WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 3, 338-346. Research Article ISSN 2277– 7105

IN VITRO ANTIPROTOZOAL ACTIVITY OF FIVE PLANT EXTRACTS FROM ALBAHA REGION

Nasser A. Awadh Ali[1]*, Saeed S. Al-Sokari[2], Ramzi Mothana[3], Mahmoud Hamed[1], Mohamed Wagih[2], Paul Cos[4], and Louis Maes[4]

[1]Pharmacognosy Department, Faculty of Clinical Pharmacy Albaha University, P.O. Box 1988, KSA.

[2]Department of Biology, Faculty of Science, Albaha University, KSA.

[3]Department of Pharmacognosy, College of Pharmacy, King SaudUniversity, P.O. Box 2457, Riyadh 11451, Saudi Arabia.

[4]Laboratory for Microbiology, Parasitology and Hygiene (LMPH), Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, Antwerp University, Universiteitsplein 1, 2610 Antwerp, Belgium.

Article Received on 13 Jan 2016,

Revised on 04 Feb 2016,
Accepted on 24 Feb 2016

***Correspondence for Author**
Prof. Nasser A. Awadh Ali
Pharmacognosy Department, Faculty of Clinical Pharmacy Albaha University, P.O. Box 1988, KSA.

ABSTRACT

Five methanol extracts obtained from five plant species selected according ethnobotanical data were screened for their antiprotozoal activity against Plasmodium falciparum, Leishmania infantum, Trypanosoma cruzi and Trypanosoma brucei. Cytotoxicity was evaluated on MRC-5 cells. *Withania somnifera* methanol extract showed low activity against P. falciparum (IC_{50} 17.7 µg ml^{-1}), but with non-selective antimalarial activity. Most of the extracts tested against L. infantum exhibited low activity with IC_{50} ranging from 20 to 32 µg ml^{-1}, except W. somnifera which showed a remarkable but non-selective leishmanicidal activity (IC_{50} of 2.0 µg ml^{-1}). W. somnifera methanol extract also exhibited a marked activity against T cruzi (IC_{50} 0.6 µg ml^{-1}, SI=3.5).

KEYWORDS: In vitro, Plasmodium, Leishmania, Trypanosoma; cytotoxicity; plant extracts.

1. INTRODUCTION

Parasitic diseases produced by Trypanosoma, Leishmania and Plasmodium species are some of the major causes for high mortality and morbidity in developing countries. According to the latest estimates, released in 2014 by WHO, there were about 198 million cases of malaria in 2013 and an estimated 584, 000 deaths.[1] Human African trypanosomiasis (HAT) is a major disease with half a million people in sub-Saharan Africa at risk of developing the disease.[2] Leishmaniasis is distributed worldwide, especially in tropical and subtropical areas and more than 12 million people are currently infected. About 20,000 to 30,000 deaths occur annually. Cutaneous leishmaniasis (CL) is the most common form of leishmaniasis and causes skin lesions, mainly ulcers. About 95% of CL cases occur in the Americas, the Mediterranean basin, the Middle East, and Central Asia.[3] The chemotherapy of these tropical protozoal diseases has been undermined by the fact that the presently used drugs are relatively toxic or become ineffective by the spreading of drug resistance. Hence, there is a continuous need to explore new therapeutic agents, and ethnobotanical knowledge may be useful to open new ways in this field. Considerable attention has been given to search for new anti-infective and in particular antiprotozoal drugs. Several antiprotozoals in the market today were directly obtained from plants, such as quinine or structurally modified plant compound artemisinin.[4,5,6,7] Some reports were already published about the antiprotozoal activity potential of Saudi medicinal plants.[8,9,10,11,12] The Albaha region in Southern Saudi Arabia is very rich in flora with a great diversity of native and naturalized medicinal plants that have yet been very poorly explored. For this reason, this region was chosen for collecting the specimens.[13] To the best of our knowledge, this study represents the first report on antiprotozoal activity for some of the tested methanol extracts, obtained from five plant species growing in Albaha region, KSA, and used in traditional medicine for the treatment of infectious diseases.

MATERIAL AND METHODS

Plant Material

The plant material was collected between March – April 2014 from different locations in Albaha town and outskirts of Albaha (Table 1). The plants were taxonomically identified at the Faculty of Science, Department of Botany, Aden University, Yemen. Voucher specimens of the plant material are deposited at the Pharmacognosy Department, Faculty of Clinical Pharmacy, Albaha University, KSA.

