Potential Antileishmanial Effect of Three Medicinal Plants

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The antileishmanial activity of three organic solvent extracts and water residue of the plants: *Acacia nilotica* (Mimosaceae) (husk), *Ambrosia miratima* (Astraceae) (aerial shoot) and *Azadarichta indica* (Meliaceae) (leaves) were tested *in vitro* against *Leishmania donovani* promastigotes. The study revealed that the extracts of *A. nilotica* and *A. miratima* have effectious antileishmanial activity at concentrations (IC_{50}) less than 8 μg/ml, while the extracts of *A. indica* lack antileishmanial activity. The chromatographic analysis of the ethyl acetate extract of *A. nilotica*, the most potent extract, resulted in four TLC fractions. Three of these fractions possessed antileishmanial activity. Phytochemical study of the potent fractions revealed the presence of poly hydroxyl compounds.

Key words: *Acacia nilotica*, *Ambrosia miratima*, antileishmanial activity, *Azadarichta indica*

Leishmaniasis is an infection due to protozoa belonging to the genus Leishmania. Leishmaniasis is considered as a major public health problem in developing countries such as Sudan[1,2]. In Sudan where the primary health care is not always affordable, the use of herbal medicine is a common practice. However, very few studies were carried out to investigate the potential use of these medicinal plants in treatment of parasitic diseases[3,4].

The plant materials were collected according to their traditional use in folk medicine and a voucher specimen was kept in the Department of Pharmacognosy, Faculty of Pharmacy, University of Khartoum. The aqueous solution of the dried methanol extracts of the test plants were partitioned by liquid-liquid partitioning using petroleum ether, dichloromethane and ethyl acetate. The three organic solvent fractions and water residue were dried and dissolved in RPMI-1640 medium to prepare test concentrations of 0.5, 5, 25, 50 and 100 μg/ml.

Promastigotes of the identified strain of *Leishmania donovani* archidadi Mon 82 were grown in RPMI-1640 medium enriched with 20% foetal calf serum. Two hundred microlitre of each test extract (concentration 0.5-100 μg/ml) was added to 96 well plate (Nunc microtitre plate). To one raw, 200 μl of plane RPMI-1640 medium was added and this was kept as control. Then 50 μl of promastigotes suspension (3×10^6/ml) was added to all wells. Parasites were allowed to multiply at 24º for 24 h. Then promastigotes were harvested in a counting slide and counted under light microscope in comparison with control cells.

The ethyl acetate extract of *A. nilotica* (husk) was fractionated by TLC on silica gel (GF) plates using a solvent system of acetone and petroleum ether (1:1). The isolated TLC fractions were detected for their purity using Knauer HPLC machine in a Hypersil column (reversed phase C18) 5 μm, 15×4.6 mm. Mobile phase consisted of water and acetonitrile (30:70); flow rate was 1 ml/min and detection was on UV detector at 271 nm.

The UV spectrum of the aqueous solution (20%) of the TLC fractions having potent antileishmanial activity was measured at 120 nm/min scan speed on Perkin Elmer Lamda 2 UV/Vis spectrophotometer. FTIR machine (Shimadzu-800) was used to study the FTIR spectrum of KBr discs of dried crystals of the three above fractions.

Human peripheral blood mononuclear cells from heparinized blood were isolated using lymphoprep solution (Pharmacia Biotech). Each cell of 96 well round bottomed microtitre plate received 0.3×10^6/ml peripheral blood mononuclear cell (PBMC) in 200 μl of RPMI 1640 containing the plants extracts, concentration of 0.5, 5, 25, 50 and 100 μg/ml; 10 μl of phyto-haemaggulitinin (PHA, 47 μg/ml, Murex diagnostic, England) was added into all microplate wells except blank control ones, the positive control ones receive PHA but no plant extract was added to those wells.

