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ORIGINAL ARTICLE

Biologically-guided isolation of leishmanicidal secondary metabolites from *Euphorbia peplus* L.

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Abstract Leishmaniasis is a worldwide health problem, highly endemic in developing countries. Moreover, the severe side effects and the reported drug resistance make it an urgent need to search for effective drugs that can replace or supplement those currently used. In a research program designed to investigate the antileishmanial activity of plants collected from the Egyptian flora, twenty extracts from fifteen plants growing in Egypt have been investigated for *in vitro* leishmanicidal activity against *Leishmania donovani* promastigotes. Among the tested extracts, the methanol extract of *Euphorbia peplus* aerial parts exhibited a significant antileishmanial activity as it produced 100% inhibition of growth with activity similar to amphotericin B. The total extract was subjected to liquid-liquid fractionation using solvents of different polarities, followed by testing the antileishmanial activity of the successive fractions. Phytochemical exploration of the active *n*-hexane fraction (which produced 75% inhibition of growth) led to isolation of four compounds: simiarenol **(1)**, 1-hexacosanol **(2)**, β-sitosterol **(3)**, and β-sitosterol-3-*O*-glucoside **(4)** from the biologically active sub-fractions. Structure elucidation was aided by 1D and 2D NMR techniques. In conclusion, *E. peplus* plant has many non-polar secondary metabolites that can be used as drug leads for treatment of leishmaniasis.

1. Introduction

Leishmaniasis is a vector-borne disease which is transmitted by sandflies. It is caused by about 20 different species of the genus *Leishmania* (Habtemariam, 2003). This disease has a wide range of clinical symptoms that ranges from self-healing ulcers, which is called cutaneous leishmaniasis, to progressive nasopharyngeal infections (mucocutaneous leishmaniasis) and fatal disseminating visceral leishmaniasis (Ahua et al., 2007). Due to the occurrence of visceral leishmaniasis as an opportunistic infection in HIV-infected patients, the expansion of the AIDS pandemic makes the emergence of leishmaniasis/HIV co-infection a serious problem (Rocha et al., 2005). Since long time attention was paid toward the promising potential of medicinal plants for treatment of prevailing ailments. Moreover, the high cost and limited availability of effective pharmaceutical products suggest the use of native plants for symptomatic treatment of leishmaniasis in areas where it is

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endemic. In traditional medicine, the treatment of this disease usually consists of oral administration of crude plant extracts for visceral leishmaniasis, and topical preparations for the treatment of skin infections (Chan-Bacab and Peña-Rodríguez, 2001).

The genus *Euphorbia* comprises the largest among genera of the family Euphorbiaceae. It includes about 1600 known species (Ali et al., 2013), ranging from annuals to trees; all contain latex and have unique flower structure. Some of the reported folk medicinal uses of *Euphorbia* include treatment of skin diseases, gonorrhea, migraines, intestinal parasites, and warts (Jassbi, 2006). In Iran some species are used as purgative (Upadhyay et al., 1976). In addition, different species of *Euphorbia* contain macrocyclic diterpenoids with antibacterial, anticancer, PGE2-inhibitory, anti-HIV, and analgesic activity (Jassbi, 2006). *Euphorbia peplus* L. is originally native to Europe and North Africa (Zhi-Qin et al., 2010). The plant has a milky sap that is used in traditional medicine for treatment of non-melanoma skin cancer; the active compounds have been determined to be diterpene esters (Ramsay et al., 2011). In our search for leishmanicidal secondary metabolites, twenty extracts from fifteen plants growing in Egypt have been investigated for *in vitro* leishmanicidal activity against *Leishmania donovani* promastigotes. Among the tested extracts, the methanol extract of *E. peplus* aerial part exhibited a powerful leishmanicidal activity. Moreover, biologically-guided isolation of four compounds from the aerial parts of *E. peplus* is presented.

2. Materials and methods

2.1. General experimental

1D and 2D NMR spectra were recorded on a Bruker Avance III 400 MHz with BBFO Smart Probe and Bruker 400 MHz AEON Nitrogen-Free Magnet (Bruker AG, Switzerland) using the chemical shift of $CDCl_3$ solvent peak at 7.24 (s) ppm in ^1H and 77.2 (t) ppm in ^{13}C NMR as an internal reference standard. Data were analyzed using Topspin 3.1 Software. LC-ESIMS was obtained using a Bruker Bio Apex FT-MS in ESI mode.

2.2. Material for chromatography

Thin layer chromatography (TLC), pre-coated silica gel 60 F_{254} plates (Fisher Scientific, Suwanee, GA) for TLC; developing system: *n*-hexane-EtOAc (8:2 and 7:3) and visualization using 10% H_2SO_4 in MeOH. Column chromatography (CC) was performed with silica gel (230–400 mesh) and Sephadex LH-20 (Pharmacia Biotech, Uppsala).

2.3. Plant materials

All plant materials were collected in Egypt and were identified by Dr. M. Elgebaly, Faculty of Science, Cairo University. Botanical names and plant parts are listed in Table 1. Voucher samples were deposited in the Pharmacognosy Department, Faculty of Pharmacy, Beni-Suef University, with voucher numbers listed in Table 1. *E. peplus* L. was collected on March, 2013, from El-Nil public garden, Beni-Suef, Egypt.

Table 1 Plant list and their primary antileishmanial screening result.

Family and plant name (voucher no.)	Part used	% of inhibition
Acanthaceae		
Adhatoda vasica (BUPD-45)	Leaf	45
	Stem	14
Aizoaceae		
Mesembryanthemum crystallinum (BUPD-22)	Aerial part	0
Mesembryanthemum forsskaolii (BUPD-21)	Aerial part	14
Aizoon canariensis (BUPD-46)	Aerial part	17
Trianthemum portulacastrum (BUPD-47)	Aerial part	20
Asteraceae		
Tagetes patula (BUPD-48)	Leaf	12
	Stem	10
	Flower	15
Brassicaceae		
Brassica rapa rapa (BUPD-49)	Aerial part	8
Chenopodiaceae		
Anabasis setifera (BUPD-50)	Aerial part	5
Atriplex lindleyi (BUPD-51)	Aerial part	6
Euphorbiaceae		
Euphorbia helioscopia (BUPD-52)	Aerial part	48
Euphorbia peplus (BUPD-53)	Aerial part	100
Gramineae		
Sorghum bicolor (BUPD-54)	Seedlings	19
Solanaceae		
Solanum nigrum (BUPD-55)	Leaf	14
	Stem	14
	Flower	18
Zygophyllaceae		
Zygophyllum coccineum (BUPD-56)	Aerial part	14
Zygophyllum decumbens (BUPD-57)	Aerial part	6

2.4. Extraction

The collected samples (200 g each) were dried under controlled temperature not exceeding 45 °C, pulverized and then extracted with 80% methanol (300 ml × 3) by percolation. The extracts were then dried under reduced pressure at temperature not exceeding 45 °C. Preliminary testing of the prepared extracts used for antileishmanial activity against the protozoan *L. donovani* (National Center for Natural Products Research NCNPR, University of Mississippi, Oxford, MS, USA), revealed significant activity for the alcohol extract of *E. peplus* (Family Euphorbiaceae). The air-dried aerial part of *E. peplus* (1 kg) was pulverized using a laboratory mill and extracted