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Article in Phytoteraphy Research · December 1998
Impact Factor: 2.66 · DOI: 10.1002/(SICI)1099-1573(199812)12:8<576::AID-PTR354>3.0.CO;2-#

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The Potential Antileishmanial Activity of some Sudanese Medicinal Plants

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A preliminary examination of the crude methanol extracts of eight plant species collected from Sudan, revealed that only three plant species had a considerable in vitro antileishmanial activity on Leishmania major promastigotes at a concentration < 0.5 μg/mL. The plants Azadirchta indica, Maytenus senegalensis and Eucalyptus globulus gave IC50 values of 11.5, 55 and 78 μg/mL, respectively. Extracts of Pseudocedrela kotischeff and Balanites aegyptiaca had a moderate biological activity, whereas extracts of Sonchous cornatus, Khaya senegalensis and Tamarindus indica failed to exhibit any significant antileishmanial activity against L. major at concentrations <100 μg/mL. Liquid–liquid partitioning of the methanol extracts indicated that fractions of M. senegalensis in dichloromethane and ethyl acetate had the highest antileishmanial activity at 5 μg/mL; IC50 values were 5.01 and 29.7 μg/mL, respectively. Preliminary phytochemical analysis of the dichloromethane fraction revealed terpenoids and traces of phenolic principles but no alkaloid, tannins or flavonoids were detected. As lymphocyte proliferation was inhibited by P. kotischeff and A. indica at higher concentrations (<50 μg/mL) in the presence of phytohaemagglutinin (PHA), M. senegalensis had no significant toxic effect whereas S. cornatus, T. indica and K. senegalensis had a stimulatory impact on lymphocyte cells. © 1998 John Wiley & Sons, Ltd.


Keywords: Sudanese plants; antileishmanial activity; phytochemical analysis; lymphocyte proliferation.

INTRODUCTION

Leishmaniasis is considered as a major public health problem (WHO, 1989,1990) causing significant morbidity and mortality in Africa, Asia and Latin America. About 350 million are currently threatened by the disease, whereas 2–3 million are affected annually (WHO, 1990).

Few antileishmanial drugs, e.g. sodium stibogluconate (Pentostam), are currently prescribed for treatment of the disease but these drugs are in general toxic and administered as long-term treatment in a hospitalized setting. Such arrangements could not be maintained in many developing countries including Sudan where several outbreaks of leishmaniasis and epidemics have been reported recently (Proefschrift, 1995). In particular, southern Sudan where the state of civil war had predominated for several years, was seriously threatened by an epidemic of visceral leishmaniasis that affected >25% of the population (Chen, 1994).

However, inhabitants of many endemic areas of leishmaniasis in Sudan relied on traditional herbal preparations to treat themselves. In fact, WHO recently advocated the use of traditional medicine where appropriate health services become inaccessible.

This study therefore, aims to investigate the in vitro potential of antileishmanial activity of some medicinal plants claimed to be effective in Sudanese folk medicine.

MATERIALS AND METHODS

Plant preparations. Eight plant species (Table 1) that represent five families of Sudanese flora commonly used in traditional medicine were collected and identified in comparison with authenticated specimens at the herbarium of the Department of Botany, Faculty of Science, University of Khartoum. Voucher specimens were deposited and samples of each plant (100 g) were dried, coarsely powdered and soaked in 80% methanol over-night with continuous shaking at 37°C and filtered. The extracts were then completely dried by rotary evaporation and freeze drying and stored at −20°C.

Liquid–liquid separation of plant methanol extracts. The methanol extract of M. senegalensis (80% w/v) was...
separated by liquid–liquid partitioning using petroleum ether, dichloromethane, ethyl acetate and water to obtain four fractions. All fractions were concentrated to complete dryness and stored at −20°C.

**Parasite culture.** Promastigotes of the WHO reference vaccine strain of *Leishmania major* (5AKSH) were cultivated in medium 199 supplemented with gentamycin (0.02 mg/mL), HEPES (2 mM), L-glutamine (4 mM) and 10% heat inactivated fetal calf serum. Incubation of parasites was carried out at 26°C. Promastigotes were harvested on day 4 or 5 of the culture and used for the evaluation of antileishmanial activity of some plant preparations using a modified *in vitro* method based on Pearson *et al.* (1984).

