INTRODUCTION

Scientific evaluation of medicinal plants has, in the past, provided the basis of modern medicine. Several compounds from higher plants are currently being evaluated in various laboratories as potential antiparasitic agents, namely in malaria, trypanosomiasis and leishmaniasis. It is clear that the major part of this research is dedicated to malaria. Much less effort is devoted to leishmaniasis although these diseases threaten about 12 million people around the world and incidence of leishmaniasis is currently increasing (Croft, 1988; Balana-Fouce et al., 1998). This is all the more a cause for concern nowadays, since AIDS and other immnosuppressive conditions and malnutrition are important risk factors, particularly in the Mediterranean areas (Durand et al., 1997) and in Africa. The development of synthetic chemical drugs over the last years has not led to any new drugs able to effectively cure leishmaniasis. The chemotherapeutic agents used for the treatment of leishmaniasis such as sodium stibogluconate (Pentostam®), N-methylglucamine antimonial (Glucantime®), pentamidine (Pentacarinat®) and Amphotericin B (Fungizone®, Ambisome®) are not active orally and require long-term parenteral administration. They also have serious side effects such as cardiotoxicity and renal toxicity and are expensive (Croft, 1988; Who, 1996). A rising problem is the changing patterns of sensitivity in antimonials (Olliaro et al., 1994). These compounds belong the following groups: alkaloids, terpenes, quinones, coumarins, chalcones, acetogenins, lactone, lignan.

Summary:
The active compounds obtained from some medicinal plants used traditionally worldwide for the treatment of leishmaniasis are reviewed. Among these active molecules described in recent literature are quinoline alkaloids such as alkyl-2 quinoline and aryl-2 quinoline from Galipea longiflora, isoquinoline alkaloids such as isoguattouregidine from Guatteria foliosa, indole alkaloids such as conodurine and gabunine from Pescheiera van heurki, terpenes such as jatrogrossidione from Jatropha grossidentata, acetogenins such as senegalene from Annona senegalensis and lignans such as (+)-nyasol from Asparagus africanus. Other natural compounds with antileishmanial activity are coumarins, chalcones, lactones, tetralones and saponins. Some of them are known antiprotozoal natural products. These compounds could be used as templates to discover new and effective drugs against leishmaniasis.

KEY WORDS: antileishmanial, leishmaniasis, natural products, alkaloids, terpenes, quinones, coumarins, chalcones, acetogenins, lactone, lignan.

Résumé: DONNÉES RÉCENTES SUR LES COMPOSÉS ACTIFS DE PLANTES Médicinales utilisées dans le traitement de la leishmaniose
Les composés actifs obtenus à partir de quelques plantes médicinales utilisées en médecine traditionnelle dans le monde pour le traitement de la leishmaniose sont répertoriés. Parmi ces molécules actives recensées au travers de la littérature depuis 1994, on trouve : les alcaloïdes quinoléiniques tels les 2-alkyl et les 2-aryl quinoléines isolées de Galipea longiflora, les alcaloïdes isoquinoléiques telle que l'isoguattouregidine isolée de Guatteria foliosa, les alcaloïdes indoléiques comme la conodurine et la gabunine isolées de Pescheiera van heurki, les terpènes telle que la jatrogrossidione isolée de Jatropha grossidentata, les acetogénines avec le senegalene isolé d'Annona senegalensis et les lignanes comme le (+)-nyasol isolé d'Asparagus africanus. Les autres composés naturels à activité leishmanicide appartiennent aux différentes classes chimiques suivantes : coumarines, chalcones, lactones, tetralones et saponines. Certains de ces composés naturels ont des propriétés antiprotozoaires bien connues. Ces structures utilisées comme modèles pourraient conduire à l'obtention de nouveaux médicaments actifs contre la leishmaniose.

MOTS CLÉS: antileishmanien, leishmaniose, produits naturels, alcaloïdes, terpènes, quinones, coumarines, chalcones, acetogénines, lactone, lignane.
marins, acetogenins of annonaceae, chalcones, tetralones, lignans and saponins.

This review is an update from 1994 and its aim is to outline the various classes of compounds, recently isolated from higher plants, which are active against leishmaniasis.

ALKALOIDS

Alkaloids are the most important natural compounds with antileishmanial activity. They occur in many plant families and interesting activities were found in several groups: quinoline alkaloids, isoquinoline alkaloids and indole alkaloids.

Quinoline alkaloids

The quinoline alkaloids seem to be very active for the treatment of parasitic diseases. Quinine, a quinoline alkaloid isolated first from *Cinchona succirubra*, was the first effective antimalarial drug to be discovered.

During an ethnopharmacological investigation in Bolivia, aryl and alkyl-2 quinoline alkaloids active against leishmaniasis were isolated from *Galipea longiflora*, through a bioactivity-guided fractionation (Fournet et al., 1993).

