Annals of Clinical Cytology and Pathology Antileishmanial Potential of Crude Plant Extracts Derived from Medicinal Plants in Palestine

Article - June 2017

CITATIONS
0

READS
472

10 authors, including:

Omar Hamarsheh
Al-Quds University
53 PUBLICATIONS 506 CITATIONS

Kifaya Azmi
Al-Quds University
60 PUBLICATIONS 652 CITATIONS

Ahmad Amro
Al-Quds University
39 PUBLICATIONS 384 CITATIONS

Ziad Abdul Muhsen Abdeen
Al-Quds University
278 PUBLICATIONS 6,662 CITATIONS

Some of the authors of this publication are also working on these related projects:

Lipopolysaccharide influence on leptin hormone and tumor necrosis factor-alpha release from human adipose tissue View project

the Case of Community Acquired Methicillin-Resistant Staphylococcus aureus and Multi-Drug-Resistant Streptococcus pneumoniae View project
INTRODUCTION

Through much of human history, plants have been used in medical treatments; such traditional medicine is still widely practiced today. Moreover, a huge number of novel drug components have been isolated from natural plant sources, where many of these plants and their extracts were used in traditional medicine [1,2]. This natural secretes, in the form of herbal remedies, could be explored based on information collected from local residents and traditional practitioners in different parts of the world [3-5]. Investigations carried out on folk medicinal plants with a potential of being curative have provided many clinical drugs for various infectious diseases. It has been reported that many plant-derived compounds regarded as important drugs currently in use, the majority of these compounds were derived from traditional medicines [6-9]. Leishmaniasis is a disease caused by different species of Leishmania parasites and is transmitted by the bite of a female phlebotomine sand fly. Depending on the parasite species, three different forms of the disease existed, ranging from the mild self-limiting cutaneous form, but causing disfigurement of the skin and lifelong scars, to more severe and life threatening mucucutaneous and visceral forms. Leishmaniasis is a disease with a large worldwide distribution, endemic in 98 countries where about 1/3 of the cutaneous cases occur in the Americas, the Mediterranean basin and western Asia with 70-75% of the cases registered in Afghanistan, Algeria, Colombia, Brazil, Iran, Syria, Ethiopia, Costa Rica, Peru and northern Sudan [10]. Incidence of the disease is increasing worldwide due to the expansion of international travel, especially among countries that are at war and/or where there are no effective vaccines for humans [11,12]. Additional problems are the emergence of strains resistant to...
Chemotherapy of parasitic diseases including leishmaniasis is still challenging, the drug efficacy is mostly limited by the inability of the pharmaceuticals to reach its target in a sufficient concentration and for a sufficient duration. The available drugs currently in use for the treatment of leishmaniasis include the followings: pentavalent antimonials; N-methylglucamine and antimoniate are considered the first-line treatment and, as a second option, amphotericin B or pentamidine. However, these drugs are disadvantaged by emergence of resistant parasites, parental administration, lethal side effects, high price and low availability especially in low-income and developing countries. The absence of vaccines and other effective prophylactic measures indicates the need for new therapies against leishmaniasis to cure people in endemic areas. Natural products of plant origin are potential preparations have been used for centuries to treat empirically parasitic diseases including leishmaniasis for people around the world which stimulating clinical and laboratory research [17,18].

Palestine is distinguished for its availability of medicinal plants because of the unique geographical location and biodiversity; these plants have been used for a long period of time to treat various illnesses. The Palestinian mountains are rich in plant species; about 2953 species are found with more than 700 species being mentioned in published ethnobotanical data as medicinal herbs or as botanical pesticides [19-21].

In the present study, we investigated the in vitro antileishmanial activity of crude extracts from 20 medicinal plants from various regions in Palestine. These plants were used by the local people to treat many infectious diseases and have never evaluated for their activity against Leishmania parasites.

