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An Overview of Some Sudanese Medicinal Plants having or Expected to have a Potential Antileishmanial Activity

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ABSTRACT

Infections caused by protozoa of the genus Leishmania are a major worldwide health problem with high morbidity and mortality in developing countries especially Sudan. The incidence of the disease has amplified since the emergence of AIDS. Since the discovery of the first drugs for Leishmaniasis treatment (i.e., pentavalent antimonials), up to now the search for substances with antileishmanial activity, without toxic effects, and able to overcome the emergence of drug-resistant strains still remains as the current goal. Moreover, in the absence of a vaccine there is a crucial need for effective drugs to replace/supplement those in current use. The plant kingdom is undeniably treasured as a source of new medicinal agents. A thorough review of the literature on Sudanese plant extracts of natural origin either showing antileishmanial activity or expected to have, depending on the phytochemical screening is presented in this article. The review include 12 plants, their geographical distribution, the parts utilized, the type of extract, and the organism tested. The level of activity exhibited by the extracts depended largely on the type of solvent used for the extraction and the plant part used. Tannins demonstrated to be the key element of the antileishmanial properties and it is highlighted as potential source through the search for new Sudanese antileishmanial medicinal plants. The array of Sudanese medicinal plants that have demonstrated antileishmanial activity suggests that the hope to discover novel antileishmanial drugs is high.

Keywords: Leishmania, Sudanese medicinal plants, Tannins

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INTRODUCTION

Leishmaniasis is caused by the protozoan *Leishmania* parasites which are transmitted by the bite of infected female phlebotomine sandflies[1], in which they differentiate from proliferative promastigotes in the sand fly vector gut into infective metacyclic promastigotes in the insect foregut. Parasites are inoculated by the vector as the flagellate promastigotes infect the mammalian host, where they invade macrophages, forming the nonmotile amastigotes and multiplying as such [2]. Leishmaniases has three main forms, visceral (kala-azar, VL) which is the most severe form of the disease, cutaneous (CL) the most common, and mucosal leishmaniasis (ML). [1] visceral leishmaniasis is fatal if left untreated. It is characterized by irregular bouts of fever, weight loss, enlargement of the spleen and liver, and anemia. It is highly endemic in the Indian subcontinent and in East Africa. An estimated 200 000 to 400 000 new cases of VL occur worldwide each year [3].

The disease affects some of the poorest people and it is associated with malnutrition, population displacement, poor housing, a depressed immune system and lack of financial resources. Leishmaniasis is linked to environmental changes such as deforestation, the building of dams, irrigation schemes, and urbanization. An estimated 0.9–1.3 million new cases and 20 000 to 30 000 deaths occur annually worldwide. Over 90% of visceral leishmaniasis cases occur in six countries: Bangladesh, Brazil, Ethiopia, India, South Sudan and Sudan. This trend is slowly changing due to the progress in elimination in South-East Asia, where Bangladesh has reported an average of some 600 new cases in 2014−2015[1].

Over 95% of Visceral leishmaniasis cases are fatal if left untreated. It is characterized by irregular bouts of fever, weight loss, enlargement of the spleen and liver, and anemia. It is highly endemic in the Indian subcontinent and in East Africa. An estimated 200 000 to 400 000 new cases of VL occur worldwide each year [3].

The pentavalent antimonials drugs have been the suggested drugs used for the treatment of all forms of leishmaniasis [4], they were discovered over 60 years ago [5]. Although three new drug formulations, liposomal amphotericin B (AmBisome®), miltefosine and paromomycin were discovered for treatment of VL, all have been reported to suffer from limitations of cost, specific toxicities or parenteral administration [5].

Beside the new oral and topical treatment alternatives for the treatment of CL that have been developed within the past few years, a vaccine is yet to be established [4].

