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ANTILEISHMANIAL ACTIVITY OF SOME PLANTS GROWING IN ALGERIA: *JUGLANS REGIA*, *LAWSONIA INERMIS* AND *SALVIA OFFICINALIS*.

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Abstract

The current study was undertaken to evaluate *in vitro* the antileishmanial activity of three plants growing wild in Algeria : *Juglans regia*, *Lawsonia inermis* and *Salvia officinalis*. The hydroalcoholic extracts of these plants were tested on the growth of the promastigotes of *Leishmania major*. The plant extract effects were compared with three controls : CRL1 composed of 1 ml RPMI inoculated with 10^6 of promastigotes, CRL2 composed of 1 ml RPMI inoculated with 10^6 of promastigotes and 100 µl of hydroalcoholic solvent, CRL3 composed of 1 ml RPMI inoculated with 10^6 of promastigotes and 100 µl of Glucantim as a reference drug in the management of leishmaniasis. The results showed that both *J. regia* and *L. inermis* extracts reduced the promastigotes number significantly (P<0.01). however, *S. officinalis* showed a total inhibition of the *Leishmania major* growth.

Key words : anti-leishmanial activity ; *Leishmania major* ; *Juglans regia* ; *Lawsonia inermis ; Salvia officinalis*.

Introduction

Leishmaniasis is endemic disease in 88 countries, 72 of which are developing countries. It is caused by a trypanosometed protozoan which is transmitted by small biting sandflies (*Phlebotomus* spp) (Sabina et al, 2005). Various antileishmanial agents are readily available in the market although none of these chemotherapy drugs are free from harmful side effects and toxicity (Kedzierski et al., 2009 ; Murray, 2001). In addition, most of the drugs currently in use are expensive and require long-term treatment. Currently, the development of new safer and more efficacious drugs against leishmaniasis is a need. In this context, several studies have focused medicinal plants as natural produits to search for new molecules with antileishmanial activity.

Lawsonia inermis L. is a biennial dicotyledonous herbaceous shrub commonly known as Henna or Mhendi belonging to family Lythraceae (Lavhate and Mishra, 2007). It is abundantly available in tropical and subtropical areas. A native of North Africa and South-West Asia, the plant is now widely cultivated through out the tropics as an ornamental and dye plant (Chaudhary et al., 2010). Henna leaves, flowers, seeds, stem bark and roots are used in traditional medicine to treat a variety of ailments as rheumatoid arthritis, headache, ulcers, diarrheoa, leprosy, fever, leucorrhoea, diabetes, cardiac disease, hepatoprotective and colouring agent (reviewed by Chaudhary et al., 2010).

Juglans regia L. (*Juglandaceae*), known as walnut tree, is native in southeastern Europe, Asia Minor, India and China. *J. regia* is being widely cultivated for nut production in the temperate part of the northern Hemisphere (Thakur, 2011). It is reported to have medicinal properties against ring worm, fungal, bacterial and viral infections and is used as cure for heat stroke. It is also reported to have antioxidant potential (Labucka et al., 2008), a purgative action and in finely powdered form it is an effective sternutator (Erdemoglu et al., 2003). It has been found to be nematicidal to root-knot nematodes (Dama, 2002). Leaf extracts of the walnut showed also an acaricidal activity on *Tetranychus viennensis* and *Tetranychus cinnabarinus* (Wang et al., 2007). The beneficial action of walnut oil on skin is known for centuries and it is widely used in cosmetic manufacturing industry (Espin et al., 2000).

Salvia officinalis (Sage) has been an important medicinal plant since earliest times ; the infusion of the plant is commonly used for the haemostatic, estrogenic, antiperspiration, anti-neuralgic, antiseptic, hypoglycemic and many other therapeutic effects, while the essential oil from this plant is applied in the treatment of a large range of diseases such as nervous system, heart and blood circulation, respiratory, digestive, metabolic and endocrine diseases (Istudor, 2001).

In the present study, the antileishmanial activity of extracts from three plants (*S. officinalis*, *L. inermis* and *J. regia*) was tested *in vitro* against *Leishmania major*.

