

## In Vitro Antileishmanial Activity of *Helianthus annuus* and *Pulicaria crispa* (Asteraceae)

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### Abstract

Leishmaniasis which is a neglected tropical disease is a global health problem. The treatment options are limited. They are either toxic or expensive. This study was conducted to test some medicinal plants belonging to Asteraceae family against *Leishmania donovani* promastigotes. Five plants have been selected: *Helianthus annuus* leaves, *Pulicaria crispa* aerial parts, *Geigeria alata* aerial parts, *Acanthospermum hispidum* aerial parts and *Sonchus oleraceus* leaves. *H. annuus* and *P. crispa* petroleum ether and chloroform extracts showed significant antileishmanial activity at 50 µg/mL in the general screening test, whereas the other three plant extracts showed significant antileishmanial activity in only one solvent system. *H. annuus* and *P. crispa* were further tested at doses range of 50, 25, 12.5, 6.25 and 3.125 µg/mL. The IC<sub>50</sub> for petroleum ether and chloroform extracts of *H. annuus* were 4.5 and 3 µg/mL respectively. While the IC<sub>50</sub> for petroleum ether and chloroform extracts of *P. crispa* were 4.9 and 3.75 µg/mL respectively. Thin layer chromatography for the active extracts of *H. annuus* and *P. crispa* has revealed the high similarity in their chemical composition which explains the similarity of their antiparasitic activity.

**Keywords:** Asteraceae; Antileishmanial activity; *Leishmania donovani* promastigotes; *Helianthus annuus*; *Pulicaria crispa*

### Introduction

Leishmaniasis is one of the neglected tropical diseases which is widely distributed parasitic disease, it is caused by the protozoan of the genus *Leishmania* [1]; also, it is regarded as a major public health problem with high incidence of morbidity and mortality in Africa, Asia and Latin America [2].

According to WHO estimates, approximately 200,000 to 400,000 visceral leishmaniasis (VL) cases and 700,000 to 1.2 million cutaneous leishmaniasis (CL) cases occur each year; more than 90% of global VL cases occur in just six countries, namely India, Bangladesh, Sudan, South Sudan, Brazil and Ethiopia. Cutaneous leishmaniasis is more widely distributed, with about two-thirds of cases occurring in three regions, the Americas, the Mediterranean basin and Western Asia; the ten countries with the highest estimated cases are Afghanistan, Algeria, Colombia, Brazil, Iran, Syria, Ethiopia, Sudan, Costa Rica and Peru, altogether account for 70 to 75% of global estimated CL incidence [3].

Sudan is one of the countries which suffered and still suffering from leishmaniasis. Epidemiological studies in Sudan showed that both *L. major* and *L. donovani* are the main causative organisms of CL and VL respectively [4,5]. Also, Sudan is characterized by the presence of Post Kala-azar Dermal Leishmaniasis (PKDL) which is the complicated form of VL; it is developed from about 50% of patients who recovered from VL [6-8]. PKDL patients may play an important role in transmission and are presumed to be the cause of severe outbreaks after periods of low transmission [9].

Recently, treatment of both VL and CL depends on limited chemotherapeutic agents, some of them were obtained from natural products (like amphotericin B), and these limited drugs are either toxic, expensive, need parenteral administration or become useless due to parasite resistance [10]. Therefore, screening for new drugs with potent leishmanicidal activity is highly recommended, as most new developed drugs are still originated from natural sources [11].

*Helianthus annuus* L. is widely distributed plant which is cultivated mainly for the production of edible oil from its seeds. It represents the

second most important edible oil in the world. Traditionally, it is used as anti-inflammatory, anti-pyretic, diuretic, renal disorders, emollient, expectorant, astringent, anti-asthma, disinfectants and vermifuge purposes [12].

*Pulicaria crispa* L. is also widely distributed over the world especially in arid and semi-arid areas, it has many traditional uses which are used for treatment of various ailments, the most important uses are in treatment of some heart diseases, gastro intestinal disorders, antibacterial, antifungal, anti-inflammatory and antiepileptic activities. Also, it possesses high antioxidant activity [13].

### Materials and Methods

#### Plant materials

The plants were collected from different regions in Sudan from July 2012 to February 2013 (Table 1). They were identified and authenticated in Medicinal and Aromatic Plants Research Institute, National Center for Research in Khartoum.

#### Extraction process

The plants were shade dried and powdered; then they were extracted successively with petroleum ether, chloroform and methanol by soxhlet apparatus. The extracts were dried under reduced pressure and the yield percentages were calculated with the reference to dry powders. The extracts were kept in well closed containers in the refrigerator at 4°C.