**In vitro test.** The dry methanol extract of *M. senegalensis* and its fractionation products were dissolved or microcentrifuged in culture medium 199 using ultrasonication. Aliquots of each plant preparation with stock concentrations of 1 mg/mL were prepared in sterile glass bottles and then diluted with culture medium to working concentrations of 0.5, 5, 25, 50 and 100 μg/mL.

Volumes of 20 μL of each plant preparation (0.5–100 μg/mL) were added into cells of a 96-well Nunc microtitre plate containing 160 μL of culture medium enriched with promastigotes (3 × 10⁶/mL). Control wells received 180 μL of culture medium only. Promastigotes were then incubated at 26°C. After 2 h, 20 μL of ³H thymidine (New England Nuclear, Boston, MA, USA) was added into each well and incubated for another 24 h. The PBMC were harvested using a Skatron harvester and ³H thymidine incorporation was measured in a scintillation counter. The effect of crude extracts on human PBMC lymphocytes was estimated as previously calculated by Kemp *et al.* (1991).

**Lymphoproliferation assay for toxicity.** Human peripheral blood mononuclear cells (PBMC) from heparinized blood were isolated using lymphoprep solution (Nyegaard, Oslo, Norway). They were washed three times in RPMI 1640 medium (Gibco) supplemented with 15% pooled human serum and penicillin (20 IU) plus streptomycin (20 μg/mL) (Kemp *et al.*, 1991). Each cell of a 96-well round bottomed microtitre plate received 0.33 × 10⁶/mL PBMC in 150 μL of RPMI 1640. The plant extracts were diluted in the complete medium and 20 μL of each extract was added to PBMC culture to make final test concentrations of 0.5, 5, 50 and 100 μg/mL; 20 μL of phyto-haemagglutinin (47 μg/mL). (Difco Lab., USA) was added into all microplate wells except control ones.

The cultures were incubated at 37°C in a humidified atmosphere (5% CO₂) for 24 h and then 20 μL of ³H thymidine was added into each well and incubated for another 24 h. The PBMC were harvested using a Skatron harvester and ³H thymidine incorporation was measured as cpm in a liquid scintillation counter. The effect of crude extracts on human PBMC lymphocytes was estimated as previously calculated by Kemp *et al.* (1991).

### RESULTS

The preliminary examination of crude methanol extracts of eight plant species (Table 1) revealed that only three plant species namely, *A. indica*, *M. senegalensis* and *E. globulus* had a considerable antileishmanial activity in vitro against *L. major* promastigotes at concentrations >0.5 μg/mL; I⁵⁰ values were 11.5, 55 and 78 μg/mL, respectively (Table 2).

Extracts of *P. kotscifye* stem bark and *B. aegyptiaca* had a moderate biological activity, whereas extracts of *S. cornatus*, *K. senegalensis* and *T. indica* failed to exhibit

### Table 1. Medicinal plants screened for potential antileishmanial activity

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Family</th>
<th>Part used</th>
<th>Vernacular name</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Azadirachta indica</em></td>
<td>Meliaceae</td>
<td>Stem bark</td>
<td>Neem Tree</td>
</tr>
<tr>
<td><em>Balanties aegyptiaca</em></td>
<td>Balanitaceae</td>
<td>Seeds, stem bark</td>
<td>Hajleeg</td>
</tr>
<tr>
<td><em>Eucalyptus globulus</em></td>
<td>Myrtaceae</td>
<td>Seeds</td>
<td>El Ban</td>
</tr>
<tr>
<td><em>Khaya senegalensis</em></td>
<td>Mielaceae</td>
<td>Seeds</td>
<td>El Mahogany</td>
</tr>
<tr>
<td><em>Maytenus senegalensis</em></td>
<td>Celasteraceae</td>
<td>Stem bark</td>
<td>Dublab</td>
</tr>
<tr>
<td><em>Pseudocedrela kotscifye</em></td>
<td>Mielaceae</td>
<td>Stem bark</td>
<td>—</td>
</tr>
<tr>
<td><em>Sonchous cornatus</em></td>
<td>Asteraceae</td>
<td>Aerial shoots</td>
<td>Molaeta</td>
</tr>
<tr>
<td><em>Tamarindus indica</em></td>
<td>Caesalpiniaceae</td>
<td>Seeds</td>
<td>Aradddeb</td>
</tr>
</tbody>
</table>