On the other hand, plant-derived compounds are possible to provide a great source of new medicinal agents [6,7]. The burning need for new and alternative treatments has led to screening of natural products for potential use as therapeutic agent for leishmaniases treatment. In fact, the WHO advocated the use of traditional medicine where suitable health services are inaccessible [8]. Furthermore, the leads obtained from the search for natural products with antileishmanial activity give a new source for adding valuable synthetic compounds to the current status[6].

The present review inspects the *in vitro* antileishmanial activity of crude extracts from 12 plants that grow in Sudan. These plants were already evaluated for their antileishmanial activity. The study also suggests a number of Sudanese medicinal plants that are highly suspected to have antileishmanial properties.

RESULTS AND DISCUSSION

Exploring of several literature sources resulted in the summarization of a list of natural products evaluated for antileishmanial activity (Tables 1) and their preliminary phytochemical screening (table 2). For details on the models or mechanism-based bioassays used for selecting crude plant extracts, pure compounds, and fractions, against the Leishmania parasite, the original references should be asked.

Sudanese crude plants extracts with antileishmanial activity

An *in vitro* assessment of Anti–*L major* (amastigote form, CL) activity of some Sudanese plants evaluated a total of 4 Sudanese plants: *Azadirachta indica* (Neem), *Acacia nilotica* (Laloob), *Balantis aegyptiaca* (Garad), and *Allium sativa* (Garlic). The different statistical measures using the standard agents (pentostam) showed that garlic and neem are the most active agents against *L. major* promastigotes compared to other extracts. They showed 44.04 & 46.667 means of growth inhibition respectively after 72 hours. Also, they showed the lowest mean differences with the standard 4.08 & 1.5 respectively. Garlic was the only extract that displayed insignificant difference with the standard agent (pentostam) (P=0.10) this reflects that garlic has powerful anti-leishmanial activity. Garlic also showed LC50 at concentration 4.94 µg/ml, (the standard showed LC50 equal 3.07µg/ml), *Acacia nilotica* and *Balantis aegyptiaca* both showed moderate antileishmanial properties [9].

The preliminary phytochemical screening of methanolic extract of *Acacia nilotica* showed that it contain saponins, flavonoids, anthraquinones , tannins, carbohydrates, alkaloids, and cardiac glycosides while proteins, amino acids, oils and fixed fats were not present [10]. *Balanitis aegyptiaca*

screening showed that it contains Saponins, tannins, phenols and anthraquinones [11]. while *Allium Sativum* phytochemical screening showed positive results for phenolic and tannins [12].

Methanolic extract of *Ambrosia maritima* screening showed the presence of Sterols, alkaloids and cardenolides [13] and preliminary phytochemical screening of the ethyl acetate extract of *A. nilotica* husk resulted in the presence of phenolic compounds and the tannins blue-black color was observed when ferric chloride reagent was added to a solution of the extract ([14]). Both of the aerial shoot extracts of *Acacia nilotica* husk and *Ambrosia miratima* showed a potent antileishmanial activity *in vitro* against *L. donovani* promastigotes t concentrations (IC_{50}) less than 8 µg/ml [14].

A mixture of tannin fractions and four other compounds (ellagic acid, gallagic acid, punicalins, and punicalagins) of *Punica granatum* showed antioxidant, antiplasmodail, and antimicrobial activities in a cell-based assays evaluation [15] *Punica granatum* also inhibited the growth of intracellular *Leishmania amazonensis* amastigotes, with IC50 values of 69.6 µg/mL [16]. Phytochemical screening tests of *Punica granatum revealed the presence of* phenols, glycosides, flavonoids, and tannins.

A preliminary *in vitro* examination of the crude methanol extracts of some Sudanese medicinal plants showed three plant species with considerable antileishmanial activity on *L major* promastigotes. The plants methanolic extracts: *Eucalyptus globulus, Maytenus senegalensis, and Azadirchta indica* gave IC50 values of 78, 55 and 11.5 mg/mL, respectively. The two extracts of *Pseudocedrela kotscifye* and *Balanites aegyptiaca* revealed a moderate antileishmanial activity[17]. The phytochemical screening of the five plants is shown in table 2.