**MATERIALS AND METHODS**

**Selection and collection of plant materials**

The list of medicinal plants in Palestine was reviewed with the aid of local traditional practitioners and botanists. Literature survey has been performed for specific reports on traditional, medical and therapeutic importance of Palestinian medicinal plants. Plant names were selected based on information available in the literature about their anti-leishmanial, anti-protozoal, anti-parasitic, antimicrobial or anti-oxidant activities, other plants were selected based on their uses in the Palestinian traditional medicine to treat bacterial, fungal or parasitic infections. Plant materials were collected during spring and summer of the year 2011, identified by a botanist and the voucher numbers were deposited at Al-Quds University Gardens (AQUG) and available upon request. Based on the traditional reports on the plant part used medicinally [17,22,23], the whole plant (except roots), flowers, fruits or seeds were collected, washed with distilled water, air dried in the shade for 20 days and then powdered in an electric grinder. The list of plant names used in this study can be found in Table (1).

**Preparation of the crude extracts**

Aqueous and organic extractions were done for each plant; each powdered plant material was extracted by maceration of plant powder in absolute ethanol and dimethyl sulfoxide (DMSO) separately for 72 hours at room temperature with gentle shaking. The quantity of solvent used for each extraction was 10 times the quantity of plant material. The filtrate obtained through Whatman No. 1 filter paper was concentrated under reduced pressure in a rotary evaporator at 30 °C. The extraction yields were calculated and the plant crude materials were dissolved in their respective solvents to a concentration of 160 mg/mL. All crude were kept at room temperature and protected from light until further processing.

**Preparation of Leishmania major promastigotes**

Promastigotes of *L. major* (*1 × 10^6* parasites/well) were cultured in micro plates with 96 wells (Corning) containing Schneider’s medium with 10% heat inactivated fetal bovine serum (FBS), 100 IU/mL Penicillin and 100 μg/mL streptomycin. Promastigotes were then washed 3 times with phosphate-buffered saline (PBS) by centrifugation at 1500 rpm for 10 min at room temperature.

**In vitro test for anti-leishmanial activity**

The *in vitro* test was performed using Alamar Blue bioassay [24] and it includes the following: cultured promastigotes at the log phase (*1 × 10^6* parasites/mL) were seeded in 125 μl Schneider’s medium in 96-well flat-bottom micro-plate, and then, 1 μl of each crude extract dissolved in DMSO and EtOH were mixed separately in 124 μl culture medium and transferred into the well. The final concentration of EtOH and DMSO was less than 0.1% (v/v) as this concentration will not affect the parasite growth rate, mobility and morphology. Amphotericin B (0.5 μg/mL) was used as drug positive control while parasites only in culture media were used as growth control. Negative control was cultured in media only. Each crude extract was tested in triplicate. The micro plate was incubated at 26°C in 5% CO₂ for 24 h in which 10% Alamar Blue (Sigma) was added to each well and the plates were incubated at 26°C for another 24 h. Optical density values (test wavelength 450 nm; reference wavelength 630 nm) were measured using a micro plate reader. The decrease of fluorescence (which indicated inhibition) was expressed as the percentage of the fluorescence of the control cultures.

**Determination of the 50% effective concentration (IC₅₀)**

The IC₅₀ values at the 95% confidence interval were calculated using sigmoid dose-response curves (Graph Pad Prism version 5.01 software Inc., San Diego, CA).

**Phytochemical analysis**

The crude extracts that have anti-leishmanial activity were screened for the presence of different phytochemicals; alkaloids, anthocyanins and betacyanin, quinones, flavonoids, phenols, saponins, tannins, sterols, triterpenoids, terpenoids and acids, following the standard methods of analysis [25-27].