2-propenyl quinoline (1) and 2-trans epoxypropyl quinoline (3) isolated from the leaves showed antileishmanial activity *in vitro* against several strains of *Leishmania* species promastigotes with an IC₉₀ value around 25 μg/ml (147 μM and 135 μM respectively). 2-propyl quinoline (2) isolated from the leaves and 4-methoxy 2-phenyl quinoline from bark, root and leaves are less active, with an IC₉₀ value of 50 μg/ml (292 μM and 212 μM respectively). The other alkaloids isolated, such as 2-phenyl quinoline, phenyl ethyl quinoline, 2-pentyl quinoline showed antileishmanial activity with an IC₉₀ at 100 μg/ml.

In the mouse footpad model infected with *Leishmania amazonensis* or *L. venezuelensis*, animals were treated one day after infection with 2-propyl quinoline by the oral route for two weeks at 100 mg/kg/day. The reference drug, Glucantime®, was administrated by subcutaneous injections at 200 mg/kg/day. Under these conditions 2-propyl quinoline showed the same efficacy as the reference drug in reducing the size of the lesion and the parasite burden. By the same protocol, 2-trans epoxypropyl quinoline appeared slightly more active than Glucantime®. Treatment with 2-propenyl quinoline by the oral route or intraleisionally for four to six weeks post infection, at the same dose, reduced the parasite load by 95 %.

More interestingly, on a model of visceral leishmaniasis, oral administration of propyl quinoline and epoxy propyl quinoline to mice infected with *L. donovani* at 50 mg/kg/day for five days led to reductions of parasitic load of 87 % and 70 % respectively. A ten-days treatment with 2-propyl quinoline showed a reduction of the parasite burden by 99 %.

In the same group, dictyolomide A (4) and dictyolomide B (5), 4-quinolinone alkaloids isolated from the stem bark of *Dictyoloma peruviana* have *in vitro* antileishmanial activity against the promastigote forms of *L. amazonensis* at 50 μg/ml (177 μM) and 100 μg/ml respectively, furthermore dictyolomide A shows also an activity at 25 μg/ml (88 μM) (Lavaud et al., 1995). Unfortunately no assay was indicated on amastigote forms.

Isoquinoline alkaloids

Isoquinolines which are active against various species of *Leishmania* have been isolated from plants belon-
ging to Annonaceae, Berberidaceae, Hernandiaceae and Menispermaeae.

Some compounds like anonaine and liriodenine isolated from the trunk bark of *Annona spinos escens* have shown in vitro activity against promastigote forms of *Leishmania* species at 10 μg/ml (37 μM) and 25 μg/ml (90 μM) respectively (Queiroz et al., 1996). Isoquatourregidine isolated from the stem bark of *Guatteria foliosa* is active against *L. donovani* and *L. amazonensis* at 100 μg/ml (Mahiou et al., 1994).

**Indole alkaloids**

Indole alkaloids isolated from several plants used in African traditional medicine to treat leishmaniasis have shown antileishmanial activity. Two bis-indole alkaloids, conodurine (6) and N-demethyl-conodurine (gabunine) (7), recently isolated from leaves and stem bark of *Peschiera van beurkii*, are active in vitro against the promastigote forms of *L. braziliensis* as well as on amastigote forms of *L. amazonensis*. Gabunine, the most potent, remain active against *L. braziliensis* at 10 μg/ml (15 μM). On *L. amazonensis* amastigotes, it exhibited considerable activity with a survival index (SI) of 3 % at 25 μg/ml (39 μM). On the same model, conodurine led to a SI of 47 % at 100 μg/ml. *In vivo*, conodurine at 40 mg/kg/day administered intraperitoneally was less active than Glucantine® on the development of *L. amazonensis* lesions in BALB/c mice whereas gabunine was devoid of activity (Munoz et al., 1994).

**Terpenes**

Diterpenes from various species possess powerful anti-leishmanial activity. Two terpenes: jatrogrossidione (8) isolated from *Jatropha grossidentata* and jatrophe from *Jatropha isabellii*, are active against *Leishmania* promastigotes with an IC₅₀ of 0.75 μg/ml (2.4 μM) and 5 μg/ml (16 μM) respectively. Under the same conditions, the IC₅₀ of pentamidine against *Leishmania* strains is 1 μg/ml (1.6 μM). The IC₅₀ of jatrogrossidione is below 0.25 μg/ml (0.8 μM) against amastigote forms of *Leishmania* within macrophages. *In vivo*, jatrophe at 25 mg/kg/day administered subcutaneously is significantly active against the virulent strain of *L. amazonensis* (PH8), but proved to be too toxic for a clinical use (Schmeda-Hirshmann et al., 1995).

Amarogentin (9), a secoiridoid glycoside isolated from the aerial parts of *Swertia chirata* is a potent inhibitor of DNA topoisomerase 1 from *Leishmania* at 30 μM (Ray et al., 1996).

**Coumarins**

5-methyl-coumarin, cycloisobrachycoumarinone epoxide (10) and its 2'-epimer (11) isolated from the roots of *Vernonia brachycalyx* have shown the same level of growth inhibition towards *L. major* promastigotes with IC₅₀ values of 13.4 μg/ml (39 μM) and 12.7 μg/ml (37 μM) respectively. In the same test, the IC₅₀ of Pentostam® was 67.3 μg/ml (199 μM) but comparisons of antipromastigote activity with pentavalent antimons (Pentostam) are pointless as these drugs are poorly active against this stage of the parasite life cycle. These two coumarins do not affect the immune system at the concentrations effective against the parasites (Oketch-Rabah et al., 1997a).

