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ORIGINAL ARTICLE

In vitro Antileishmanial Activity of Methanolic Extracts for Some Selected Medicinal Plants

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ABSTRACT

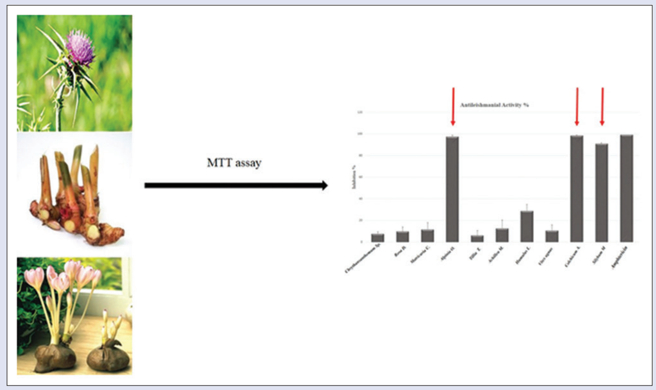
Objective: The aim of this study is to evaluate the antileishmanial activity of selected medicinal plants; ten well-known medicinal plants cultivated and growing under African environmental conditions were studied. **Materials and Methods:** The methanolic extracts of these plants were screened for their antileishmanial activity against *Leishmania major* using 3-(4.5-dimethylthiazol-2yl)-2.5-diphenyltetrazolium bromide assay. **Results:** The methanol extract of *Colchicum autumnale* and *Alpinia officinarum* showed potent antileishmanial activity at inhibition% value of 98.29% ± 0.75% and 97.25 ± 1.63%, respectively, while *Silybum marianum* exhibited inhibition% value of 90.97% ± 1.13%, compared with the standard amphotericin B (89.31% ± 2.08%). **Conclusion:** Considering these results, medicinal plants from African environment could constitute a developer source for antileishmanial compounds.

Key words: 3-(4.5-dimethylthiazol-2yl)-2.5-diphenyltetrazolium bromide assay, *Alpinia officinarum*, amphotericin B, antileishmanial, *Rosa damascene*, *Silybum marianum*

SUMMARY

- The medicinal plants from African environment such as *Colchicum autumnale* and *Alpinia officinarum* could establish a developer source for antileishmanial compounds.

Abbreviations used: FBS: Fetal bovine serum; MTT: 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium; DMEM: Dulbecco's modified Eagle's medium; DMSO: Dimethyl sulfoxide.



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INTRODUCTION

Leishmania is a genus of trypanosomatid protozoa and is the parasite responsible for the disease leishmaniasis. It spreads through sandflies. Their primary hosts are vertebrates; *Leishmania* commonly infects hyraxes, Canidae, rodents, and humans and currently affects more than 12 million people. Leishmaniasis is a disease with a prolonged worldwide distribution in 98 countries.[1] Leishmaniasis is considered as a serious health problem worldwide, especially in Africa, where it is significant morbidity and mortality.[2,3] Chemotherapy of leishmaniasis is still inspiring, due to the limitation of the efficiency of the drug, for example, miltefosine was the first oral antileishmanial drug that is considered for the treatment of visceral leishmaniasis in India and Germany and for cutaneous leishmaniasis in Colombia. *In vitro Leishmania promastigotes* resistant to miltefosine concentrations of up to 40 μM were easily produced and resistance was conferred to the intracellular amastigote stage.[4,5] Amphotericin B was originally extracted from *Streptomyces nodosus*. Amphotericin B deoxycholate (Fungizone®), a micellar formulation, is highly effective. It is used as first-line treatment in zones with high rates of unresponsiveness to antimonials and second-line treatment elsewhere.[6] In addition to many other drugs such as pentavalent antimonials, paromomycin, sitamaquine, 2-substituted quinoline alkaloids, buparvaquone, and 8-aminoquinolines, solid nanoparticles of amphotericin B deoxycholate have shown activity against *Leishmania donovani*. However, many parasites are resistant to these drugs. Medicinal plants are a good source of bioactive phytochemicals that showed several pharmacological properties such as antibacterial,[4,5] antioxidant,[7] antitumor,[8] antifungal,[9] anti-litholytic,[10,11] and antileishmanial activities.[12,13] These secondary metabolites are complex molecules with various functional structures such as polyphenols, flavonoids, terpenoids, and coumarins.[14] In this way, recent studies that focused on antileishmanial activities of medicinal plant products showed the success of these products in the inhibition of growth of several *Leishmania* species such as *Leishmania major* (cutaneous leishmaniasis) and *Leishmania infantum* (visceral leishmaniasis).[15] However, there is an under exploitation of the explored medicinal plants such as *Alpinia*