**RESULTS**

Twenty plant species belonging to 14 families were examined for their *in vitro* antiparasitic effect against *L. major* using the Alamar Blue bioassay method. Local names in Palestine, their
scientific names and medicinal and traditional uses are listed in Table (1). Antileishmanial activities and IC$_{50}$ results are listed in Table (2). Among the total crude extracts tested; five were found to have various levels of activities (20%), some extracts having significant antileishmanial activity with IC$_{50}$ values ranging from 8.83 to 100 µg/mL. Extracts with IC$_{50}$ less than 100 µg/mL were considered active. One prominent extract, Artemisia  \textit{inculta} of the ASTERACEAE (COMPOSITAE) family, was the most potent (activity 84.1% and IC$_{50}$= 8.8 ± 2.3µg/mL). This was considered as promising activity, as shown in Table (2). Others have moderate to very little activities with IC$_{50}$ values between 19.5 - 100 µg/mL, these include extracts of \textit{Malva sylvestris} of MALVACEAE family with leishmanicidal activity of 90.1% and IC$_{50}$ of 19.5 ± 16.3 µg/mL (Table 2). On the other hand, three plant extracts including \textit{Trigonella berythea}, \textit{Carthamus tinctorius}, and \textit{Paronychia argentea} showed very low or even negligible activities with IC$_{50}$ values between 37.01 – 77.84 µg/mL. Three plant extracts, \textit{Sinapis arvensis}, \textit{Crataegus aronia}, and \textit{Calotropis procera} may be considered inactive with IC$_{50}$ > 100 µg/mL. Twelve plant extracts including \textit{Moderatum capitatus}, \textit{Arum palustre}, \textit{Dittrichia viscosa}, \textit{Punica granatum}, \textit{Rosmarinus officinalis}, \textit{Nigella ciliaris}, \textit{Hibiscus sabdariffa}, \textit{Ficus carica}, \textit{Citrullus colocynthis}, \textit{Origanum majorana}, and \textit{Pimpinella anisum} were found inactive.

### Table 1: Selected plant species used in this study with scientific and popular names, plant parts used and their medical importance.