### Table 2. *In vitro* biological activity of methanol plant extracts (0.5–100 μg/mL) on the promastigotes of *L. major*. The activity was estimated as growth inhibition percent

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Concentration of extract (μg/mL)</th>
<th>I⁵⁰ (μg/mL)⁸</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. indica</em></td>
<td>0.5</td>
<td>12%</td>
</tr>
<tr>
<td><em>B. aegyptiaca</em></td>
<td>0.5</td>
<td>20%</td>
</tr>
<tr>
<td><em>E. globulus</em></td>
<td>0.5</td>
<td>20%</td>
</tr>
<tr>
<td><em>K. senegalensis</em></td>
<td>0.5</td>
<td>2%</td>
</tr>
<tr>
<td><em>M. senegalensis</em></td>
<td>0.5</td>
<td>20%</td>
</tr>
<tr>
<td><em>P. kotscifye</em></td>
<td>NT</td>
<td>7%</td>
</tr>
<tr>
<td><em>S. cornatus</em></td>
<td>NT</td>
<td>0</td>
</tr>
<tr>
<td><em>T. indica</em></td>
<td>NT</td>
<td>1%</td>
</tr>
</tbody>
</table>

a I⁵₀ is the concentration of plant extract that caused 50% inhibition of parasite growth. NT, not tested.
any significant antileishmanial activity against *L. major* at concentrations >100 μg/mL.

As presented in Table 3, liquid–liquid partitioning of methanol extracts indicated that fractions of *M. senegalensis* in dichloromethane and ethyl acetate had the highest antileishmanial activity at 5 μg/mL; IC$_{50}$ values were 5.01, 29.7 μg/mL, respectively. The dichloromethane fraction of *M. senegalensis* apparently caused a parasite growth inhibition of 90% at a concentration of 25 μg/mL, but fractions partitioned in petroleum ether and ethyl acetate showed less growth inhibition at concentrations >50 μg/mL (Table 3).

Figure 1 presents the effect of crude extracts on the proliferation of lymphocytes with the addition of PHA stimulator. About 50% of lymphocyte proliferation was suppressed by extracts of *P. kotscifye* and *A. indica* (>50 μg/mL), whereas *M. senegalensis* had no significant effect at the tested concentration, 50 μg/mL. Interestingly, *S. cornatus*, *T. indica* and *K. senegalensis* significantly stimulated the lymphocytes at a concentration >50 μg/mL, *p* > 0.01 (Fig. 1).

**Table 3. In vitro antileishmanial activity of liquid–liquid fractionation products of the methanol extract of *M. senegalensis* (5.0–100 μg/mL)**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Concentration (μg/mL)</th>
<th>IC$_{50}$a (μg/mL)</th>
<th>PIb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether</td>
<td>1% 25 50 100</td>
<td>1% 1% 80% 97% 97%</td>
<td>35</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>51% 90% 94% 99%</td>
<td>5.01 25 46% 66%</td>
<td>2.9</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>42% 46% 66% 83%</td>
<td>29.7 2.9</td>
<td></td>
</tr>
<tr>
<td>Aqueous</td>
<td>26% 28% 33% 21%</td>
<td>&gt;100 &gt;100</td>
<td></td>
</tr>
</tbody>
</table>

a IC$_{50}$, the concentration of plant extract that caused 50% inhibition of parasite growth.

b PI, Pentostam index

**Figure 1. Effect of methanol crude extracts on phytohaemagglutinin stimulated human lymphocyte cells. cpm: mean count per minute of stimulated culture minus mean count per minute of unstimulated culture. ♦ *A. indica* stem bark; ■ *B. aegyptiaca*; ▲ *K. senegalensis* seed; × *M. senegalensis* stem bark; * *P. glabrum* leaves; ● *P. kotscifye* stem bark; + *S. cornatus* aerial part; - *T. indica* seeds.**

**DISCUSSION**

The use of herbal medicine is a common practice worldwide particularly in many developing countries including Sudan where primary health services are not always accessible.

