0.78 µg/ml of crude extracts to be tested. The plates were incubated in the dark at 22^{o}C for 72 hrs on an orbital shaker. After 5 days exposure, drug activity (IC_{50}) was assessed microscopically using improved Neubauer–counting chamber programme [11].

Phytotoxicity assay

The Lemna bioassay was carried out using the modified protocol of Mclaughlin [12, 13]. The *Lemna minor* (Duckweed) were cultivated under optimum conditions for 1 or 2 days, briefly washed in water and transferred into the E-medium nutrient (a mixture of various constituents adjusted to pH 5.5-7 to provide nutrients for growth of plant) prior to use. The flasks used for the bioassay were initially inoculated with 10, 100 and 1000 µL in each of three replicates of the stock solution of the extracts (30 mg of crude dissolved in 1.5 mL MeOH / EtOH). The solvents were left to evaporate overnight, thus yielding 10, 100 and 1000 µgml^{-1} medium flasks to which 20ml of E-medium and 10 plants of *L. minor*- each containing a rosette of 2-3 fronds was introduced. Other flasks containing solvent and reference/standard drug paraquate served as negative and positive controls, respectively.

The flasks were placed in growth cabinets maintained at 28 ± 1^{o}C for 7 days and examined daily during incubation. The number of fronds per flask was counted on day 7 to determine the growth inhibition or proliferation of fronds in the flasks. The percentage growth regulation was therefore analysed with reference to the negative control [14].

RESULTS AND DISCUSSION

Leishmanicidal activity. Concentrations of *A. hispida, A. torta* and *A. wilkesiana* methanolic stem and leaf extracts ranging from 0.78 µgml^{-1} to 100 µgml^{-1} in triplicates were tested for their antileishmanial activity. The leaf methanolic extract of *Acalypha hispida* as shown in Table 1, was found to be leishmanicidal at an IC_{50} value of 71.75 µgml^{-1}. $IC_{50} \leq 100$ µgml^{-1} for extracts was considered significant [11, 15]

Phytotoxicity. 10, 100 and 1000 µgml^{-1} in each of three replicates of the stock solution of the stem and leaf methanolic extracts of *A. hispida, A. torta* and *A. wilkesiana* (30 mg of crude dissolved in 1.5 mL MeOH / EtOH) were tested for their inhibitory or proliferatory activity on *Lemna* fronds. The leaf methanolic extracts of *A. torta* exhibited significant dose dependent phytotoxicity, with low activity (21.7 %inhibition) at 10 µgml^{-1}, moderate activity (41.6 %inhibition) at 100 µgml^{-1}and significant activity (80 %inhibition) at 1000 µgml^{-1}. The leaf extract of *A. hispida and* the stem extract of *A. wilkesiana* also displayed marked activity (80 and 70 %inhibition, respectively) at 1000 µgml^{-1} as indicated in Table 1. The leaf extract of *A. wilkesiana* and the stem extract of *A. torta* on the other hand, displayed only moderate phytotoxicity (53.8 and 55.8%inhibition, respectively) at 1000 µgml^{-1}.

Table 1 Antileishmaniasis and phytotoxicity assays of methanolic extracts of stem and leaves of *A.hispida, A. torta* and *A. wilkesiana*

Methanolic Extracts/ Standards Concentration	% Growth Inhibition[a,b] (*Lemna minor*)				% Inhibition IC_{50} (µg/ml)[a,c] (Leishmaniasis)
	1000 µg/ml	100 µg/ml	10 µg/ml	0.0176 µg/ml	
A.hispida					
Stem	31	-20.7	-27.6		>100
Leaves	80	10	0		71.75± 0.25
A. torta					
Stem	55.8	-1.9	-5.8		>100
Leaves	80	41.6	21.7		>100
A.Wilkesiana					
Stem	70	16.7	0		>100
Leaves	53.8	-5.8	-2.5		>100
Paraquate				100	
Amphotericin B					0.12 ± 0.01

[a]*Values are mean ±S.E.M (n = 3), p ‹ 0.05 (Student's t-test)*
[b]*Negative inhibition means growth promoter / proliferation*
[c]*Assay in 96 well micro titer plates (serial dilutions from 100 to 0. 78 µg/ml)*

CONCLUSION

The present findings demonstrate that the leaf methanolic extract of *A. hispida* has leishmanicidal activity while the leaf methanolic extracts of *A. torta*, *A. hispida* and stem extract of *A. wilkesiana* exhibit significant phytotoxicity. The use of *A. hispida*, *A. torta* and *A. wilkesiana* extracts in the treatment of chronic skin ailments, as well as their anti-inflammatory property [5-7] might be explained in the light of these results. In addition, the phytotoxicity exhibited by all the extracts in significant inhibition of the growth of *Lemna minor* is indicative of the presence of biologically active natural products in all the extracts. Their use can particularly become more important when standardized therapies induce resistance to various infections. Further studies will be necessary to investigate the effect of these active plant extracts when combined with antileishmanial agents commonly used.

Acknowledgements
The authors are grateful to Mr Felix Usang of the Forest Research Institute (FRIN), Ibadan, Nigeria, for authenticating the plant material. P. A Onocha acknowledges with thanks the Senate Research Grant of the University of Ibadan, Nigeria and H.E.J. Research Institute of Chemistry, International Centre for Chemical Sciences, University of Karachi, Karachi, Pakistan for collaborative research.

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