<table>
<thead>
<tr>
<th>No</th>
<th>Plant scientific name</th>
<th>English name</th>
<th>Parts used</th>
<th>AQUUG voucher number</th>
<th>Medical importance</th>
<th>Antimicrobial activities</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>\textit{Artemisia inculta} Deile</td>
<td>White Wormwood</td>
<td>Shoots</td>
<td>PS-Ai10</td>
<td>Anti-Helicobacter pylori</td>
<td></td>
<td>[40]</td>
</tr>
<tr>
<td>2</td>
<td>\textit{Coridothymus capitatus} (L.) Rehβf.</td>
<td>Capitate Thyme</td>
<td>Leaves</td>
<td>PS-Cc19</td>
<td>Antioxidant</td>
<td></td>
<td>[41]</td>
</tr>
<tr>
<td>3</td>
<td>\textit{Sinapis arvensis} L.</td>
<td>Mustard/wild</td>
<td>Shoots</td>
<td>PS-Sa11</td>
<td>Antibacterial</td>
<td></td>
<td>[19]</td>
</tr>
<tr>
<td>4</td>
<td>\textit{Arum palustre}</td>
<td>Arum</td>
<td>Leaves</td>
<td>PS-Ap20</td>
<td>Anticancer</td>
<td></td>
<td>[42]</td>
</tr>
<tr>
<td>5</td>
<td>\textit{Malva sylvestris} L.</td>
<td>Common Mallow</td>
<td>Leaves</td>
<td>PS-Ms50</td>
<td>Antileishmanial, Antibacterial, Anti-inflammatory</td>
<td></td>
<td>[43]</td>
</tr>
<tr>
<td>6</td>
<td>\textit{Carum carvi} L.</td>
<td>Caraway</td>
<td>Seeds</td>
<td>PS-Cc12</td>
<td>Treatment of Gastrointestinal disorders, increase flow of breast milk</td>
<td></td>
<td>[44]</td>
</tr>
<tr>
<td>7</td>
<td>\textit{Trigonella berythea} Boiss. &amp; Blanche</td>
<td>Fenugreek</td>
<td>Seeds</td>
<td>PS-Tb21</td>
<td>Anti-diabetic activity</td>
<td></td>
<td>[45]</td>
</tr>
<tr>
<td>8</td>
<td>\textit{Carthamus tinctorius} L.</td>
<td>Safflower</td>
<td>Flowers</td>
<td>PS-Ct33</td>
<td>Treatment of dysmenorrhoea, amenorrhea, postpartum abdominal pain</td>
<td></td>
<td>[46]</td>
</tr>
<tr>
<td>9</td>
<td>\textit{Paronychia argentea} Lam.</td>
<td>Silvery Whitlow</td>
<td>Flowers</td>
<td>PS-Pa15</td>
<td>Aphrodisiac, Diuretic</td>
<td></td>
<td>[47]</td>
</tr>
<tr>
<td>10</td>
<td>\textit{Dittrichia viscosa} (L) Greuter</td>
<td>Inula</td>
<td>Leaves</td>
<td>PS-Dv22</td>
<td>Antidiabetic, antiphlogistic, antiviral, antifungal, antibacterial, and antiseptic properties</td>
<td></td>
<td>[48]</td>
</tr>
<tr>
<td>11</td>
<td>\textit{Crataegus aronia} (L) Bosc ex DC</td>
<td>Hawthorn, Azarole</td>
<td>Leaves</td>
<td>PS-Ca55</td>
<td>Antioxidant, treatment of cardiovascular diseases</td>
<td></td>
<td>[49]</td>
</tr>
<tr>
<td>12</td>
<td>\textit{Punica granatum} L.</td>
<td>Pomegrante</td>
<td>Peel</td>
<td>PS-Pg70</td>
<td>Anticancer, anti-inflammatory, treatment of Osteoarthritis, traditional remedies against diarrhea, dysentery and intestinal parasites.</td>
<td></td>
<td>[50]</td>
</tr>
<tr>
<td>13</td>
<td>\textit{Rosmarinus officinalis} L.</td>
<td>Rosemary</td>
<td>Shoots</td>
<td>PS-Ro80</td>
<td>Anti-inflammatory, antitumor, antioxidant, antimicrobial, Antileishmanial, and Antitryptosomosal</td>
<td></td>
<td>[51]</td>
</tr>
<tr>
<td>14</td>
<td>\textit{Nigella ciliaris} DC.</td>
<td>Black cumin</td>
<td>Seeds</td>
<td>PS-Nc30</td>
<td>Anticancer, anti-oxidant</td>
<td></td>
<td>[21]</td>
</tr>
<tr>
<td>15</td>
<td>\textit{Hibiscus sabdariffa} L.</td>
<td>Roselle</td>
<td>Flowers</td>
<td>PS-Hs17</td>
<td>Treatment of Melanoma</td>
<td></td>
<td>[52]</td>
</tr>
<tr>
<td>16</td>
<td>\textit{Ficus carica} L.</td>
<td>Fig tree</td>
<td>Leaves</td>
<td>PS-Fc40</td>
<td>Antimicrobial, antifungal, antioxidant, antiviral, anti-inflammatory</td>
<td></td>
<td>[53]</td>
</tr>
<tr>
<td>17</td>
<td>\textit{Citrus colocynthis} (L) Schrader</td>
<td>Colocynth</td>
<td>Leaves</td>
<td>PS-Cc18</td>
<td>Antidiabetic</td>
<td></td>
<td>[54]</td>
</tr>
<tr>
<td>18</td>
<td>\textit{Origanum majorana} L.</td>
<td>Sweet-Marjoram</td>
<td>Leaves</td>
<td>PS-Om60</td>
<td>Antimicrobial, antioxidant</td>
<td></td>
<td>[56]</td>
</tr>
<tr>
<td>19</td>
<td>\textit{Calotropis procera} (Aiton) Dryand.</td>
<td>Apple of Sodom</td>
<td>Leaves</td>
<td>PS-Cp22</td>
<td>Anti-inflammatory</td>
<td></td>
<td>[57]</td>
</tr>
<tr>
<td>20</td>
<td>\textit{Pimpinella anisum} L.</td>
<td>Anis</td>
<td>Seeds</td>
<td>PS-Pa02</td>
<td>Antiviral, antioxidant, muscle relaxant, analgesic and anti-convulsant activity, hypoglycemic and hypolipidemic effect</td>
<td></td>
<td>[58]</td>
</tr>
</tbody>
</table>