**Chalcones**

Licochalcone A (12) isolated from *Glycyrrhiza glabra*, *G. uralensis* and *G. inflata* roots at 0.5 μg/ml (1.4 μM) reduced the infection rate of human peripheral blood monocyte-derived macrophages with *L. major* amastigotes and exhibited a strong intracellular killing of parasite without to be toxic to host cells. These data show that intracellular *Leishmania* amastigotes are more susceptible than promastigotes to licochalcone A (Chen M. et al., 1993). On a model of visceral leishmaniasis, the compound administered intraperitoneally at 20 mg/kg/day and orally at 5 to 150 mg/kg/day for six days led to reductions of parasite load in the liver and in the spleen of 96 % and 65-85 % respectively. Its intraperitoneal administration at 2.5 mg/kg/day completely prevented lesion development in BALB/c mice infected with *L. major* (Chen et al., 1994). E-1-(2,4-dihydroxy-3-(3-methyl-2-butenyl)phenyl)-3-(4-hydroxy-3-(3-methyl-2-butenyl)phenyl)-2-propen-1-one, a chalcone (13) isolated from roots bark of *Glycyrrhiza inflata* showed potent in vitro antileishmanial activity against *L. donovani* promastigotes with an IC₅₀ of 10 μg/ml (25 μM) (Brogger Christensen et al., 1994).

**Acetogenins from Annonaceae**

Acetogenins isolated from *Annona senegalensis* seeds possess antileishmanial activity. The mono-tetrahydrofuranic acetogenin, senegalone (14), has been shown to be active against *L. major* and *L. donovani* at the concentrations of 50 μg/ml (80 μM) and 25 μg/ml (40 μM) respectively. In the bis-tetrahydrofuranic series, squamocine (15) and molvizarine are also effective at the same level. Pentamidine, the reference drug, was approximately twice as efficient as these acetogenins (Sahpaz et al., 1994). Nevertheless this class of compounds is known to be cytotoxic. Toxicity on macrophages and assay on amastigote form are necessary to show their interest on leishmania.

**Miscellaneous**

(+)-Nyasol (16), a lignan isolated from the roots of *Asparagus africanus* showed, *in vitro*, inhibition of the growth of *L. major* promastigotes with an IC₅₀ value of 12 μM, but this concentration also slightly affected
the proliferation of human lymphocytes (Oketch-Rabah et al., 1997b).

Muzunzagenin (17), a sapogenin isolated from *Asparagus africanus* has been shown to possess moderate in *vitro* leishmanicidal activity, the IC_{50} against *L. major* promastigotes being 70 µM (Oketch-Rabah et al., 1997b).

Argentilactone (18), an α-β unsaturated δ-lactone isolated from the roots of *Annona baematantha*, exhibited in *vitro* activity against various strains of *Leishmania* at 10 µg/ml (51 µM). In *vivo*, administered by the subcutaneous route at 25 mg/kg/day for 14 days, the compound produced the same effect as N-methyl-glucamine antimonate, the reference drug, decreasing the size of lesions in mice infected with *L. amazonensis* (Waechter et al., 1997).

4-hydroxy-1-tetralone (19) isolated from the stem bark of *Ampelocera edentula*, has shown in *vitro* activity against promastigote forms of various strains of *Leishmania* with an IC_{90} of 10 µg/ml (61 µM). In *vivo* at 25 mg/kg/day in mice infected with *L. amazonensis* (PH8) or *L. venezuelensis* this compound was slightly less active than Glucantime® (Fournet et al., 1994).
Diospyrin, a bisnaphthoquinone isolated from the stem-bark of Diospyros montana, active against L. donovani promastigote forms at 1 μg/ml (Phillipson et al., 1991b) was tested in vitro against intracellular amastigotes in macrophages (Yardley V. et al., 1996). This compound was inactive at 10 μM but synthetic derivatives obtained by minor changes in the functional groups of diospyrin had improved the antileishmanial activity.

2-benzoxazolinone isolated from the leaves of Acanthus ilicifolius possesses a leishmanicidal activity in vitro against L. donovani promastigotes with an IC$_{50}$ of 40 μg/ml (296 μM) (Kapil et al., 1994).

CONCLUSION

Various natural chemical compounds possess antileishmanial activity. Several of them are active against promastigote forms, nevertheless to confirm their interest, activity assays on amastigote forms as well as the evaluation of their toxicity on macrophages are necessary. Furthermore, in vitro assays, by different routes allow to confirm the biological interest of these compounds on leishmaniasis. All these compounds could be interesting leads for further development of antileishmanials drugs. Most of them have been isolated from medicinal plants used in traditional medicines using bioassay-guided fractionation. In this review, a few, such as alkyl quinoline and licochalcone, are quite simple to synthesize, thus allowing the development of low-cost drugs for chemotherapy of leishmaniasis in developing countries.

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