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No.	Plant (Latin name)	Local name	Source
1	<i>Helianthus annuus</i> leaves	Zahrat Elshams	Eldamazeen (Blue Nile state)
2	<i>Pulicaria crispa</i> (aerial parts)	Taghar, Rabul	Omdurman
3	<i>Geigeria alata</i> (aerial parts)	Gudgad	Om-rowaba (North Kordofan)
4	<i>Acanthospermum hispidum</i> (aerial parts)	Herab Elhawsa	Kass (South Darfur)
5	<i>Sonchus oleraceus</i> (aerial parts)	Elmoleata	Shambat (Khartoum North)

Table 1: The plants and their sources.

## Parasite culture

*Leishmania donovani* parasite was obtained from The Institute of Endemic Diseases, University of Khartoum. The parasite was isolated from visceral leishmaniasis Sudanese patient in NNN media. The cultures were transferred into 50 ml tissue culture flasks and cultured in M-199 medium supplemented with 10-12% Fetal Calf Serum (FCS) and 100 units of benzyl penicillin and streptomycin. The parasites were maintained in this medium and the flasks were examined every two days for the growth and contamination. The parasites (at stationary phase) were routinely sub cultured when good growth was observed almost every three days.

## Preparation of the plant extracts for antileishmanial activity screening

Twenty mg from each plant extract was taken and dissolved with minimum amount of DMSO (almost 0.2 mL), and then the volume was completed to 1 mL by adding M-199 complete media, to prepare 20 mg/mL stock solution. Different concentrations were prepared for the general antileishmanial screening up to a final concentration of 50 µg/mL. Promising extracts will be further tested at lower concentrations.

## Antileishmanial activity

Antileishmanial activity screening was carried according to Attar-Rahman et al. method [14]. In a 96 well micro titer plate, 90 µL of the parasite culture with ( $2 \times 10^6$  *L. donovani* promastigotes/ml) were taken into each well. 10 µL of each plant extract with final concentration of 50 µg/mL were put in each well and mixed well. Amphotericin B was set as positive control with final concentration of 10 µg/mL, while 10 µL of M-199 complete media containing 5% DMSO was set as negative control. The plate was incubated inside the incubator at 25°C. Parasites counting were done after 48 hours of incubation to determine the inhibition percentages; also, morphology and motility of the parasites were checked. Highly active extracts of *H. annuus* and *P. crispa* were further tested at concentrations (50, 25, 12.5, 6.25 and 3.125 µg/mL) in order to determine their  $IC_{50}$  which was obtained by using excel sheet.

## TLC profiling of *H. annuus* and *P. crispa* active extracts

Petroleum ether and chloroform extracts of both *H. annuus* and *P. crispa* were subjected to TLC using precoated aluminum plates, several mobile phase systems were attempted, *n*-hexane: ethyl acetate (4:1) was found to be the most suitable mobile phase system which had showed good separation of the compounds, the plates were seen under UV light, exposed to iodine and finally were sprayed by vanillin-*o*- phosphoric acid as an indicator and heated in an oven at 110°C for 10 minutes.

## Preliminary phytochemical screening of *H. annuus* and *P. crispa*

The above plant powders were screened for the presence of different phytochemical groups. Various chemical tests were performed: flavonoids (NaOH), anthraquinones (Borntrager's test), cyanogenic glycosides (sodium picrate paper), alkaloids (Mayer's, Wagner's and Dragendorff's),

saponins (froth test), cardiac glycosides (Baljet's, Keller-Killiani's and Liebermann-Burchard's tests), steroids (Liebermann-Burchard's test), tannins (FeCl<sub>3</sub>) and coumarins (NaOH and fluorescence).

## Results and Discussion

### Antileishmanial activity

The results of the inhibition percentages for all plant extracts used in general antileishmanial screening test at 50 µg/ml are shown in Tables 2 and 3.

In general, nonpolar and moderately polar extracts were found to be more active than polar extracts, except *S. oleraceus* which exhibited the highest activity for methanol extract and low activity for petroleum ether and moderate activity for chloroform. Both *H. annuus* and *P. crispa* showed very strong activity for both petroleum ether and chloroform extracts, but with weak activity for methanol extracts. *G. alata* and *A. hispidum* showed very strong antileishmanial activity for chloroform extracts and moderate activity for petroleum ether extracts; in addition, the methanolic extract of *A. hispidum* has shown moderate antileishmanial activity.