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Materials and methods

Plant material

The plant material was obtained from plants growing in their natural habitat or from herbalists. Their identification was done by a taxonomist confirmed, and vouchers specimens were deposited in the herbarium of the Laboratory of medical botany, Pharmacy Department, Faculty of Medicine, Mentouri Constantine University (Algeria). Three plants were used : bark of *Juglans regia* L. (AB04-109), leaves of *Lawsonia inermis* L. (AB04-111) and leaves of *Salvia officinalis* L. AB04-118). The freshly harvested plant material was cleaned and dried in an oven at a temperature not exceeding 35 °C. Properly labeled samples were stored awaiting analysis in a closed, protected from light and moisture.

Preparation of plant extracts

The dried plants were sprayed just prior to extraction using a knife mill. The extraction of the powders obtained (30 g) was made by cold maceration in a hydroalcoholic solution of 70% ethanol (1:5, w / v), stirred on a magnetic plate (700 r / min for 30 min). The solutions were then clarified by filtration through Whatman paper, concentrated by evaporation of alcohol under *vacuum* and then lyophilized to give a crude extract texture more or less dry powder. They are stored until analysis at low temperature (-10 °C).

Strains and anti-leishmanial activity

Leishmania major MON-25 / LIPA / 32/06 has been provided by the Pasteur Institute of Algiers, the promastigote form will be used to evaluate the effect of our anti-leishmanial plant material. The primary *inoculum* of the strain used is prepared in Falcon tubes from strains preserved in culture medium NNN (Novy, Mac Neal, Nicoll), the primary *inoculum* is adjusted to a final concentration of 10^6 *Leishmania*/ml in RPMI (Roswell Park Institute Park Memerial) -1640 enriched of 10 % of fetal calf serum and inoculated in the presence of hydro-alcoholic extracts of the three plants at the concentration of 100µl for each extract. The *inoculum* is distributed in the wells of cell culture plates at 12 wells per plate at 1 ml per well and each plate contained the control 1, 2, 3 and the extract of a plant; tests were repeated three times to assess reproducibility. Counts of promastigotes were done daily to cells after fixation Thomas promastigote forms of formalin, compared to three control wells: the CRL 1 is composed of 1 ml of RPMI and 1 ml of *inoculum* was 10^6 of promastigotes per well. The control 2 is composed of 1 ml of RPMI, 1 ml of *inoculum* with 10^6 promastigotes per well over 100µl of water-alcohol solution used to dissolve the plant extracts to control the possible effect of the solvent. The control 3 is composed of 1 ml of RPMI and 1 ml of *inoculum* with 10^6 of promastigotes per well over 100 µl of meglumine antimoniate (Glucantim) substance used in the treatment of leishmaniasis.

Statistical analysis

Data represented the mean ± SD of three repetitions. The differences between the mean values were evaluated by the *Student's t test*. The level of significance was set at 0.05.

Results and discussion

Many natural products have been proven to exhibit anti-parasitic activity. The present study provided data on the activity of hydro-alcoholic extracts of three plants against *Leishmania major*. The results of the cultures of promastigotes of this parasitic strain in the presence and absence of the different plant extracts are summarized in table 1 and figure 1.

Table 1 shows an increase in the number of promastigotes of *Leishmania major* from day 1 to day 4 in the absence or presence of the hydroalcoholic solution and Glucantim. Between 5^{th} and 7^{th} day, the promastigotes were decreased. The growth inhibition on *L. major* was well marked in CRL 3 (with Glucantim) when compared with the other controls; however, Glucantim is reported to be active only against amastigote stage (Fournet, 1991).

In the presence of the hydroalcoholic extract of *Junglans regia*, the number of promastigotes was reduced significantly ($P < 0.01$) compared to the different controls from the day 1 to the 6th day (Fig. 1-A). According to scientific reports, this plant is frequently used for the management of the parasitic diseases in central Italy (Guarrera, 1999), Morocco (El-Rhaffari et al., 2002). In addition, Urban et al. (2008) have demonstrated the anthelmintic activity of *J. regia* from Czech Republic. It has been reported also by Nancy et al. (2011) that *Juglans regia L.* contains naphthaquinones, tannins, flavonoids and tannic acids, and that it showed good anti plaque activity when tested against four microorganisms related to dental caries (*Streptococcus mutans, Streptococcus sobrinus, Actinomyces viscosus*).