Preparation of Extracts

The harvested plant parts were air-dried at ambient temperature and powdered with a blender. The powdered plant material (40 g) was extracted in methanol (powdered material:

solvent = (1:10). The extraction was done at room temperature (29-32 °C) with constant shaking of the extraction set-up. Thereafter, the extracts were filtered, and evaporated to dryness in vacuo at 40 °C to obtain the dried extracts. The yields of each dried extract were calculated in %. The resulting dried crude extracts were stored at 4 °C.

Antiprotozoal activity

Standard Drugs

For the different tests, appropriate reference drugs were used as positive control: tamoxifen for MRC-5, chloroquine for P. falciparum, miltefosine for L. infantum, benznidazole for T. cruzi and suramin for T. brucei. All reference drugs were either obtained from the fine chemical supplier Sigma-Aldrich (tamoxifen, suramin) or from WHO-TDR (chloroquine, miltefosine, benznidazole).

Biological Assays

The integrated panel of microbial screens and standard screening methodologies were adopted as previously described.[14] All assays were performed in triplicate at the Laboratory of Microbiology, Parasitology and Hygiene at the University of Antwerp (Belgium). Plant extracts were tested at 5 concentrations (64, 16, 4, 1 and 0.25 μg/mL) to establish a full dose-titration and determination of the IC_{50} (inhibitory concentration 50%). The final in-test concentration of DMSO did not exceed 0.5%. The selectivity antiprotozoal potential was assessed by simultaneous evaluation of cytotoxicity on a lung fibroblast normal cell line (MRC-5). The criterion for activity was an IC_{50}<10 μg/mL and a selectivity index (SI) of >4.

Antiplasmodial Activity

Chloroquine-resistant P. falciparum K 1-strain was cultured in human erythrocytes O^+ at 37 °C under a low oxygen atmosphere (3% O_2, 4% CO_2, and 93% N_2) in RPMI-1640, supplemented with 10% human serum. Infected human red blood cells (200 μL, 1% parasitaemia, 2% hematocrit) were added to each well and incubated for 72 h. After incubation, test plates were frozen at –20 °C. Parasite multiplication was measured using the Malstat assay, a colorimetric method based on the reduction of 3-acetylpyridine adenine dinucleotide (APAD) by parasite- specific lactate-dehydrogenase (pLDH).[15]

Antileishmanial Activity

L. infantum MHOM/MA(BE)/67 amastigotes were collected from the spleen of an infected donor hamster and used to infect primary peritoneal mouse macrophages. To determine in-vitro antileishmanial activity, 3×10^4 macrophages were seeded in each well of a 96-well plate. After 2 days outgrowth, 5×10^5 amastigotes/well, were added and incubated for 2 h at 37 °C.

Pre-diluted plant extracts were subsequently added and the plates were further incubated for 5 days at 37 °C and 5% CO_2. Parasite burdens (mean number of amastigotes/macrophage) were microscopically assessed after Giemsa staining on 500 cells, and expressed as a percentage of the blank controls without plant extract.

Antitrypanosomal Activity

Trypanosoma brucei Squib-427 strain (suramin-sensitive) was cultured at 37 °C and 5% CO_2 in Hirumi-9 medium[16], supplemented with 10% fetal calf serum (FCS). About 1.5×10^4 trypomastigotes/well were added to each well and parasite growth was assessed after 72 h at 37 °C by adding resazurin[17]. For Chagas disease, T. cruzi Tulahuen CL2 (benznidazole-sensitive) strain was maintained on MRC-5 cells in minimal essential medium (MEM) supplemented with 20 mML-glutamine, 16.5 mM sodium hydrogen carbonate and 5% FCS. In the assay, 4×10^3 MRC-5 cells and 4×10^4 parasites were added to each well and after incubation at 37 °C for 7 days, parasite growth was assessed by adding the β-galactosidase substrate chlorophenol red β-D-galactopyranoside[18]. The color reaction was read at 540 nm after 4 h and absorbance values were expressed as a percentage of the blank controls.