The butanol, aqueous, and ethyl acetate fractions of *Anogeissus leiocarpus* were screened for *in vitro* leishmanicidal activity using four strains of promastigotes of *L ethiopica*, the best leishmanicidal activity was showed by butanolic and aqueous fractions; Castalagin isolated from these fractions displayed the best leishmanicidal activity among all the isolated compounds, followed by flavogallonic acid (both of the compounds are hydrolysable tannins)[8] . *Anogeissus leiocarpa* preliminary phytochemical screening revealed the presence of tannins, flavonoids, Terpenes, and saponins [18].

Table 1: Plant extracts summary showing antileishmanial activity

Plant species	Part used	Organism tested	Preparation	IC50 µg/ml [1]	Reference
Azadirachta indica	LF	L. major promastigotes	Methanol extract	10.21	(9)
Acacia nilotica	FR	*L. major* promastigotes	Methanol extract	191.17	(9)
Acacia nilotica	HU	*L. donovani* promastigotes	Methanol extract	8	(14)
Balantis aegyptiaca	SD	*L. major* promastigotes	Methanol extract	89.38	(9)
Allium sativum	BU	*L. major* promastigotes	Methanol extract	4.94	(9)
Ambrosia miratima	AP	*L. donovani* promastigotes	Methanol extract	8	(14)
Punica granatum	LF	*L. amazonensis* amastigotes	Ethanol extract	29.0	(16)
Eucalyptus globulus	SD	*L. major promastigotes*	Methanol extract	78	(17)
Maytenus senegalensis	SB	*L. major promastigotes*	Methanol extract	55	(17)
Azadirchta indica	SB	*L. major promastigotes*	Methanol extract	11.5	(17)
Pseudocedrela kotscifye	SB	*L. major promastigotes*	Methanol extract	>100	(17)
Balanites aegyptiaca	SD, SB	*L. major promastigotes*	Methanol extract	>100	(17)
Anogeissus leiocarpa	SB	*L. ethiopica promastigotes*	Methanol extract	62.5	(8)

[1] In most cases the reference compound (positive control) were either glucantime, pentamidine or pentostam. AP, aerial part; BC, branches; BK, bark; EP, entire plant; FL, flowers; FR, fruits; IF, inflorescence; LF, leaves; PE, pericarp; RB, root bark; RT, roots; RZ, rhizome; SB, stem bark; SD, seeds; SM, stem; TG, twig; BU, Bulb; HU, husk.

Table 2: The phytochemical screening of selected Sudanese medicinal extracts with antileishmanial activity

Plant species	alkaloids	Saponins	flavonoids	Anthraquinones	tannins	steroids	phenols	glycosides	Terpenoids	reference
Balantis aegyptiaca	_	+	_	+	+	_	+	_	_	(11)
Allium sativum	_	_	_	_	+	_	+	_	_	(12)
Ambrosia miratima	+	_	_	_	+	+	_	_	_	(13)
Acacia nilotica	_	_	_	_	+	_	+	_	_	(14)
Acacia nilotica	+	+	+	+	+	_	_	+	_	(10)
Punica granatum	_	_	+	_	+	_	+	+	_	(19)
Eucalyptus globulus	+	_	+	_	+	_	_	_	_	(20)
Maytenus senegalensis	+	_	+	_	+	_	_	_	+	(17)
Azadirchta indica	+	+	+	_	+	_	_	+	+	(21)
Pseudocedrela kotschyi	+	+	+	_	+	+	_	+	+	(22)
Balanites aegyptiaca	+	+	_	_	+	_	+	_	+	(23)
Anogeissus leiocarpa	_	+	+	_	+	_	_	_	+	(18)

The preliminary screening of the 12 plants extracts showed that tannins were present in all the plants; alkaloids and flavonoids showed positive results in 7 plants; flavonoids were present in 7 plants; while saponins and phenols were detected in 6; phenols and terpenoids revealed their presence in two plants; glycosides were detected in 4 plants while anthraquinones and steroids were present in only two plants extracts.

