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Antiamoebic and phytochemical screening of some Congolese medicinal plants

L. Tona [a], K. Kambu [a], N. Ngimbi [b], K. Cimanga [c,*], A.J. Vlietinck [c]

[a] *Faculty of Pharmacy, University of Kinshasa, B.P. 212, Kinshasa XI, Congo*
[b] *Faculty of Medicine, Institute of Tropical Medicine, Faculty of Medicine, University of Kinshasa, B.P. 836, Kinshasa XI, Congo*
[c] *Department of Pharmaceutical Sciences, University of Antwerp (U.I.A.), Universiteitsplein 1, B 2610, Wilrijk, Antwerp, Belgium*

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Abstract

Results from the in vitro antiamoebic activity of some Congolese plant extracts used as antidiarrhoeic in traditional medicine indicated that of 45 plant extracts tested, 35 (77.78%) exhibited an antiamoebic activity and 10 (22.22%) were inactive. The highest activity (MIC < 100 μg/ml) was obtained with extracts from root bark of *Paropsia brazzeana*, *Cryptolepis sanguinolenta*, *Alchornea cordifolia*, *Hensia pulchella*, *Maprounea africana*, *Rauwolfia obscura* and *Voacanga africana*, leaves and stem bark of *Psidium guajava*, stem bark of *Dialum englerianum*, *Harungana madagascariensis* and *Mangifera indica*, mature seeds of *Carica papaya*, and leaves of *Morinda morindoides* and *Tithonia diversifolia*. Metronidazole used as reference product showed a more pronounced activity than that of all plant extracts tested.

Keywords: Plant extracts; Antiamoebic; *Entamoeba histolytica*; Diarrhoea; Congo

1. Introduction

Medicinal plants are considered as an important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the biological and phytochemical screening of plant extracts and/or extracts from traditional preparations used in popular medicine (Alonso Paz et al., 1995; Sohni et al., 1995). Successful strategies for investigation these preparations involve the selection of test crude extracts based on a combination of ethnopharmacology and daily healer's practices. Several traditional preparations from various medicinal plant species are used in Congolese traditional medicine as antidiarrhoeal agents. These remedies have been included in a programme for biological evaluating to justify their use.

* Corresponding author.

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However, intestinal amoebiasis is one of the current diseases in tropical regions causing diarrhoea. Traditional preparations from medicinal plants are still used with success for the treatment of amoebiasis. Extensive phytochemical study of some plants involved antiamoebic activity have resulted in the isolation and characterization of active constituents (Gillin et al., 1982; Keene et al., 1986; Sharma and Bhutani, 1987; Wright et al., 1988, 1992). Other investigations on pure natural products from different plant species allowed identification of some constituents with an interesting antiamoebic activity (Ohiri et al., 1983; Keene et al., 1987; Phillipson and Wright, 1991; Wright et al., 1991; Marshall et al., 1994).

The present work reports the effects of 45 crude extracts from medicinal plants against *Entamoeba histolytica* in vitro. These plants were selected on the basis of their use for the treatment of diarrhoea in Congolese traditional medicine (Kambu, 1990).

2. Material and methods

2.1. Plant materials

All plants were collected in Kinshasa Congo in March 1986. They were identified by M. Nlandu from the Institut National d'Etudes et de Recherches en Agronomie (INERA), University of Kinshasa where a voucher specimen of each plant had been deposited.

2.2. Preparation of crude extracts

The traditional preparations were obtained according to daily traditional healers' practises. The amount of each plant part/100 ml water and the method of preparation are given in Table 2. Briefly, an amount of fresh plant material was macerated with water for 24 h. The mixture was filtered and the filtrate evaporated to dryness under 40°C to yield a dried extract. On the other hand, decoctions were prepared by boiling an amount of plant material with water for 15–30 min. The mixture was cooled and treated as described for the macerate yielding a corresponding dried extract.