The results of the present study reveal that, the extracts of *A. nilotica* husk and *A. miratima* aerial shoot have a potent antileishmanial activity *in vitro* against *L. donovani* promastigotes at concentrations (IC_{50}) less than 8 μg/ml (Table 1). On the other hand the extracts of *A. indica* leaves did not give significant antileishmanial activity. As shown in Table 2 the thin layer chromatography of the ethyl acetate extract of *A. nilotica* husk indicated the presence of three fractions 1, 2 and 3 of R_f values: 0.70, 0.41 and 0.24, these fractions possessed potent antileishmanial activity with IC_{50} concentration less than 5 μg/ml. Where as the fourth fraction R_f value 0.00 (at base line) exhibited poor antileishmanial activity IC_{50} concentration more than 20 μg/ml. The performance of the different sensitivity and toxicity tests in this study were analyzed using SPSS computer software using

TABLE 1: THE ANTILEISHMANIAL EFFECT OF THE MEDICINAL PLANTS EXTRACTS

Plant extract solvent	Y=a+bx	IC_{50} (µg/ml)	IC_{90} (µg/ml)	R^2
Acacia nilotica/Ethyl acetate	Y=-2.13+2.413x	7.65	62.28	0.924
Acacia nilotica/Dichloromethane	Y=-0.69+1.03x	4.6	624.0	0.625
Ambrosia maritima/Ethyl acetate	Y=-0.45+0.761x	4.01	325.0	0.590
Ambrosia maritime/Dichloromethane	Y=-1.1+1.65x	4.65	100.0	0.999
Ambrosia maritime/Petroleum ether	Y=-1.43+2.63x	3.49	23.9	0.687
Ambrosia maritima/Water residual	Y=-0.45+0.98x	2.9	520.0	0.188
Azadarichta indica/Ethyl acetate	Y=-4.05+0.90x	31900.0	8857169	0.835
Azadarichta indica/Dichloromethane	Y=-2.84+0.68x	14865.0	>25432544	0.410
Azadarichta indica/Petroleum ether	Y=-3.36+0.81x	14095.0	>7323899	1.000
Azadarichta indica /Water residual	Y=-3.34+0.93x	3818.9	>871619	0.939

TABLE 2: THE ANTILEISHMANIAL ACTIVITY OF TLC FRACTIONS (1-4) OF *ACACIA NILOTICA* DRIED ETHYL ACETATE EXTRACT

TLC Fractions of *A. nilotica* ethyl acetate extract	Y=a+bx	IC_{50} (µg/ml)	IC_{90} (µg/ml)	R^2
Fraction (1) R_f=0.70	Y=-1.39+2.04x	4.80	57.80	0.496
Fraction (2) R_f=0.41	Y=-0.819+1.83x	2.80	44.80	0.832
Fraction (3) R_f=0.24	Y=-0.3+1.46x	1.60	51.66	0.966
Fraction (4) R_f=0.00	Y=-1.497+1.46x	22.39	712.20	0.525

TABLE 3: IR ANALYSIS RESULTS OF TLC FRACTIONS OF THE ETHYL ACETATE EXTRACT OF *ACACIA NILOTICA* HUSK

Fraction 1	Fraction 2	Fraction 3	Putative chemical group	Absorption band (cm^{-1})
+	+	+	O-H	3500-3200
+	+	+	C-H(aliphatic origin)	2950
+	-	+	-C=O	1750-1650
+	+	+	-C=C-	1650-1600
+	+	+	$-CH_3$ and $-CH_2-$	1450-1400
+	+	+	C-O (different environments)	Multiple bands 1300-1000
+	-	-	C-Cl	600

the standard agent (pentostam) which exhibit relatively high IC_{50} of 156 µg/ml.

The HPLC chromatogram of fraction 1 showed a single principle peak at R_t of 3.28 min with minor impurities, while chromatograms of fraction 2 and 3 show multiple peaks. The spectroscopic analysis of the TLC fractions of the ethyl acetate extract of *A. nilotica* husk provided the following results: The UV spectrum of aqueous solutions of fraction 1 showed absorption bands at 278.6, 262.3 and strong end absorption at 228.3 nm. With fraction 2 the UV spectrum revealed strong end absorption at 210 nm and shoulders at 423.2, 378 and 276 nm. The UV spectrum of fraction 3 gave strong end absorption with a single shoulder at 277.7 nm in aqueous solution. The IR spectrum absorption bands of TLC fractions of the ethyl acetate extract of *Acacia nilotica* husk are listed in Table 3.