Cytotoxicity against MRC-5 Cells

MRC-5 SV2 cells were cultivated in MEM, supplemented with L-glutamine (20 mM), 16.5 mM sodium hydrogen carbonate and 5% FCS. For the assay, 10^4 MRC-5 cells/well were seeded onto the test plates containing the pre-diluted sample and incubated at 37 °C and 5% CO_2 for 72 h. Cell viability was assessed fluorimetrically after 4 h of addition of resazurin. Fluorescence was measured (excitation 550 nm, emission 590 nm) and the results were expressed as % reduction in cell viability compared to control and CC_{50} was calculated using Origin program.

RESULTS AND DISCUSSION

Five methanol extracts of five plant species belonging to five families collected from Albaha region (Table 1) were investigated for their antiprotozoal and cytotoxic activity against P. falciparum, L. infantum, T. cruzi, T. brucei and MRC-5 cells, respectively (Table 2). The listed IC_{50} values are the means of three determinations. Particularly for natural products, evaluation of selectivity is essential and represented as the selectivity index (SI): ratio (CC_{50} MRC-5 cells/IC_{50} protozoa). An acceptable criterion for activity was an IC_{50}<10 µg ml^{-1} and a selectivity index (SI) of >4. Indeed, in evaluating the selectivity against protozoan versus mammalian cells, it is noted that the antiprotozoal activities of most active extracts may be due to their cytotoxicity to mammalian cells, as is demonstrated by the moderate, low or very low SI-values between <1 and 3.5.

Among the extracts tested, W. somnifera and L. dentata extracts displayed a low non-selective antiplasmodial activity with IC_{50} values of 17.7 and 23.4 µg ml^{-1} respectively. Most of the plant extracts showed non-selective low antileishmanial activity with IC_{50} values ranging from 20.3 to 32.0 µg ml^{-1}, except W. somnifera which showed a non-selective antileishmanial activity with an IC_{50} of 2.0 µg ml^{-1}. The latter also showed the highest activity against T. cruzi with an IC_{50} of 0.6 µg ml^{-1} and moderate SI of 3.5, followed by extract of L. dentata with an IC_{50} of 7.1 µg ml^{-1} and SI of 3.4. Similar inhibitory activity of L. dentata extract was also recorded against T. brucei (IC_{50}: 8.1 µg ml^{-1}, SI: 3).

In our study, L. dentata possessed moderate antitrypanosomal activity against both T. cruzi and T. brucei with moderate SI of 3.4 and 3.0. This result partially agrees with literature data on L. dentata published by[10,11,12], who reported antiprotozoal activity of the methanol extract of L. dentata with better selectivity (in comparison to our results. This activity is probably linked to the presence of linalool-rich essential oil that has been reported in L. dentata[7,19], and the difference in the antiprotozoal activity may be due to variation in the time and area of plant collection and other ecological factors which exert great influence on the quality and quantity of the active constituents, in particular of essential oil components.[7]

In the present study, W. somnifera (IC_{50} of 2.0 µg ml^{-1}) exhibited more potent anti-leishmanial activity than those reported previously and collected from Oman (IC_{50}: 22.10 ± 1.62, SI: 0.89)[20] and India (IC_{50}: 63 ± 6).[21] It was suggested that withaferin A, the inhibitor of protein kinase C, is responsible for the high anti-leishmanial activity.[22,23] E. helioscopia has been subjected to abundant phytochemical and biological investigations, but no antiprotozoal activity has been found in the literature. Several compounds have been isolated from E. helioscopia, such as diterpenoids, triterpenoids, that may be responsible for the antitrypanosomal activity.[24] Several triterpenoids isolated from E. resinifera and E. officinarum were reported to have antitrypanosomal activity.[25]

Table: 1. Selected plants studied: ethnobotanical information and characteristics.

Species	Plant Family (Voucher specimen No.)	Part tested[a] (%) (Yield in %)	Local name	Traditional uses
Euphorbia helioscopia L.L.	Euphorbiaceae (CP-051)	AP (4.2)	Al-dehin	Antiseptic[2]
Lavandula dentata L.	Lamiaceae (CP-041)	AP (2.9)	Al-shiah	As antispasmodic, antiseptic[1] when the leaves chewed[1]
Rumex nervosus Vahl	Polygonaceae (CP-001)	L (4.8)	Ah-athrub	Paste of leaves used for treating wounds[2]
Ruta chalepensis L.	Rutaceae (CP-121)	L (5.2)	Al-shathab	Antimicrobial[2]
Withania somnifera L. (L.) Dunal	Solanaceae (CP-011)	F (4.6)	Alobeb	Chronic dermatitis[2]

a AP, aerial parts; F: fruits; L: leaves. 1 information obtained from reference [13]. 2Interviewing with local people.