**Abbreviations:** AQUUG: Al-Quds University Gardens; PS: Palestine
Among the active extracts, four were extracted with DMSO and only one with ethanol. Therefore, DMSO extracts were generally more active than the ethanol ones. The results of the qualitative phytochemical analysis of the five active extracts showed that anthocyanins and betacyanins are found in only one extract, *Paronychia argentea*, which also contain phenols, saponins, tannins, and acids. Flavonoids were found only in the most active extract, *Trigonella berythea* contains saponins, tannins, and triterpenoids. *Trigonella berythea* contains saponins, tannins, and acids. *Carthamus tinctorius* which has moderate activity contains saponins, tannins, terpenoids, and acids. Although none of the studied extracts contain alkaloids, quinones, or sterols, each of the studied plant extract contained at least two classes of secondary metabolites. The detailed phytochemical composition is shown in Table (3). The presence of these phytoconstituents is thought to be responsible for antileishmanial activity.

**DISCUSSION**

Medicinal plants have been known throughout history as most appropriate sources of active chemicals and their derivatives to be used as templates for designing and developing more effective compounds, preferably with fewer side effects. Most plants that have medicinal properties in Palestine have not yet been thoroughly evaluated for their biological activities. In vitro screenings of various medicinal plants currently used in traditional medicine are essential and important first steps to prove the efficacy and safety of these plants in the treatment of infectious diseases, especially leishmaniasis, in poor and developing countries.

This study is designed to obtain preliminary results on the antileishmanial effects of selected medicinal plants from Palestine on *L. major*. Our results strongly suggest that *Artemisia inculta* and *Malva sylvestris* could be promising for treatment of leishmaniasis demanding a search for new chemotherapeutic agents. However, further studies need to be carried out, in order to isolate, purify and characterize active ingredients in pure forms and to understand the mechanisms of action and to evaluate the highly active crudes for further drug development. Toxicity against human cells should be done once active materials have been purified. The cytotoxicity was not carried out at this level for two reasons; firstly the plants that showed activity against *Leishmania* promastigotes are edible and traditionally used as medicinal plants for the treatment of various illnesses, and