2.3. Phytochemical screening

Five grams of each powdered plant material was exhaustively macerated and percolated with 80% ethanol or water. The macerate and percolate were combined and evaporated to dryness. The identification of each major chemical group in the 80% ethanol extracts was carried out by TLC on silica gel Merck $60F_{245}$ (layer thickness 0.2 mm) as follows: for terpenes and sterols, hexane/ethyl acetate: 1:1 was used as mobile phase and Liebermann-Burchard as reagent, a range of colors are produced after heating sprayed plates for 10 min at 100°C. Alkaloids were detected in the alkaloid fraction obtained by a classical acid/base extraction procedure for alkaloids and analyzed by TLC in chloroform/methanol/ammonia solution 25% 8:2:0.5 as solvent system, spots were detected after spraying with Dragendorff's reagent. To detect flavonoids, TLC was developed in n-butanol/acetic acid/water 4:1:5 (top layer), spots were visualized with 1% aluminium chloride solution in methanol under UV (366 nm; Harborne, 1974). Aqueous extracts were used for the identification of tannins with 1% gelatin solution, saponins by froth test and anthraquinones with 10% potassium hydroxyde solution in methanol (Tanira et al., 1994).

2.4. Preparation of samples for testing

One-hundred milligrams of each dried extract were separatively dissolved in 10 ml distilled water to obtain stock solutions of 10 mg/ml. These stock solutions were diluted two-fold with culture medium to obtain a series of concentrations of the test sample. Metronidazole from the Laboratoire d'Analyse et de Contrôle des Médicaments et des Denrées Alimentaires (LACOMEDA) of the Faculty of Pharmacy (University of Kinshasa) was used as an antiamoebic reference product. Ten milligrams of metronidazole was dissolved in 2 ml MeOH and treated in the same manner as described for extracts.

2.5. In vitro antiamoebic testing

2.5.1. Entamoeba histolytica

Entamoeba histolytica strain used in this investigation is a laboratory strain isolated from patients with acute amoebic dysentery, and kindly provided by Professor Dr N. Ngimbi, of the Institute of Tropical Medicine, Faculty of Medicine, University of Kinshasa.

2.5.2. Growth medium

The growth medium is a diphasic medium (medium N of Institut Pasteur) called Dobbel and Laidlaw's medium. It is constituted with inactivated horse serum: 1 l, Ringer solution ($CaCl_2$: 200 mg/l, KCl: 200 mg/l, NaCl: 2000 mg/l) and rice starch. Streptomycin (0.8 ml) and penicillin (0.5 ml) were added to prevent contaminations. The culture was stored at 4°C. Adjustment to pH 6–9 was made prior to use by adding of $NaHCO_3$ 2 M.

2.5.3. Maintenance of Entamoeba histolytica cultures

Entamoeba histilytica was cultured in sterile tubes containing 9 ml of culture medium. The mixture was stirred and incubated for 1 week at 37°C. The content was daily examined through a microscope in order to monitor the parasite growth and to detect eventual contamination. The counting of amoebae was made with the aid of Neubauer's cell. Uncontaminated tubes containing an average number of amoebae 2.5 millions/ml of culture medium were selected as test tubes.

2.5.4. Antiamoebic testing

The antiamoebic activity of plant extracts was assessed in vitro according to the procedure described by Ndir and Pousset (1981). Briefly, in a series of test tubes, 1 ml of test samples was added. The tubes were filled up with sterile cotton and the mixture stirred. Each test was performed in duplicate. Two sets of controls were used. One control was the *Entamoeba histolytica* control cultured in medium with no plant extract, and the second consisted of plant extract in cultured medium for the sterility control. All tubes were plugged with sterile cotton and incubated at 37°C for 1 week. The counting of dead and alive amoebae was done daily through a microscope with the aid of Neubauer's cell. The test was considered as positive if vegetative and cystic forms were not microscopically observed. The minimum inhibitory concentration ranging from 500 to 2.5 μg/ml in culture medium was determined for each plant extract tested.