Petroleum ether and chloroform extracts of both *H. annuus* and *P. crispa* were tested in dose range of 50, 25, 12.5, 6.25 and 3.125 µg/mL and the results are shown in Figures 1 and 2, reflecting that chloroform extract is more active for both species with slight differences in the results.

The  $IC_{50}$  for all extracts showed that *H. annuus* was more active than *P. crispa*, but the differences are relatively low. Moreover, according to excel sheet, about 23.5 µg/mL was the minimum concentration needed to cause 100% leishmanicidal activity and was seen in the chloroform extract of *H. annuus* (Figure 1); while about 47 µg/mL was the minimum concentration needed to cause 100% leishmanicidal activity which was observed in the petroleum ether extract of *P. crispa* (Figure 2). Generally, the active extracts of both species revealed that more than 90% of leishmanicidal activity is exhibited between 20- 50 µg/mL.

### Thin layer chromatography

The picture shows that there are some associated compounds in the two extracts for the two plants reflecting the convergence in the results. This means that these compounds have almost the same nucleus, may be identical (for the same plant) or may be much related (for the two plants).

Many previous studies proved that the antileishmanial activity of most plants belonging to Asteraceae family are attributed to terpenoidal compounds, these compounds like monoterpenes, sesquiterpenes, sesquiterpene lactones, diterpenes and triterpenes are very frequently present in Asteraceae plants and are mainly extracted via nonpolar solvents; the family Asteraceae species are reputed for their content of sesquiterpene lactones, a group of compounds with wide range of biological activities [15]. Our findings which are compatible with these findings depicted that the activity of these two plants mainly can be probably due to their content of sesquiterpene lactones. On the other

No.	Plant	Inhibition %		
		Petroleum ether	Chloroform	Methanol
1	<i>Helianthus annuus</i> leaves	+++	+++	-
2	<i>Pulicaria crispa</i> (aerial parts)	+++	+++	-
3	<i>Geigeria alata</i> (aerial parts)	++	+++	+
4	<i>Acanthospermum hispidum</i> (aerial parts)	++	+++	++
5	<i>Sonchus oleraceus</i> leaves	+	++	+++

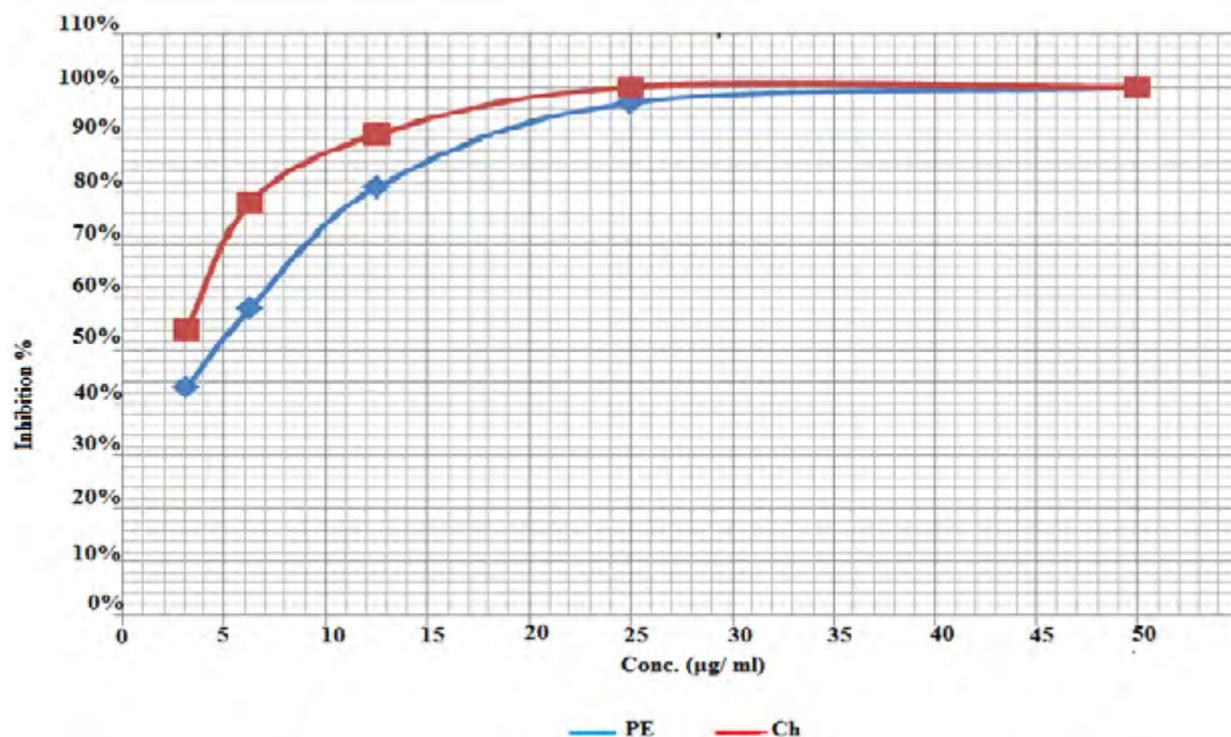
(-): No or very weak activity; (+): Inhibition % < 50%; (++) : Inhibition % between 50-75%; (+++): Inhibition % > 75%