The occurrence of antileishmanial activity is related to the biological activity of the plant, in our concern, the antileishmanial activity of the Sudanese crude plants extracts could be due to their contents of the tannins bioactive ingredients. This is supported by the previous study, the study of Herbert Kolodziej in which a series of 67 tannins was tested for activity against promastigotes and amastigotes of *Leishmania donovani* and L. major, with few exclusions, e.g., caffeic acid derivatives, these tannins showed minor direct toxicity for extracellular promastigotes Leishmania donovani or *L. major* strains. While, the other tannins appreciably decreased the survival of the intracellular amastigotes parasite form in an *in vitro* assays, this indicates that tannin has the potential to induce the release of TNF, NO and IFN-like activity in macrophage-like RAW cell [24].

Suspected Sudanese plants with antileishmanial properties

Exploration of Sudanese medicinal plants with antiparasitic, antiplasmodail, antimicrobial and antiprotozoal together with bringing up phytochemical screening resulted in a list of medicinal plants with potential antileishmanial activity (table 3).

Diospyros mespiliformis is limited to tropical and subtropical areas remarkably in central Africa [25 26]. Several ethno pharmacological applications have been known for *Diospyros mespiliformis*, the leaf decoction was used as an unusual remedy for fever, wounds, whooping cough [27]. Different parts of the tree are used against skin infections, diarrhea, as a psycho-pharmacological drug , toothache and similar pains and headache [26]. The phytochemical screening of the ethanolic crude extracts revealed the presence of steroids, alkaloids, glycosides, Saponins, flavonoids, tannins and terpenoids [28].

Croton zambesicus roots are used in Sudan as aperients [29] and for menstrual pain [30]. The root is moreover used in some areas of Nigeria as antimalarial and anti-diabetic. Phytochemical screening of the crude root extract proved the presence of Terpenes, flavonoids, tannins, saponins, anthraquinones, reducing sugars, alkaloids and cardiac glycosides [31].

Ethanolic extract of the different part of *Bauhinia variegate* shows various activities, leaves extract shows anti-diabetic activity ; woody aerial part shows anti-inflammatory activity; stem extract shows immune modulatory, antitumor, hepatoprotective activity, antibacterial activity, hematinic activity, antimicrobial activity, antiulcer activity and anticarcinogenic activity; while the crudes seeds show

haemagglutinating activity[32]. Qualitative phytochemical analysis of *Bauhinia variegata* bark powder indicated the presence of Tannins , Alkaloids, Saponins, Cardiac Glycosides, Steroids, terpenoids and Flavonoids [33].

Quercus infectoria ethanolic extracts showed both antimicrobial and antiprotozoal activity, the antimalarial properties have IC50 OF 2.51µg/mL against the chloroquine sensitive strain of Plasmodium falciparum and IC_{50} 2 µg/mL respectively against chloroquine-resistant strain of Plasmodium falciparum, phenolic acids, and tannins components of these plants are suggested to be responsible for the properties through carbonic anhydrase inhibition[34].

Trachyspermum ammi crude extract showed macrofilaricidal properties, the corresponding IC_{50} values were 0.067 and 0.019 mg/mL at two incubation stages 24 and 48 hrs. respectively [35]. Phytochemical analysis of *Trachyspermum ammi revealed the presence of alkaloids*, saponins, flavonoids, and tannins [35].

A vitro assessment of the antiviral and antibacterial activities of some Sudanese medicinal plants evaluated a total of 23 Sudanese plants, three standard bacterial pathogens, K. *pneumoniae*, *S. Aureus* and E. *coli* was used in the preliminary antibacterial evaluation. Among the extracts tested about 50% showed antibacterial activity against at least one bacterial strain, only 11 of them have shown the presence of tannins: *Ziziphus spina-christi, Tribulus terretris, Abutilon figarianum, Aristolochia bracteolata, Maytenus senegalensis, Combretum glutinosum, Acacia nilotica, Prosopis chilensis, Cassia obtusifolia, Tamarindus indica, Diospyros mespiliformis and Cassia obtusifolia* [36]. Previous studies have proved the antileishmanial activity of two plants extract: *Acacia nilotica*[9], *Maytenus senegalensis*[37].