3. Results

Table 1 shows the major constituents present in different extracts. Steroids and terpenoids are present in all extracts. Flavonoids are mainly found in the leaves and rarely in the stem or root extracts. These later extracts contain tannins as predominant constituents. Alkaloids were detected in *Alchornea cordifolia*, *Crossopteryx febrifuga*, *Datura arborea*, *Dracaena reflexa*, *Cryptolepis sanguinolenta*, *Harungana madagascariensis*, *Hymenocardia acida*, *Rauwolfia obscura* and *Voacanga africana* extracts while anthraquinones were only identified in extracts from *Harungana madagascariensis*, *Morinda morindoides* and *Cassia siamea*. The inhibitory potency of *E. histolytica* growth by extracts as indicated by their MIC values is summarized in Table 2. Results indicate that of 45 extracts tested, 35 inhibited *E. histolytica* growth and 10 were inactive. The most inactive extracts were from leaves. As the criterions depend of each searcher group, the degree of the antiamoebic effect of each plant extract in our screening was appreciated by its minimum inhibitory concentration (MIC) as follows:

$MIC \leq 20$ μg/ml: more active;
$20 < MIC < 100$ μg/ml: active;
$100 < MIC \leq 250$ μg/ml: moderately active;
$MIC = 500$ μg/ml: weakly active;
$MIC > 500$ μg/ml: inactive.

4. Discussion

Forty-five extracts from Congolese medicinal plants were tested in vitro against *Entamoeba histolytica*. Results in Table 2 indicate that the decoction form is more used than the macerate

Table 1
Phytochemical screening of crude extracts from selected plants

Plant species and parts		Flav.	Tan.	Ster/terp.	Sap.	Alk.	Anthraq.
Alchornea cordifolia Mull. Arg. (P86030NL)	L	+	+	+	+	+	−
Bridelia ferruginea Bernth (P860302NL)	L	+	−	+	+	−	−
	Sb	+	+	+	+	−	−
Cajanus cajan (L.) Millsp (P860351NL)	L	+	−	+	−	−	−
Cassia siamea Lam. (P860343NL)	Sb	−	+	+	+	−	+
Carica papaya L. (P860325NL)	Seeds	−	−	−	−	−	−
Ceiba pentandra Gaertn (P860310NL)	Sb	−	+	+	+	−	−
Cissius areloides Planch (P860308NL)	L	−	−	+	−	−	−
Costus afer Ker Grawl (P860306NL)	Juice	−	−	+	−	−	−
Crossopteryx febrifuga L. (P860342NL)	L	+	+	+	−	+	−
Cryptolepis sanguinolenta (Lindl.) Schlecther (P86039NL)	Rb	−	−	+	+	+	−
Datura arborea mettel (P860316NL)	L	−	−	+	−	+	−
Dialum englerianum H. (P860315NL)	Sb	−	+	+	+	−	−
Draceana reflexa var. Nittens Wew ex Back (P860318NL)	L	+	−	+	+	+	−
Euphorbia hirta L. (P860320NL)	Wp	+	+	+	+	−	−
Garcinia kola Heckel (P860322NL)	Sb	+	+	+	−	−	−
Harungana madagascariensis Lam. ex Poir. (P860321NL)	Sb	−	+	+	+	+	+
Hensia pulchella K. Schum. (P860325NL)	Rb	−	+	+	+	−	−
Hymenocardia acida Tull (P860324NL)	Rb	−	+	+	+	+	−
	Sb	−	+	+	+	−	−
Mangifera indica L. (P860319NL)	Sb	−	+	+	+	−	−
Maprounea africana Mull. Arg. (P860317NL)	L	+	−	+	−	−	−
	Rb	−	+	+	+	+	−
Morinda morindoides (Baker) Milne Redhead (P860326NL)	L	+	−	+	+	−	+
Myrianthus arboreus P. Beauv (P860327NL)	L	−	−	+	−	+	−
Nauclea latifolia Smith (P860329NL)	L	+	−	+	−	+	−
	Rb	−	+	−	+	+	−
Ongokea goré (Hua) Pierre (P860330NL)	Sb	+	+	+	−	+	−
Paropsia brazzeana Baill. (P860332NL)	Rb	−	+	+	+	−	−
Pentaclectra macrophylla Benth. (P860335NL)	Sb	−	+	+	+	−	−
Psidium guajava L. (P860337NL)	L	+	+	+	+	−	−
	Sb	−	+	+	+	−	−
Phytolacca dodecandra H. (P860341NL)	L	−	−	+	−	−	−
Pteridium aquilum Kuhln (P860339NL)	T	−	−	−	+	−	−
Quassia africana Baill. (P860343NL)	Sb	−	+	+	+	+	−
Rauwolfia obscura K. Schum (P860338NL)	Rb	−	+	+	−	+	−
Sida rombifolia L. (P860344NL)	L	−	−	+	−	+	−
Tetraceara poggei Gilg. (P860345NL)	L	+	−	+	−	−	−
Tithonia diversifolia (Hasmel) A. Gray (P860350NL)	L	+	+	+	+	−	−
Vitex madiensis L. (P860354NL)	L	+	−	+	+	−	−
Voacanga africana Stapf (P860347NL)	Rb	+	+	+	−	+	−
Justicia insularis Mull. Arg. (P860355NL)	L	−	−	+	−	−	−
Jathropha curcas L. (P860352NL)	L	+	−	+	+	−	−