---

**Table 2:** In vitro antileishmanial activity and cytotoxicity of the plant extracts used in this study.

<table>
<thead>
<tr>
<th>No.</th>
<th>Plant Scientific name</th>
<th>Family name</th>
<th>AQUG voucher number</th>
<th>Solvent</th>
<th>Parts used</th>
<th>Antileishmanial activity (%)</th>
<th>IC50, µg/ml</th>
<th>Average ± Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Artemisia inculta</em> Delile</td>
<td>ASTERACEAE (COMPOSITAE)</td>
<td>PS-Ai10</td>
<td>DMSO</td>
<td>Shoots</td>
<td>84.1</td>
<td>8.83± 2.3</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td><em>Cordotymus capitatus</em> (L.) Rcb.f.</td>
<td>LAMIACEAE (LABIATAE)</td>
<td>PS-Cc19</td>
<td>Leaves</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td><em>Sinapis arvensis</em> L.</td>
<td>BRASSICACEAE (CRUCIFERAE)</td>
<td>PS-Sa11</td>
<td>Ethanol</td>
<td>Shoots</td>
<td>20</td>
<td>&gt;100</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td><em>Arum palaoestimuim</em> Boiss.</td>
<td>ARACEAE</td>
<td>PS-Ap20</td>
<td>Leaves</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td><em>Malva sylvestris</em> L.</td>
<td>MALVACEAE</td>
<td>PS-Ms50</td>
<td>DMSO</td>
<td>Leaves</td>
<td>90.1</td>
<td>19.50±16.3</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td><em>Carum carvi</em> L.</td>
<td>APIACEAE (UMBELLIFERAE)</td>
<td>PS-Cc12</td>
<td>Seeds</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td><em>Trigonella berythea</em> Boiss. &amp; Blanche</td>
<td>FABACEAE (LEGUMINOSAE)</td>
<td>PS-Th21</td>
<td>Ethanol</td>
<td>Seeds</td>
<td>30</td>
<td>77.84±46.2</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td><em>Carthamus tinctorius</em> L.</td>
<td>ASTERACEAE (COMPOSITAE)</td>
<td>PS-Ct33</td>
<td>DMSO</td>
<td>Flowers</td>
<td>82.1</td>
<td>37.0±0.001</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td><em>Paronychia argentea</em> Lam</td>
<td>CARYOPHYLLACEAE</td>
<td>PS-Pa15</td>
<td>DMSO</td>
<td>Shoots</td>
<td>74.5</td>
<td>77.80±46.3</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td><em>Dittrichia viscosa</em> (L.) Greuter</td>
<td>ASTERACEAE (COMPOSITAE)</td>
<td>PS-Dv22</td>
<td>Leaves</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td><em>Crataegus aronia</em> (L.) Bosc ex DC</td>
<td>ROSACEAE</td>
<td>PS-Ca55</td>
<td>DMSO</td>
<td>Leaves</td>
<td>31.9</td>
<td>&gt;100</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td><em>Punica granatum</em> L.</td>
<td>PUNICACEAE</td>
<td>PS-Pg70</td>
<td>Peel</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td><em>Rosmarinus officinalis</em> L.</td>
<td>LAMIACEAE (LABIATAE)</td>
<td>PS-Ro80</td>
<td>Shoots</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td><em>Nigella ciliaris</em> DC.</td>
<td>RANUNCULACEAE</td>
<td>PS-Nc30</td>
<td>Seeds</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td><em>Hibiscus sabdariffa</em> L.</td>
<td>MALVACEAE</td>
<td>PS-Hs17</td>
<td>Leaves</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td><em>Ficus carica</em> L.</td>
<td>MORACEAE</td>
<td>PS-Fc40</td>
<td>Leaves</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td><em>Citrus coloynthis</em> (L.) Schrader</td>
<td>CUCURBITACEAE</td>
<td>PS-Cc18</td>
<td>Leaves</td>
<td>Fruits</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td><em>Origanum majorana</em> L.</td>
<td>LAMIACEAE (LABIATAE)</td>
<td>PS-Om60</td>
<td>Leaves</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td><em>Calotropis procera</em> (Alton) Dryand.</td>
<td>APOCYNACEAE</td>
<td>PS-Cp22</td>
<td>Ethanol</td>
<td>Leaves</td>
<td>3</td>
<td>&gt;100</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td><em>Pinpinella anisum</em> L.</td>
<td>APIACEAE (UMBELLIFERAE)</td>
<td>PS-Pa02</td>
<td>Seeds</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** AQUG: Al-Quds University Gardens; PS: Palestine; DMSO: Dimethyl Sulfoxide
Table 3: Phytochemical composition of the five active plant extracts.

<table>
<thead>
<tr>
<th>Plant sample</th>
<th>Alkaloids</th>
<th>Anthocyanines &amp; Betacyanin</th>
<th>Quinones</th>
<th>Flavonoids</th>
<th>Phenols</th>
<th>Saponins</th>
<th>Tanins</th>
<th>Sterols</th>
<th>Triterpenoids</th>
<th>Terpenoids</th>
<th>Acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artemisia inculta Deile</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malva sylvestris L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trigonella berythea-Boiss. &amp; Blanche</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Carthamus tinctorius L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paronychia argentea Lam</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

secondly, the extracts are solely in crude and not in pure form and the active ingredient that confers toxicity for Leishmania is mixed up with many others that may have toxicity against human cells; therefore, cytotoxicity tests at this level may lead to exclusion of active crude extracts that may have toxic ingredients. The most active extracts come from the use of DMSO as extraction solvent, suggesting that the less polar compounds are responsible for the observed activity. Plants found active were also extracted with dichloromethane with almost the same activity, however dichloromethane is recommended since it has a boiling temperature much less than DMSO and hence easily evaporated and removed.