In the presence of *Lawsonia inermis* extract, the number of promastigotes was reduced significantly ($P < 0.01$) compared to CRL1, CRL 2 and CRL3 from day 1 to day 6. We mention that this plant has been classified among medicinal plants with antiparasitic potential in Ivory Coast; it is also demonstrated to possess trypanocidal activity (Okpekon et al., 2004). The hydroalcoholic extract of *Salvia officinalis* showed a total leishmanicidal activity on L. major promastigotes from the 1st day of the assay (Table 1, Fig. 1-B). This efficiency can be explained by the presence of flavones and flavonols which are synthesized from different flavonoids such as quercetin are known for their anti-inflammatory and antibacterial (Treki Amina et al., 2009), and the presence of ketones organic compounds from the family of carbonyls such as thujone known mainly for their regenerating and healing properties. Farcasanu and Oprea (2006) reported that the extracts obtained with aqueous ethanol of various concentrations of *salvia officinalis L.* showed different antifungal effect against the *Saccharomyces cerevisiae* cells. The strongest growth inhibitory capacity was noted for the extracts obtained in 90% ethanol demonstrating that under these conditions the yeast cells were more susceptible to metabolic or structural damage.

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Table 1: Effect of plant extracts and controls on promastigotes growth of *Leishmania major*.

Days post inoculation	Number of promastigotes in million (Mean ± SD)					
	Controls			Plant extracts		
	CRL 1	CRL 2	CRL 3	JRE	SOF	LIN
0	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0
1	2.8 ± 0.33	3.5 ± 0.45	2.22 ± 0.64	1.6 ± 0.34^{¶¶,ΦΦ,‡‡}	0 ± 0$^{♠♠,↓↓,◊◊}$	1.52 ± 0.38$^{**,◆◆,❤❤}$
2	4.3 ± 01.01	5.8 ± 0.55	3.6 ± 0.58	1.94 ± 0.62^{¶¶,ΦΦ,‡‡}	0 ± 0$^{♠♠,↓↓,◊◊}$	1.8 ± 0.46$^{**,◆◆,❤❤}$
3	5 ± 0.77	7.18 ± 0.88	5 ± 0.47	2.7 ± 0.78^{¶¶,ΦΦ,‡‡}	0 ± 0$^{♠♠,↓↓,◊◊}$	1.96 ± 0.40$^{**,◆◆,❤❤}$
4	6.8 ± 0.6	7.8 ± 0.59	5.32 ± 0.67	2.1 ± 0.48^{¶¶,ΦΦ,‡‡}	0 ± 0$^{♠♠,↓↓,◊◊}$	2.56 ± 0.78$^{**,◆◆,❤❤}$
5	4.6 ± 0.63	5.1 ± 0.7	3.72 ± 0.4	1.48 ± 0.48^{¶¶,ΦΦ,‡‡}	0 ± 0$^{♠♠,↓↓,◊◊}$	1.84 ± 0.53$^{**,◆◆,❤❤}$
6	2.4 ± 0.42	3.8 ± 0.85	2.6 ± 0.8	1.2 ± 0.3^{¶¶,ΦΦ,‡‡}	0 ± 0$^{♠♠,↓↓,◊◊}$	0.94 ± 0.32$^{**,◆◆,❤❤}$
7	1.3 ± 0.69	2.9 ± 0.85	0.9 ± 0.57	0.72 ± 0.29	0 ± 0$^{♠♠,↓↓,◊◊}$	0.52 ± 0.44

Legend: CRL 1: 1ml of RPMI +1 ml of SV + 1ml of promastigotes (10^6) + 100 µl of hydro alcoolic solution (70 % Methanol, 30 % water), CRL 2: 1 ml of RPMI +1 ml of SV + 1 ml of promastigotes (10^6), CRL 3: 1 ml of RPMI +1 ml of SV + 1 ml of promastigotes (10^6) + 100 µl of Glucantim, JRE: *Juglans regia*, LIN: *Lawsonia inermis*, SOF: *Salvia officinalis*, Mean: average of 3 repetitions, SD: Standard deviation, ¶ (P<0.01) [JRE extract vs CRL 1], ^{ΦΦ} (P<0.01) [JRE extract vs CRL 2], ‡‡ (P<0.01) [JRE vs CRL 3], $^{♠♠}$ (P<0.01) [SOF extract vs CRL 1], $^{↓↓}$ (P<0.01) [SOF extract vs CRL 2], $^{◊◊}$ (P<0.01) [SOF vs CRL 3], ** (P<0.01) [LIN extract vs CRL 1], $^{◆◆}$ (P<0.01) [LIN extract vs CRL 2], $^{❤❤}$ (P<0.01) [LIN vs CRL 3].