Alk.: alkaloids, Anthraq.: Anthraquinones, Flav: flavonoids, L: leaves, Rb: root bark, Sap.: saponins, Sb: stem bark, Ster/terp.: steroids and terpens, T: twigs, Tan: tannins, Wp: whole plant, +: test positive, −: test negative.

one. Of 45 extracts tested, 35 (77.78%) showed at different degrees antiamoebic activity. Among these active extracts, seven extracts (15.56%) exhibited a strong antiamoebic activity (MIC = 15.6 or ≤ 7.81 μg/ml). They include aqueous extracts from *Carica papaya* (mature seeds), *Hensia pulchella* (root bark), *Mangifera indica* (stem bark), *Cryptolepis sanguinolenta* (root bark) *Paropsia*

Table 2
Antiamoebic activity of crude extracts from some Congolese medicinal plants (MIC in μg/ml)

Family and botanical name	Part used	Quantity (g)	Meth prep.	MIC (μg/ml)
Acanthaceae				
Justicia insularis Mull.Arg.	L	20.03	decoction	>500
Agavaceae				
Draceana reflexa var. Nittens Welw. ex Bak.	L	30	decoction	62.5
Anacardiaceae				
Mangifera indica L.	Sb	66	decoction	≤7.81
Apocynaceae				
Rauwolfia obstura K.Schm.	Rb	20	decoction	31.5
Voacanga africana Staph.	Rb	57.80	maceration	62.5
Asteraceae				
Tithonia diversifolia (Hasmel) A. Gray	L	32.29	maceration	62.5
Bombacaceae				
Ceiba pentandra (L.) Gaertn	Sb	22	decoction	125
Caesalpiniaceae				
Dialum englerianum H.	Sb	20	decoction	62.5
Caricaceae				
Carica papaya L.	[a]Seeds	20	decoction	≤7.81
	[b]Seeds	20	macerate	62.5
Clusiaceae				
Garcinia kola Haeckel	Sb	50	decoction	125
Dillenaceae				
Tetracera poggei Gilg.	L	29.13	decoction	>500
Euphorbiaceae				
Alchornea cordifolia Mull. Arg.	L	16.52	decoction	125
Bridelia ferruginea Benth	Rb	16.52	decoction	62.5
	Sb	9	decoction	250
Euphorbia hirta L.	L	16	maceration	250
	Wp	20	maceration	31.25
Hymenocardia acida Tull.	Sb	21.06	decoction	31.25
	Rb	21.06	decoction	250
Jatropha curcas L.	L	20.5	decoction	31.25
Maprounea africana Mull. Arg.	L	25	decoction	62.5
	Rb	20	decoction	31.25
Fabaceae				
Cajanus cajan (L.) Millsp.	L	60	decoction	>500
Flacourtiaceae				
Paropsia brazzeana Baill.	Rb	10	decoction	≤7.81
Hypericaeae				
Harungana madagascariensis Lam. ex Poir	Sb	50	decoction	62.5
Malvaceae				
Sida rhombifolia L.	L	25.3	decoction	62.5
Mimosaceae				
Pentacletra macrophylla Benth.	Sb	33.04	decoction	250