The medicinal flora of Sudan has been described as rich and diversified but very few studies investigated the potential use in the treatment of parasitic diseases (Khalid et al., 1986; Ibrahim et al., 1992).

The study indicated that methanol crude extracts (0.5–100 μg/mL) of three plant species namely *A. indica*, *M. senegalensis* and *E. globulus* caused ≥ 60% growth inhibition of *L. major* promastigotes at IC$_{50}$ ≥ 78 μg/mL (Table 2). The potential activity of extracts from *A. indica* in the treatment of some parasitic diseases, e.g. malaria, had already been suggested in previous studies (Bray et al., 1985; Khalid et al., 1989; Rosoañairó et al., 1992).

However, human lymphocytes treated with extracts of *A. indica* (>50 μg/mL) demonstrated the minimum level of proliferation (Fig. 1) when compared with lymphocytes treated with preparations of other plant species. In contrast, the *M. senegalensis* methanol fraction had no toxic inhibitory effect upon lymphocytes at a concentration >100 μg/mL. Consequently, the methanol extract of this plant species was further partitioned. The dichloromethane fraction demonstrated the highest antileishmanial activity in vitro (IC$_{50}$, 5 μg/mL; PI, 0.5). In fact, >90% of promastigote growth was suppressed at a concentration ≥ 25 μg/mL (Table 3). It was also conceivable that some antileishmanial activity occurred in the fractions of petroleum ether and ethyl acetate with higher values of IC$_{50}$ and PI (Table 3).

Preliminary phytochemical analysis of the dichloromethane fraction of *M. senegalensis* revealed terpenoids and traces of phenolic principles but no alkaloid, tannins or flavonoids were detected (Table 4). However, spectroscopic analysis is currently being conducted to elucidate the chemical nature of the most active ingredients of *M. senegalensis*, but their antiparasitic effect and toxicity need to be verified *in vivo*.

On the other hand, *S. cornatus*, *T. indica*, *K. senegalensis* and *P. kotscifye* that were previously found to have some biological activity (Gessler et al., 1994; Hussein, 1994) failed to show any significant effect at IC$_{50}$>100 μg/mL upon *L. major* promastigotes in this study. Interestingly, extracts of these plants stimulated the proliferation of lymphocytes which implies their ability to potentiate the human immune system (Fig. 1). This perhaps justifies the use of such plant preparations...
amongst the Sudanese human population living in at risk areas for leishmaniasis.

In fact, several studies demonstrated the immunomodulatory action of many plant species. Labadie et al. (1989) for example, found that aqueous extracts of *Asparagus falcata*, *Piper longrum* and *Picrorhiza kurora*, selectively or predominantly inhibit the pathways of complement activation. As indicated by Bray et al. (1990) the beneficial therapeutic effect of many plant species of the Meliaceae family (e.g. *Cedrela odorata*, *A. indica, Guarea multiflora*) that had been claimed in the traditional treatment of malaria might be due to the antiinflammatory and immunomodulating activities recently described for these plants. Sreelekha et al. (1993) noted that a polysaccharide isolated from *T. indica* exerted an immunomodulating activity that enhanced leukocyte migration. The present study, therefore, emphasizes the need to carefully screen the Sudanese flora for plant species with possible immunomodulatory activity that may enhance recovery from leishmaniasis.

**REFERENCES**


<table>
<thead>
<tr>
<th>Principle</th>
<th>Test applied</th>
<th>Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Dragendorff reagent</td>
<td>Methanol: +, Petroleum ether: -</td>
</tr>
<tr>
<td>Phenolic</td>
<td>Ferric chloride</td>
<td>Dichloromethane: +</td>
</tr>
<tr>
<td>Tannins</td>
<td>Ferric chloride</td>
<td>Ethyl acetate: +</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Aluminium chloride</td>
<td></td>
</tr>
<tr>
<td>Saponins</td>
<td>RBCs haemolysis</td>
<td></td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Vanillin-sulphuric acid and anisaldehyde</td>
<td>++, +</td>
</tr>
</tbody>
</table>