Table: 2 Antiprotozoal activity of the extracts of the investigated plants and their cytotoxicity against MRC-5 cell line.

Plant species (part tested)[a]	P. falciparum		L. infantum		T. cruzi		T. brucei		
	IC_{50}[b]	SI[c]	IC_{50}	SI	IC_{50}	SI	IC_{50}	SI	CC_{50}[d]
E. helioscopia (AP)	>64	-	27.3	-	7.2	>1	33.7	-	7.9
R. nervosa (L)	>64	-	>64	-	32.6	-	>64	-	21.3
L. dentata (AP)	23.4	>1	20.3	>1	7.1	3.4	8.1	3.0	24.4
R. chalepensis (L)	>64	-	32.0	-	21.3	>1	36.9	-	29.2
W. somnifera (F)	17.7	-	2.0	-	0.6	3.5	8.1	-	1.9
Chloroquine	0.3 ± 0.1		-			-		-	-
Miltefosine	-		3.3 ± 0.7			-		-	-
Benznidazole	-		-			-		-	-
Suramin	-		-			0.03 ± 0.02		0.03 ± 0.02	-
Tamoxifen	-		-						11.0 ± 2.3

[a]AP, aerial parts; F: fruits; L: leaves

[b]IC_{50}: Concentration of extract causing 50% growth inhibition.

[c]SI: Selectivity index. —: Not done

[d] CC: Cytotoxic concentration of the extracts causing death to 50% of viable cells.

CONCLUSION

The in vitro antiprotozoal activity against P. falciparum, L. infantum, T. brucei and T. cruzi is reported for the first time for E. helioscopia and R. chalepensis, which all have been used traditionally for treating infectious diseases. The methanol extract of W. somnifera showed the best activity against T. cruzi and L. infantum. W. somnifera extract was selected for further bioactivity-guided fractionation in order to isolate the active pure compound(s).

ACKNOWLEDGEMENT

The authors extend their appreciation to the Deanship of High Studies & Scientific Research in Albaha University for funding the work through the project number 56/1434. The authors gratefully acknowledge that financial support.

REFERENCES

1. WHO, Media Centre, (2013). Factsheet No. 94, Malaria. Reviewed April 2015 http://www.who.int/mediacentre/factsheets/fs094/en/
2. WHO, Media Centre Factsheet 259, Trypanosomiasis, human African" updated May 2015 http://www.who.int/mediacentre/factsheets/fs259/en/
3. WHO, Leishmaniasis,WHO FactSheet 375, 2015 a ,http://www.who.int/mediacentre/ factsheets/fs375/en/
4. Cragg GM, Newman DJ, Snader KM, Natural products in drug discovery and development. J. Nat. Prod., 1997; 60: 52–60.
5. Hussain H, Al-Harrasi A, Al-Rawahi A, Green IR, Gibbons S. Fruitful decade for antileishmanial compounds from 2002 to late 2011. Chem. Rev., 2014; 114: 10369-428.
6. Newman DJ, Cragg, GM. Natural products as sources of new drugs over the last 25 years. J. Nat. Prod., 2007; 70: 461–477.
7. Monzote L, Alarcon O, Setzer WN. Antiprotozoal Activity of Essential Oils. Agric. conspec. sci., 2012; 77: 167-175.
8. Mothana RA, Al-Musayeib NM, Al-Ajmi MF, Cos P, Maes L. Evaluation of the in vitro antiplasmodial, antileishmanial, and antitrypanosomalactivity of medicinal plants used in saudi and yemeni traditional medicine. Evid Based Complement Alternat Med., 2014; 1-7.