Table 2 (*Continued*)

Family and botanical name	Part used	Quantity (g)	Meth prep.	MIC (μg/ml)
Moraceae				
Myrianthus arboreus P. Beauv.	L	23.2	decoction	>500
Myrtaceae				
Psidium guajava L.	L	54	decoction	62.5
	Sb	37.3	decoction	≤7.81
Olacaceae				
Ongokea goré (Hua) Pierre	Sb	16.3	decoction	>500
Periplocaceae				
Cryptolepis sanguinolenta (Lindl) Schlechter	Rb	20	macerate	≤7.81
Phytollacaceae				
Phytollaca dodecandra H.	L	30.5	decoction	>500
Pteridaceae				
Pteridium aquilinum (L.) Kuhn	T	35.22	decoction	>500
Rubiaceae				
Crossopteryx febrifuga Benth.	L	20	decoction	125
Heinsia pulchella K. Schum.	Rb	20	decoction	15.62
Nauclea latifolia Smith	L	20	decoction	>500
	Rb	25.6	decoction	125
Morinda morindoides (Baker) Milne Redhead	L	20	decoction	15.62
Simaroubaceae				
Quassia africana Baill.	Rb	32.14	decoction	31.5
Solanaceae				
Datura arborea mettel	L	34.92	decoction	125
Verbenaceae				
Vitex madiensis Oliv.	L	52.3	decoction	>500
Vitaceae				
Cissius areloides (Wew. ex Back.) Planck	L	30.17	decoction	>500
Zingiberaceae				
Costus afer Ker Gawl.	Juice	—	—	125
Metronidazole	—	—	—	<2.5

L: leaves, Meth. Prep.: method of preparation, Rb: root bark, Sb: stem bark, T: twigs.
[a] Mature seeds.
[b] Immature seeds.

brazzeana (root bark), *Psidium guajava* (stem bark) and *Morinda morindoides* (leaves). Another group of ten plant extracts (22.22%) exhibited an antiamoebic activity with MIC values ranging from 30 to 63 μg/ml, among other things *Euphorbia hirta*, a medicinal plant used in several African countries for its antiamoebic property (Kerharo and Adam, 1974; Kambu, 1990). The antiamoebic activity of this plant has been reported earlier (Ridet and Chartol, 1964; Ndir and Pousset, 1981). In our previous antiamoebic investigation on this species, a bioassay-guided fractionation of a 80% MeOH extract from the whole plant resulted in the localization of a more pronounced activity in the flavonoid and tannin fractions than in the saponin fractions (Tona et al., 1989). This finding was in good agreement with previous reports mentioned before. Unfortunately, active constituents are still unknown. Only the antibacterial and antiviral constituents from this plant were reported (Bakana, 1984). The aqueous extract from the root bark of *Quassia africana*