The present work aimed at screening the antileishmanial activity of some Sudanese medicinal plants by *in vitro* studies to identify their active extracts or principles. The ethyl acetate extract of *A. nilotica* husk showed a promising antileishmanial activity. The findings of the present study agree with the earlier reports where, the ethyl acetate extract of *A. nilotica* husk had showed potent antiparasitic activity against *Plasmodium falciprum*. *A. nilotica* is widely used in folk medicine to treat a variety of diseases, where the gum stem bark, leaves and fruits are used for treatment of colds, bronchitis, pneumonia, ophthalmia, diarrhea and hemorrhage[5]. In a preliminary phytochemical screening of the ethyl acetate extract of *A. nilotica* husk, the presence of tannins and phenolic compounds was confirmed when a blue-black color was observed when ferric chloride reagent was added to a solution of the extract.

Several studies stated that many plant species have immunomodulatory action[6]. The ethyl acetate extract of *A. nilotica* husk, at low concentrations revealed an increase of human lymphocytes cells count. This may be an indication of the ability of this extract to enhance human immunity. TLC fractioning of the ethyl acetate extract of *A. nilotica* husk using silica gel GF as stationary phase and a solvent system of acetone: petroleum ether (1:1) was carried out. Four fractions were isolated of R_f values of 0.70, 0.41, 0.24 and baseline spot. The first three fractions were found to posses antileishmanial activity with IC_{50} value of 4.8, 2.8, 1.6 µg/ml and IC_{90} value of 57.8, 44.8 and

51.66 µg/ml, respectively. The isolated TLC fractions were detected for their purity using Knauer HPLC machine. Fraction 1 was found to be the most pure, while fraction 2 and 3 showed multiple peaks as an indication of the presence of other compounds in each fraction. The high antileishmanial activity of fraction 2 and 3 may be attributed to synergistic action of these additional compounds.

A potent antileishmanial activity was observed when *L. donovani* was treated with *A. maritima* extracts (IC_{50} <5 µg/ml). Further *in vivo* studies are needed to confirm the bioactivity of this plant.

The present study concludes that *A. indica* leaves lack anti leishmanial activity (IC_{50} value >1 mg/ml). In a previous study, it was reported that the methanol extract of *A. indica* leaves exhibited a moderate antileishmanial activity[7]. In fact, *A. indica* preparations are used locally in treatment of many parasitic diseases. Oil extracted from the seeds is used in both man and animals as an anthelmintic, and in scabies, abdominal ulcers, rheumatism and muscular pain[8].

The extracts of the tested plants illustrates the diversity of antiprotozoal compounds found in those plants. Some of them may act on systems present in the parasite which are absent in the host cells. These are worthy of further study and perhaps semi synthetic modification in order to improve their therapeutic/toxic ratios.

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REFERENCES

1. Desjeux P. Leishmaniasis: current situation and new perspectives. Comp Immunol Microbiol Infect Dis 2004;27:305–18.
2. Anonymous. Control of the Lesishmanaisis. Report of a WHO Expert Committee. World Health Organ Tech Rep Ser 1990;793:1-158.
3. Khalid SA, Farouk A, Geary TG, Jensen JB. Potential antimalarial candidates from African plants: An *in vitro* approach using *Plasmodium falciparum*. J Ethanopharmacol 1986;15:201-9.
4. Ibrahim AM. Anthelmintic activity of some Sudanese medicinal plants. Phytother Res 1992;6:155-7.
5. Watt JM. Leguminosae. In: Watt JM, Brandwijk MG, editors. The Medicinal and Poisonous Plants of Southern Africa. Edinburgh: E and S Livingstone Ltd.; 1962. p. 546.
6. Labadie RP, van der Nat JM, Simons JM, Kroes BH, Kosasi S, van den Berg AJ, *et al*. An ethnopharmacognostic approach to the search for immunomodulators of plant origin. Planta Med 1989; 55:339-48.
7. Fatima F, Khalid A, Nazar N, Abdalla M, Mohomed H, Toum AM, *et al*. *In vitro* Assesment of anti-cutaneous leishmaniasis activity of some Sudanese plants. Turk Parazitol Derg 2005;29:3-6.
8. Fernando S. Herbal food and Medicine in Siralanka. New Delhi: Navrang Booksellers and Publishers; 1982. p. 114.

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