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officinarum, *Achillea millefolium*, *Colchicum autumnale*, *Chrysanthemum morifolium*, *Humulus lupulus*, *Matricaria chamomilla*, *Tilia tomentosa*, *Rosa damascena*, *Silybum marianum*, and *Vitex agnus-castus*. These plants have important charges of phenolic and flavonoid contents and possess significant antibacterial and antioxidant effects.[16-45] The recent studies suggested that the following *in vitro* and *in vivo* models, respectively, are the most suitable for the assessment of antileishmanial drugs: *L. major*–C57BL/6 mice (or–vervet monkey, or–rhesus monkeys), *Leishmania tropica*–CsS-16 mice, *Leishmania amazonensis*–CBA mice, *Leishmania braziliensis*–golden hamster (or–rhesus monkey).[46] The aim of our study was the screening of the antileishmanial activity of some selected plant extracts against *L. major*.

MATERIALS AND METHODS

Plant materials

The plants were collected in February 2014 from Botanical Garden, Giza, Egypt. The plant was kindly identified by Eng. Esraa Mohamed, Department of Agricultural Chemistry, Faculty of Agriculture, Minia University, Egypt. A voucher specimen of the plant was deposited in the Herbarium of the Department of Agricultural Chemistry, Faculty of Agriculture, Minia University, Egypt (Mn-Agri-1-10). The collected parts of plants [Table 1] were separated, cleaned from dust, and placed in the shade inside a well-ventilated room until were completely dried and weight was obtained. Dried parts of plants were grounded to a fine powder.

Solvents and chemicals

Dulbecco's modified Eagle's medium (SIGMA), penicillin-streptomycin (WAKO 119-00703), fetal bovine serum (FBS), and 3-(4,5 Dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium (MTT) was obtained from Nacalai Tesque.

Preparation of plant extracts

The air-dried powdered parts (250 g) of *A. officinarum*, *A. millefolium*, *C. autumnale*, *C. morifolium*, *H. lupulus*, *M. chamomilla*, *T. tomentosa*, *R. damascene*, *S. Marianum*, *V. agnus-castus* were extracted with 70% methanol (3 L × 3) till exhaustion and concentrated under reduced pressure at 50°C using a rotary evaporator to yield viscous gummy materials, and then, they subjected to drying in vacuum desiccators (oil pump) to yield 84.58, 68.67, 91.46, 56.34, 102.78, 62.56, 78.74, 86.60, 82.45, and 96.21 g, respectively. All the extracts kept in the dark bottles at 4°C (cold room).

Determination of the antileishmanial activity

The leishmanial activities of methanolic extracts were performed using the colorimetric MTT assay. Medium 199 supplemented with 10% heat-inactivated FBS and 100 μg/ml of kanamycin was used as the cell culture medium. The methanolic extracts were dissolved in dimethyl sulfoxide (DMSO) and added to each well of the 96-well micro-titration plates at 1% as the final concentration. *L. major* cells (2×10^5 cells/well) were cultured in a CO_2 incubator at 25°C for 72 h, and then, MTT solution was added to each well and the plates were incubated overnight at 25°C. The absorbance was measured at 540 nm using a Molecular Device Versamex tunable microplate reader. Amphotericin B was used as a positive control.[47] The inhibition% was calculated using the following equation:

$$\%\text{Inhibition} = [1 - (A_{sample} - A_{blank})/(A_{control} - A_{blank})] \times 100$$

Where $A_{control}$ is the absorbance of the control reaction mixture (containing DMSO and all reagents except for the methanolic extracts). IC_{50} was determined as the concentration of the sample required to inhibit the formation of MTT formazan by 50%.[47]

Data analysis

The analysis was performed using data analysis and statistical application available for Microsoft Excel (XLSTAT 2018.3.16, Florida, USA).