**Table 2:** General antileishmanial screening of the plant extracts.

Phytoconstituent	<i>H. annuus</i>	<i>P. crispa</i>
Flavonoids	+	+
Antraquinones	-	-
Cyanogenic glycosides	-	-
Alkaloids	+	+
Saponins	+	+
Cardiac glycosides	-	-
Steroids/ Triterpenes	+	**
Tannins	+	+
Coumarins	+	+

(+): present; (-): absent; (\*): green color; (\*\*): pink color

**Table 3:** Preliminary phytochemical screening of *H. annuus* and *P. crispa*.



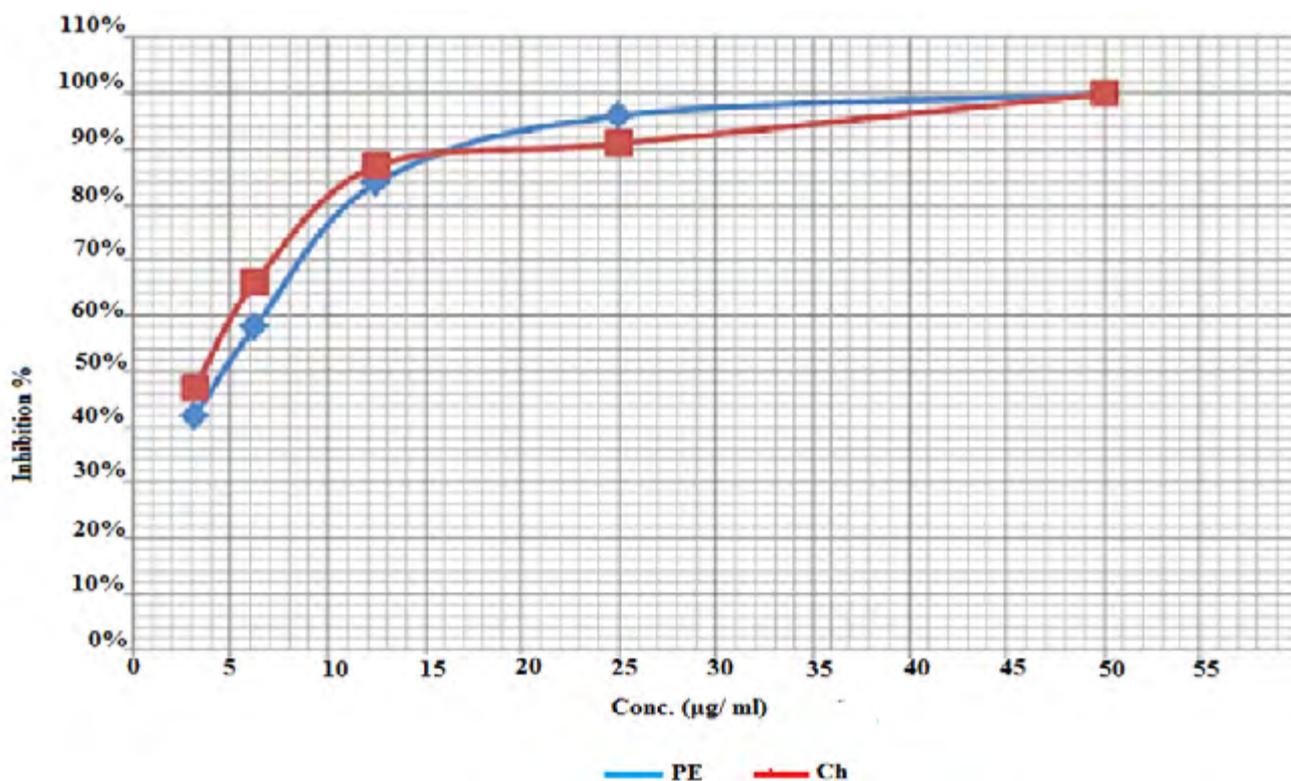
**Figure 1:** Inhibition percentage (Y- axis) versus concentration in µg/mL (X- axis) of petroleum ether (PE) and chloroform (Ch) extracts of *H. annuus* leaves; the  $IC_{50}$  of the chloroform extract is about 3 µg/mL, while the  $IC_{50}$  of the petroleum ether extract is about 4.5 µg/mL.