Sesbania sesban is used as anthelmintic, antimicrobial, astringent, demulcent, anti-inflammatory, and carminative. In vitro evaluation of the methanol stem extract was tested against different five fungal species and bacterial species. Strong inhibition was observed against the bacteria *Erwinia amylovora* followed by Escherichia coli , while *Curvularia lunata* And *Fusarium oxysporum* fungi were inhibited completely [38]. The phytochemical screening of *Sesbania sesban extracts revealed the presence of* tannins, saponins, alkaloids, phenol, and [39].

Antioxidant and antiproliferative activities of *Abrus precatorius* leaf extracts was evaluated in vitro and the work showed a high antiproliferative and antioxidant activity of this plant, [40]. *Abrus precatonus* phytochemical screening detected in the extracts alkaloids, steroids, tannins, terpenoids, and flavonoids [41].

Rhyncosia minima anthelminthic activity evaluation revealed that ethanol and aqueous extracts exhibited variable degree of activity against Pheretima posthuma earthworms and caused paralysis

followed by death at all used concentrations. Preliminary phytochemical screening revealed the presence of glycosides, alkaloids, flavonoids, tannins, and terpenoids [42].

Antimicrobial assay of *Striga hermonthica* aerial part extracts revealed antimicrobial activity, the richest extract with phytochemical constituents and most effective one was the ethanol extracts of the different parts, which give positive results for tannins after phytochemical screening. Phytochemical screening detected the presence of alkaloids, saponins, flavonoids, steroids, terpenoids [43]. A study of In vitro trypanocidal effect of methanolic extract of some Nigerian savannah plants *showed that Striga hermonthica* was strongly trypanocidal to *Trypanosoma brucei brucei* [44].

Table 3: The phytochemical screening of Sudanese medicinal extract with suspected antileishmanial activity

Plant species	Alkaloids	Saponins	flavonoids	Anthraquinones	tannins	steroids	phenols	glycosides	terpenoids	reference
Diospyros Mespiliformis	+	+	+	_	+	+	_	+	+	(28)
Croton zambesicus	+	+	+	+	+	_	_	+	+	(31)
Bauhinia variegata	+	+	+	_	+	+	_	+	+	(33)
Quercus infectoria	_	_	+	_	+	+	_	_	_	(45)
Trachyspermum ammi	+	+	+	_	+	_	_	_	_	(35)
Abutilon figarianum	_	_	+	_	+	+	_	_	+	(36)
Aristolochia bracteolata	_	+	+	_	+	_	_	_	_	(36)
Cassia obtusifolia	+	_	+	_	+	_	_	_	_	(46)
Combretum glutinosum	_	+	+	_	+	_	_	_	+	(47)
Prosopis chilensis	_	+	+	_	+	+	_	_	+	(36)
Tamarindus indica	+	_	+	_	+	_	_	_	+	(48)
Tribulus terretris	+	_	+	_	+	_	_	+	_	(49)
Ziziphus spina-christi	+	_	_	_	+	_	_	+	_	(50)
Sesbania sesban	+	+	+	_	+	_	+	_	_	(39)
Abrus precatonus	+	_	+	_	+	+	_	_	+	(41)
Rhynchosia minima	+	_	+	_	+	_	_	+	+	(42)
Striga Hermonthica	+	+	+	_	_	+	_	_	+	(43)

CONCLUSION

This study shows a variety of plant extracts exhibit remarkable antileishmanial properties *in vitro* and other 17 plants expected to have antileishmanial activities that need to be evaluated. This represents an exciting advance in the search for novel antiprotozoal agents at a time when there is a pressing need for new innovative drug leads. This study supports the importance of natural tannins as potential sources in the search for new antileishmanial drugs.

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