RESULTS

The aim of this study was to evaluate the antileishmanial activity for ten methanolic plant extracts.

Antileishmanial activity of the plant extracts

The results are shown in Table 2 and Figure 1. The methanolic extract of *C. autumnale* and *A. officinarum* with concentration 100 μM showed antileishmanial activity at inhibition% value of 98.29% ±0.75% and 97.25% ±1.63%, respectively, compared to the standard amphotericin B (99.13% ±2.08%), while the remaining tested extracts had no antileishmanial activity.

DISCUSSION

A. officinarum, A. millefolium, C. autumnale, *C. morifolium*, *H. lupulus, M. chamomilla*, *Tilia tomentosa, R. damascena, S. marianum,* and *V. agnus-castus* were found to have multiple biological activities and broad traditional uses against infectious and non-infectious diseases. *A. officinarum* is used in folk medicine as anticancer, antioxidant, antifungal, and antimicrobial.[18-21] Conventionally, *A. millefolium* is used as antiseptic, antispasmodic, astringent, carminative, diaphoretic, digestive emmenagogue, stimulant, tonics, vasodilator, and vulnerary and also used against colds, cramps, fevers, and kidney disorders.[22,23] *C. autumnale* is used as anti-inflammatory, antimitotic, and antifibrotic activity and involved in the inhibition of microtubule formation.[24,25] *C. morifolium*

Table 1: List of the screened selected plants and their collected parts

Scientific name	Common name	Family	Part used
Alpinia officinarum	Galangal	*Zingiberaceae*	Rhizomes
Achillea millefolium	Yarrow	*Asteraceae*	flowers and stems
Colchicum autumnale	Autumn crocus	*Colchicaceae*	corms and bulbs
Chrysanthemum morifolium	Mums	*Asteraceae*	Flowers
Humulus lupulus	Hop	*Cannabinaceae*	Flowers and leaves
Matricaria chamomilla	Chamomile	*Asteraceae*	Flowers
Tilia tomentosa	Silver linden	*Malvaceae*	Flowers
Rosa damascene	Damask rose	*Roseaceae*	Flowers
Silybum Marianum	Milk thistle	*Asteraceae*	Seeds
Vitex agnus-castus	Chaste tree	*Lamiaceae*	Flowers, seeds and leaves

Table 2: Antileishmanial activity percentage and IC_{50} values for the selected plants

Plant extracts	Percentage inhibition (100 μg/ml)	IC_{50}
Alpinia officinarum	97.25±1.63	65.16±2.71
Rosa damascena	NA	>100
Silybum marianum	90.97±1.13	77.34±3.01
Colchicum autumnale	98.29±0.75	60.09±0.81
Humulus lupulus	NA	>100
Achillea millefolium	NA	>100
Chrysanthemum morifolium	NA	>100
Matricaria chamomilla	NA	>100
Tilia tomentosa	NA	>100
Vitex agnus-castus	NA	>100
Amphotericin B	99.13±2.08[a]	58.75±1.09

[a]0.5 μM. Inhibition percentage and IC_{50} results expressed as mean values±SD of triplicates. NA: Not active (<20% for antileishmanial); SD: Standard deviation