side, the activity of *S. oleraceus* (methanolic extract) may be attributed to flavonoids (or any other phenolic compounds) which are also widely distributed in Asteraceae family [16].

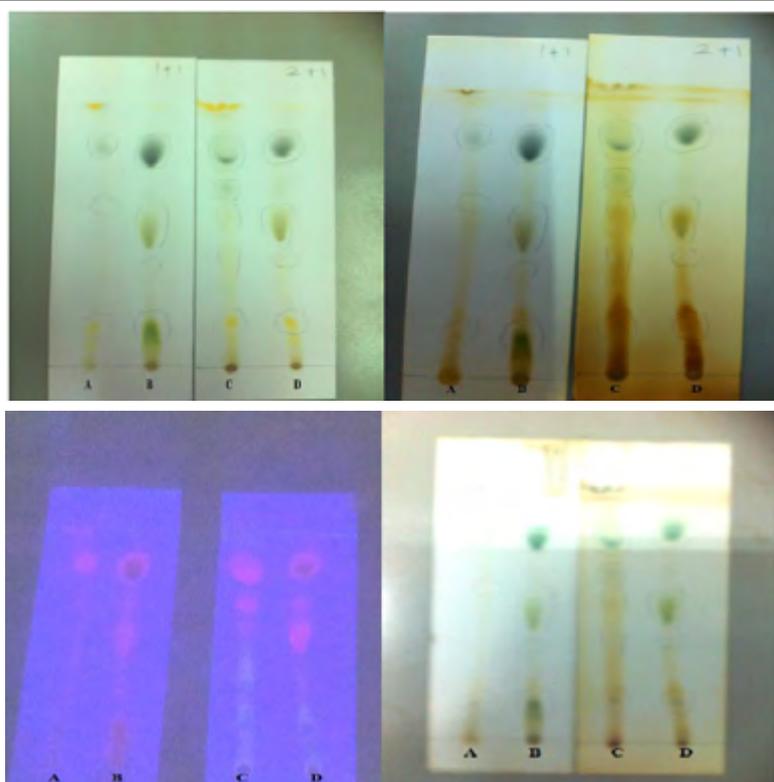
### Phytochemical screening

Both species have shown high similarity in their content of

secondary phytochemical constituents; the only difference observed was in Liebermann- Burchard's test which is used for detection of steroids and triterpenes, *H. annuus* showed the domination of green color which indicates the presence of steroidal nucleus, while *P. crispa* showed the domination of pink color which indicates the presence of triterpenes; so, these observations agree with previous studies [12,13].



**Figure 2:** Inhibition percentage (Y- axis) versus concentration in µg/mL (X- axis) of petroleum ether (PE) and chloroform (Ch) extracts of *P. crispa*; the  $IC_{50}$  of chloroform extract is about 3.75 µg/mL, while the  $IC_{50}$  of petroleum ether extract is about 4.9 µg/mL.



**Figure 3:** TLC profile of the active extracts: **A**, *H. annuus* petroleum ether extract; **B**, *H. annuus* chloroform extract; **C**, *P. crispa* petroleum ether extract; **D**, *P. crispa* chloroform extract. Upper left, original plates; Upper right, exposed to iodine; Lower left, seen under long UV light; Lower right, treated with vanillin-o-phosphoric acid and heated.

This high similarity of phytochemical constituents in both species goes along with the results obtained from antileishmanial activity (Figure 3). Also, a part of similarity can be seen from the TLC profile.

## Conclusion

The study explicit the importance of medicinal plants as a source of new lead compounds. *H. annuus* and *P. crispa* showed promising antileishmanial activity. Our future plan is to attempt fractionation to the active extracts, the fractions should again be tested which finally lead to structure elucidations of the active fractions. Also, we recommend testing the active extracts against *L. donovani* amastigotes, as amastigotes are the infective form of the parasite to humans.

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