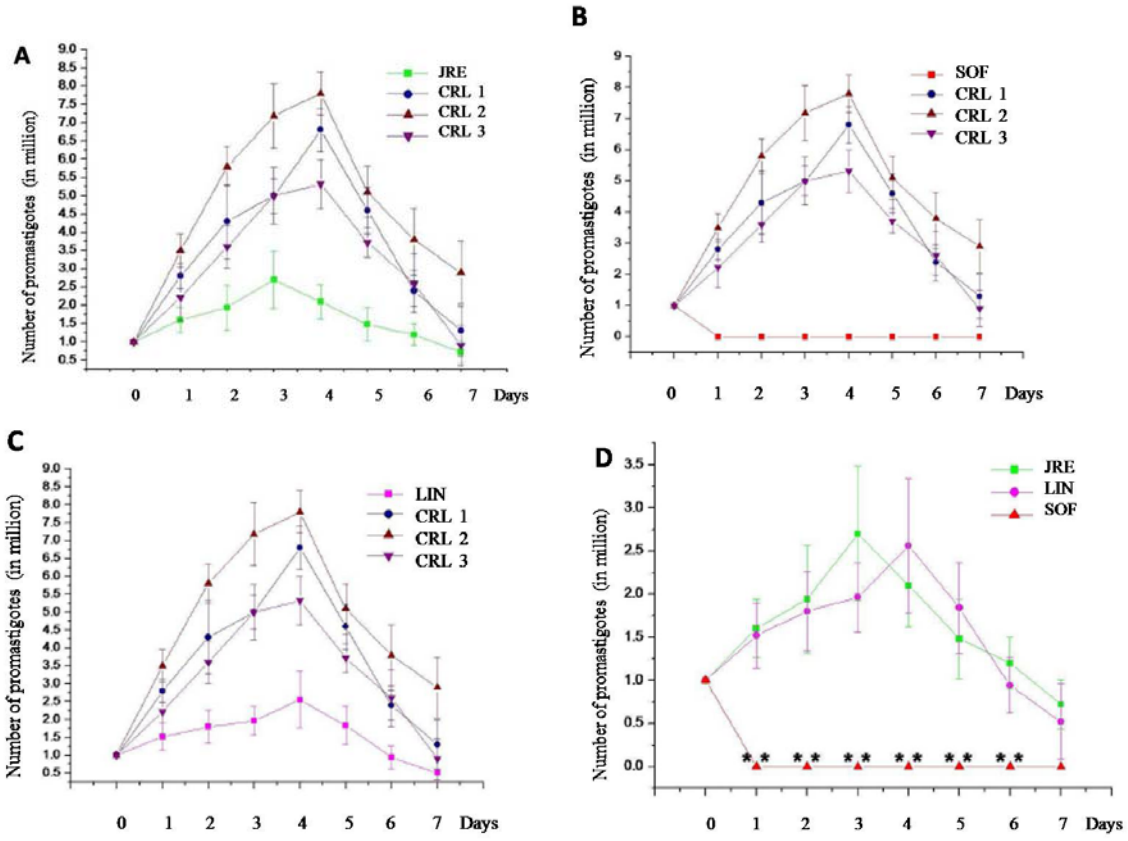

Figure 1 : Effect of plant extracts and controls on promastigotes growth of *Leishmania major*. A- *Juglans regia* activity compared with controls ; B- *Salvia officinalis* activity compared with controls ; C- *Lawsonia inermis activity* compared with controls ; D- Comparison of *J. regia*, *S. officinalis* and *L. inermis* extracts *activities ; **(P<0.01) [SOF extract vs JRE & LIN]*.

Conclusion

We conclude that the hydoalcoholic extracts of *Juglans regia, Lawsonia inermis* and *Salvia officinalis* are found to be active on promastigote forms of *Leishmania major ;* however, *S. officinalis* showed a promising leishmanicidal activity on the tested strain. Other studies are needed to identify constituents

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behind this antileishmanial property. In addition, the study will be completed by a study of the cytotoxicity of fractions / molecules, because many natural substances show an antileishmanial activity resulting from a high toxicity and are therefore unusable in practice.

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