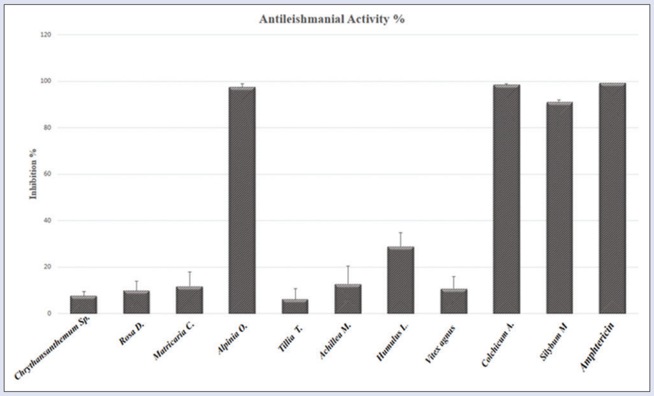

Figure 1: The antileishmanial activity at a concentration (100 μM) of the plant extracts. Inhibition percentages are expressed as mean values ± standard deviation of triplicates

possesses antihepatotoxic and antigenotoxic effects.[26] It exhibits an allelopathic activity[27] and has anti-inflammatory, immunomodulatory, humoral, and cellular and mononuclear phagocytic activities.[28] *H. lupulus* is traditionally used to relieve insomnia, anxiety, excitability, restlessness associated with tension, headache, and gastrointestinal spasms.[29,30] *M. chamomilla* showed different pharmacological activities such as anti-inflammatory, anticancer, anti-allergic activities and is used in the treatment of stress and depression.[31,32] *T. tomentosa* has been used as diuretic, diaphoretic, antispasmodic, stomachic, and sedative activities and has been taken for the treatment of flu, cough, migraine, nervous tension, ingestion problems, various types of spasms, and liver disorders.[33-35] *R. damascena* has been used as cardiotonic, mild laxative, anti-inflammatory, cough suppressant, anti-HIV, antibacterial, and antitussive.[36-41] *S. marianum* has hepatoprotective and antidepressant activities and is used in the treatment of diabetes, varicose veins, selenic congestions, amenorrhea, and uterine hemorrhage.[42-44] The essential oils of *V. agnus-castus* have antifungal and antimicrobial activities.[45]

The therapeutic targets and the mode of action for some chemotherapeutic agents such as miltefosine suggested that uptake of miltefosine into *L. donovani* is mediated by a plasma membrane P-type ATPase aminophospholipid translocase.[48] The proposed targets of miltefosine in *Leishmania* include perturbation of ether-lipid metabolism, glycosylphosphatidylinositol anchor biosynthesis and signal transduction[49] as well as inhibition of the glycosomal located alkyl-specific acyl-Co-A acyltransferase, an enzyme involved in lipid–remodeling.[50] Recently, mitochondria and specifically the cytochrome c oxidase have been implicated as a target of miltefosine in *L. donovani* promastigotes.[51] Effects on lipid metabolism, specifically phospholipid content, fatty acid, and sterol content, have also been described in *L. donovani* promastigotes.[52] However, paromomycin as a chemotherapeutic agent in *Leishmania* spp. has implicated mitochondrial membrane depolarization, ribosomes, and respiratory dysfunction in the mode of action of this molecule.[53] In the present study, the antileishmanial activity of ten plants was evaluated for the first time. The methanol extract of *C. autumnale* and *A. officinarum* showed potent antileishmanial activity at inhibition% value of 98.29% ± 0.75 and 97.25% ± 1.63%, respectively, at concentration 100 μg/ml with IC_{50} 60.09 ± 0.81 and 65.16 ± 2.71 μg/ml, respectively, while *S. marianum* exhibited inhibition % value of 90.97% ± 1.13% with IC_{50} 77.34 ± 3.01 μg/ml, compared with the standard amphotericin B (89.31% ±2.08%).

The remaining plant extracts did not show any antileishmanial activity at a concentration of 100 μg/ml.

CONCLUSION

The results demonstrate that the medicinal plants are a good source of new antileishmanial drugs. Future studies will be conducted to study the different fractions of the most effective extracts to identify the main phenolic components responsible for the antileishmanial and anticancer activities.

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Conflicts of interest

The authors declare no conflict of interest

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