showed also an interesting activity. However, it is well known that *Quassia* species contain bitter constituents characterized as quassinoids. Some of them have been shown to possess a strong antiamoebic activity in vitro and in vivo (Del Pozo and Alcaraz, 1956; De Carneri and Casinovi, 1968; Gillin et al., 1982; Wright et al., 1988; Phillipson et al., 1995), but they also exhibited a pronounced cytotoxicity. Quassin was the first compound to be isolated from Simaroubaceae species (Phillipson et al., 1995). This compound was isolated and identified from the root of *Q. africana* (Lumonadio and Vanhaelen, 1986). Unfortunately, it had been shown to be inactive against *E. histolytica* in vitro (Phillipson et al., 1995). This suggested that the observed antiamoebic activity for this species could be due to the presence of other quassinoids, probably those described by Lumonadio and Farnsworth (1994), or at a certain degree, to its alkaloid constituents. The moderate antiamoebic activity showed by *D. arborea* extract is not due to its alkaloid constituents because atropine and scopolamine were found to be inactive in vitro against *E. histolytica* (Keene et al., 1986). However, this extract would act as antidiarrhoeic by the known antispasmodic effect of alkaloid components. For the other plant extracts showing a pronounced activity, it seems very difficult to ascribe the antimoebic effect to the presence of one class of compounds.

However, some of active plant extracts are currently used in traditional medicine of some African countries for the treatment of intestinal amoebiasis. This is the case of *Euphorbia hirta*, *Mangifera indica*, *Carica papaya* and *Psidium guajava*. *Morinda morindoides* is also used frequently for the same purpose (Kambu, 1990).

The 18 remaining active extracts (40%) showed a moderate (MIC = 125 or 250 μg/ml) or a weak (MIC = 500 μg/ml) inhibitory effect of *E. hystolitica* growth (Table 2).

The comparison of the antiamoebic activity of two different parts from the same plant species showed a significant difference of activity. This is illustrated in the case of the leaves and stem bark of *Psidium guajava* and *Hymenocardia acida*, root bark and leaves of *Nauclea latifolia*, *Quassia africana*, *Maprounea africana* and *Alchornea cordifolia*. The time of plant part collection appears to play an important role in the manifestation of the antiamoebic activity. The case of the seeds from *Carica papaya* reported in the present work is an interesting example illustrating this last observation. Results of thin-layer chromatography of these crude extracts strongly differed from each other suggesting the varied antiamoebic activity. This observation applied for all extracts tested indicated that the most inactive (22.22%) were from leaves.

In general, results from our biological assessing programme of these higher plant extracts allowed us to observe that aqueous crude extracts from *C. siamea*, *C. papaya*, *C. pentandra*, *C. febrifuga*, *D. englerianum*, *G. kola*, *H. madagascariensis*, *H. pulchella*, *H. acida*, *M. indica*, *M. africana*, *N. latifolia*, *O. goré*, *P. brazzeana*, *P. guajava*, *T. diversifolia*, *C. sanguinolenta*, *E. hirta* and *M. morindoides* act as antidiarrhoeic by a triple effect: antispasmodic (Tona et al., 1987), antibacterial (Kambu et al., 1989), and antiamoebic reported here. For some plants included in our antiamoebic screening, their antidiarrhoeic activity was also reported in other pharmacological models. The antidiarrhoeic property of *E. hirta* was attributed to quercetin 3-0-rhamnoside found with a capability to decrease both the total number of faeces and the number of diarrhoeic faeces in mice (Galvez et al., 1993), while that of the leaves of *C. papaya* was due to the presence of quercetin and its glycosides quercitrin and rutin showing a pronounced antispasmodic activity (Lutterdot, 1989; Morales et al., 1994; Lozoya et al., 1994). It is interesting to note that the same quercetin derivatives mentioned above were also isolated from the leaves of *M. morindoides* (Cimanga et al., 1995) and *E. hirta* (Bakana, 1984). The presence of these compounds in these plant species more supports their antidiarrhoeic property.

Results reported in the present work constitute a rational evidence and a scientific basis to justify and support the use of these traditional preparations for the treatment of amobiasis in Congolese traditional medicine. The most active plant extracts were selected for an extensive biological and phytochemical study leading to the isolation and characterization of active principles.

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