9. Mothana RA, Al-Musayeib NM, Matheeussen A, Cos P, Maes L. Assessment of the in vitro antiprotozoal and cytotoxic potential of 20 selected medicinal plants from the island of soqotra. Molecules, 2012; 17: 14349–14360.
10. Al-Musayeib NM, Mothana RA, Al-Massarani S, Matheeussen A,Cos P, Maes L. Study of the in vitro antiplasmodial, antileishmanial and antitrypanosomal activities of medicinal plants from Saudi Arabia. Molecules, 2012; 17: 11379-90.
11. Al-Musayeib; N.M.;Mothana, R.A.;Matheeussen, A., Cos. P; Maes, L. In vitro antiplasmodial, antileishmanial and antitrypanosomal activities of selected medicinal plants used in the traditional Arabian Peninsular region. BMC Complement Altern Med., 2012; 12: 49.
12. Abdel-Sattar E, Maes L, Salama MM, In vitro activities of plant extracts from Saudi Arabia against malaria, leishmaniasis, sleeping sickness and Chagas disease. Phytother Res., 2010; 24: 1322-8.
13. A. Gashash, Plants in Mountains of Sarah and Alhajaz, Albaha University Publisher, Albaha, Saudi Arabia, 2012.
14. Cos P, Vlietinck AJ, Berghe DV, Maes L. Anti-infective potential of natural products: How to develop a stronger in vitro proof-of-concept. J. Ethnopharmacol., 2006; 106: 290–302.
15. Makler MT, Ries JM, Williams JA, Bancroft JE, Piper RC, Hinrichs DJ. Parasite lactate dehydrogenase as an assay for Plasmodium falciparum drug sensitivity. Am. J. Trop. Med. Hyg., 1993; 48: 739–741.
16. Hirumi H, Hirumi K. Continuous cultivation of Trypanosomabruceiblood stream forms in a medium containing a low concentration of serum protein without feeder cell layers. J. Parasitol., 1989; 75: 985–989.
17. Raz B, Iten M, Grether-Buhler Y, Kaminsky R, Brun, R. The Alamar Blue asssay to determine drug sensitivity of African trypanosomes (T. b. rhodesiense, T. b. gambiense) in vitro. Acta Trop., 1997; 68: 139–147.
18. Buckner FS, Verlinde CL, la Flamme AC, van Voorhis WC. Efficient technique forscreening drugs for activity against Trypanosomacruzi using parasites expressing beta-galactosidase. Antimicrob. Agents Chemother. (Bethesda), 1996; 40: 2592–2597.
19. Msaad K, Salem N, Tammar S, Hammami M, Saharkhiz MJ, Debiche F, Limam N, Marzouk N. Essential oil composition of Lavandula dentata, L. stoechas and L. multifida cultivated in Tunisia," J. Essen.Oil Bearing Plants, 2012; 15: 1030-1039.

20. Camacho MDR, Phillipson JD, Croft SL, Solis PN, Marshall SJ, Ghazanfar SA. Screening of plant extracts for antiprotozoal and cytotoxic activities. J. Ethnopharmacol., 2003; 89:185–191.
21. Sharma U, Velpandian T, Sharma P, Singh S. Evaluation of anti-leishmanial activity of selected Indian plants known to have antimicrobial properties. Parasitol. Res., 2009; 105: 1287–1293.
22. Sen N, Banerjee B, Das BB, Ganguly A, Sen T, Pramanik S, Mukhopadhyay S, Majumder HK. Apoptosis is induced in leishmanial cells by a novel protein kinase inhibitor withaferin A and is facilitated by apoptotic topoisomerase I-DNA complex. Cell Death Differ., 2007; 14: 358–367.
23. Tripathi CD, Gupta R, Kushawaha PK, Mandal C, Misra Bhattacharya S, Dube A. Efficacy of Withania somnifera chemotypes NMITLI - 101R, 118R and Withaferin A against experimental visceral leishmaniasis. Parasite Immunol., 2014; 36: 253-65.
24. Abou-El-Hamd EM, Mohamed-Elamir FH, Moustafa MFM, et al., Euphorbia helioscopia: chemical constituents and biological activities. Int. J. Phytopharmacol., 2012; 3: 78–90.
25. Mazoir N, Benharref, A, Bailén M, Reina M, González-Coloma A, Martínez-Díaz RA. Antileishmanial and antitrypanosomal activity of triterpene derivatives from latex of two Euphorbia species. Z. Naturforsch. C, 2011; 66: 360–366.