Natural products are potential sources of new and selective agents for the treatment of important tropical diseases caused by protozoans and other parasites [28-30]. The most active extract, Artemisia inculta, contains polyphenolic compounds like flavonoids and triterpenoids (Table 3) which are believed to have the ability to inhibit trypanosomal and leishmanial infections without significant toxicity to mammalian cells [31-33]. Different Artemisia species have been reported to have in vitro and in vivo activity against various Leishmania species; Artemisia annua leaves extract was proved to have leishmanicidal activity against L. donovani which causes visceral leishmaniasis [34,35]. The second promising plant is Malva sylvestris, reported in this study to have antileishmanial activity for the first time; it is an edible plant and people in Palestine used it extensivly.

The activity of these plants is believed to be structurdependent, according to literature flavonoids bind to C-terminal nucleotide-binding domain (NBD2) of the P. glycoprotein-like transporter in L. tropica which is involved in parasite multidrug resistance [36,37]. Flavonoids also inhibit important enzymes or proteins; flavonoids quercetin inhibits DNA topoisomerases, promoting site specific DNA cleavage resulting in the growth inhibition of L. donovani promastigotes and amastigotes [38]. In other parasites, flavonoids inhibit the synthesis of heat shock proteins (Hsp90, Hsp70, and Hsp27); these factors are important to protect virulent parasites from the effects of host immune responses [39].

Based on susceptibility tests using Alamar Blue Bioassay, phytochemicals are routinely evaluated for antileishmanial activity. For crude extracts, activity is considered to be significant if IC₅₀ values are below 20 μg/mL and moderate when 20 < IC₅₀ < 100 μg/mL. Therefore, the activity recorded with Artemisia inculta, Malva sylvestris, Coridothymus capitatus, Trigonella berythea, Carthamus tinctorius, and Paronychia argentea against L. major can be considered important. Previous reports documented leishmanicidal activity of Artemisia species from Iran against L. major promastigotes [35]. The genus Artemisia L. (Asteraceae) is a large, heterogeneous and widely distributed throughout the world. These species are perennial, biennial and annual herbs or small shrubs.

There was no toxicity mentioned in the literature or concerns about the use of these medicinal plants. The samples were not assayed on intracellular amastigote forms, which should be done the next step of evaluation and validation of these plants. Further analysis still to be done on the active crude; bio-guided fractionation should also be conducted and may lead to the isolation of the major components in the active crude.

CONCLUSION

Our study investigated twenty selected crude plant extracts and their antileishmanial activity. Among them, Artemisia inculta and Malva sylvestris exhibited promising results that may lead to the development of effective and affordable antileishmanial drugs. In developing countries, these results provide an alternative way to use plant-based remedies that might be safer, cheaper, and less toxic than existing prescription medications. This is an area rich in possibilities, and the world’s flora represents an enormous source of material for testing. However, further studies are needed, particularly bio-guided fractionation to identify the active faction and further chemical characterization of structure. This research belongs to the global effort carried by researchers around the world to locate compounds with antileishmanial activity by validating natural products as genuine sources for drug discovery.

ACKNOWLEDGEMENTS

The authors gratefully thank the Deutscher Akademischer Austauschdienst (DAAD) and Zamalah program for providing travel grant, IMIB - Institute for Molecular Infection Biology, for providing support to validate this work at Würzburg University, financial support by the Deutsche Forschungsgemeinschaft (SFB 630) given To HM is gratefully acknowledged.

REFERENCES

Antileishmanial activities associated with plants used in the Malian traditional medicine. | Ethnopharmacol. 2007;11:99-104.
19. Jaradat N, Masoud B, Abu-haddad M. Screening antibacterial and antifungal activities and evaluation of the exhaustive extraction yields for Verbasum sinuatum L. Int J Res Ayurveda Pharm. 2015;6:


