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IN VITRO AMOEBICIDAL ACTIVITY OF SOME MEDICINAL PLANTS OF THE BAMUN REGION (CAMEROON)

Paul F. Moundipa[1*], Kamini G. Melanie Flore[1], Charles F. Bilong Bilong[2], Iris Bruchhaus[3]

[1]Laboratory of Biochemical Toxicology and Nutrition Department of Biochemistry.[2] Laboratory of parasitology University of Yaounde I, [3]University of Hamburg, Germany , Bernhard Nocht Institute for Tropical Medicine

Email: pmoundipa@hotmail.com or pmoundipa@uycdc.uninet.cm

Abstract

Fifty five medicinal plants belonging to different families selected on the basis of their traditional use against jaundice and various liver disorders were tested for their amoebicidal activities. They were extracted and tested for their antiamoebic activity in vitro using polyxenic culture of *Entamoebic histolytica*. As the result, 14 exhibited an antiamoebic activity at a dose of 100µg/ml from the second to the fourth day of incubation. The 14 extracts selected extracts were additionnaly tested for 6 days at 10, 100 and 500µg/ml of concentration, and only the leaves extract of *Codiaeum variegatum* exhibited a clear antiamoebic activity (EC_{50}=10,74 the second day), and had a more pronounced activity than metronidazole the reference product.

Keywords: Medicinal plants; *Codiaeum variegatum*; In vitro; amoebicidal; Bamun;.Cameroon

Introduction

Amoebiasis is the infection of human gastrointestinal tract by *Entamoeba histolytica (E.histolytica)*, a protozoan parasite capable of invading the intestinal mucosa and that may spread to other organs, mainly the liver which usually leads to amoebic liver abscess. This infection remains a significant cause of morbidity and mortality world-wide. (Samuel *et al*., 2001).

Metronidazole is the drug now widely used and recommended in the treatment of amoebiasis (Townson *et al*., 1994). But It is less effective in the tissue than in the gut lumen (Bhopale *et al*., 1995). In addition it can eradicate only up to 50% of laminae infections (Tierney et al., 1999) and has no action on cysts. This suggests the

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combination of metronidazole with other drugs as diloxanide furoate or paramomycin to eliminate the parasite from the intestine and to cure *Entamoeba histolytica* carriers. However, this combination therapy is not fully effective. .

As human is the only relevant host for this parasite, an effective treatment of luminal intestinal infection is necessary to interrupt transmission of the parasite. Therefore the search of new compounds with amoebicidal activity is urgent and important.

The selection of medicinal plant as new source of drugs is based mainly on herbal remedies used in traditional medicine (Distasi, 1995). In the Bamun traditional medicine (Cameroon) certain plants are used to cure jaundice and other liver disorders (Moundipa *et al*., 2002). Some of these disorders caused by the invasion of this organ by some parasites including *E. histolytica,* lead to development of hepatic amoebiasis. Nevertheless, no investigation has been undertaken in Cameroon with a view to determine the effect of our plants extracts

The purpose of the present study was to identify some of the plants that were active *in vitro* against polyxenic culture of *E. histolytica*

Material and methods

Plant materials

The plants used in this study were havested in the Noun department (Cameroon) and dried (Moundipa et al., 2002). The dried plant material (50g) was grounded and extracted with a methanol : Methylene chloride (v/v) (200ml) by maceration for 24 hrs and *Codiaeum variegatum* was extracted in water by decoction. The solvent was evaporated using Rotavapor apparatus. The residue of each extract was stored at -41°C. All the chemical used in this study were of cell culture grade.

In vitro culture of *Entamoeba histolytica*

Trophozoites isolated from amoeba dysenteric patient faeces were used for this purpose. Boeck and Drbohlav's polyxenic and diphasic amoebic medium was used for culture according to Parija et al. (1995).

Preparation of the culture medium

The culture was done in the diphasic polyxenic medium which constituted the albumin slope and overlay solution. Albumin slope was prepared by mixing 270ml of fresh egg albumin and 75 ml of sterilised Ringer solution (0.8g NaCl, 0.2g $CaCl_2$ 0.2g KCl in 100 ml of distilled water). The mixture (2.5ml) was dispensed aseptically after filtration through sterile gause into sterile culture tubes and insipitated in slanted position at 100°C for 10 min. The overlay solution was obtained by mixing 100 ml of sterilised Lock's solution (8g NaCl. 0.2g Na_2HPO_4. 0.2g KCl 0.01$MgCl_2$, 0.4g $NaHCO_3$ and 0.3g KH_2PO_4) to 1 ml of calf serum. To complete the medium, 5 ml of overlay solution was added to each tube containing albumin.

Culture of parasites

Just before the time to sow, a loop full of sterilised rice starch (1 mg) was added

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to the medium. Then a small quantity of faeces was inoculated in the culture medium and incubated at 37°C for 48 hrs. After this time, the culture fluid in the tube was mixed and then examined on a microscope for amoebic growth. In order to renew the culture medium, culture tubes were chilled on ice for 5 min and the upper phase (around 4 ml) was discarded. The sedimented part containing the parasites was mixed and transfered to a fresh sterile culture tube containing the culture medium and rice starch. This operation repeated for a further 48 hrs permitted to maintain the amoeba strain.

Measurement of amoebicidal activities of plant extracts.

In vitro test against trophozoites of *E. histolytica* was carried out according to Chitravanshi et al. (1992). Plant extract was used at the concentration of 100µg /ml. For this assay, two controls were used ($T_{0,}T_{d)}$ and one test (T_e).

To 5ml of the medium in test tubes were added 50 µl of the plant extract (to achieve 100µg/ml, for the first screening test of 58 plants or 10, 100, or 500µg/ml for the 14 selected plants extracts) and 1ml of the amoebic inoculum containing around 10^5 trophozoites. The control tubes T_o and T_d contained 5 ml of inoculum and 50µl of DMSO respectively. Metronidazole was used as reference drug. All the tubes were incubated at 37°C for 48 hrs. After this time, 1ml of the medium was taken off from each tube for the viability count using trypan blue exclusion technique. The remaining medium was transferred as previously described in another new culture tubes containing the same quantity of extract or control, for another 48 hrs. The mortality percentages of the trophozoîtes was calculated according to the following formula and the EC_{50} was evaluated using graph pad prism 3.0.

$$\text{Mortaliy (\%) due to extract} = \frac{\text{number of dead cells in Te} - \text{number of dead cells in Td}}{\text{total number of cells}}$$

Results

The result of the *in vitro* effect of extracts on *E. histolytica* after 48 hours and 96 hours of incubation was summarised in Table 1.

At the dose of 100µg/ml after 48 hours of incubation 16 of the tested extracts showed antiamoebic activity (AA) higher or equal to 50% mortality. The highest activity (% mortality = 93.19) was obtained with the extract of *Euphorbia hirta*. After 96 hours of contact 23 extracts had an AA ≥ 50% (Table 1). The highest percentage (91%) was obtained with the extract from *Euphorbia hirta*. It was noticed that ten extracts with AA < 50% at 48 hours of incubation exhibited an AA ≥ 50% at 96 hours. These species were *Vernonia amygdalina, Sonchu oleraceus, Occimum gratissimum, Nauclea latifolia, Manihot esculenta, Kalonchoe crenata, Gladiolus dalenii, Eremomastase speciola, Costus afer* and *Citrus cinensis.* At 48 hours of incubation the extract of *Codiaeum variegatum* had an AA of 75.5% and at 96 hours no survival parasite was found. In addition there was no cyst in the medium.

At the end of this first screening, 14 plants (*Harungana madagascariensis, Dichrocephala integrifolia, Gladiolus dalenii, Costus afer, Piliiostima thonningii, Ocimum gratissimum, Senna alata, Voacanga africana, Codiaeum variegatum, Bidens*

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pilosa, Nauclea latifolia, Melinis minutiflora, Olax subcorpioideae, Polysia fulva) were selected on the basis of continuous AA ≥ 50% and from 48 to 96 hours of incubation These plant extracts were then screened at various concentration for six days and the results are show on Table 2.

These results showed the highest activity of *Codiaeum variegatum* compared to metronidazole irrespectively of the doses and the incubation times. At 6 days of contact no *E. hyistolytica* survivors were found in the culture (Table 2). Secondly, the extract of *Voacanga africana* had a good AA especially for the doses of 100µg/ml to 500µg/ml. Furthermore this AA appeared highest than that of metronidazole at 48 h (100µg/ml) and 96h (500µg/ml). C*ostus afer, Bidens pilosa* and *Senna alata* at the concentration of 10 µg/ml after 144 h there are some amoebae still alive, but the antiamoebic activity was also higher in comparison to metronidazole. Other plant extracts that exhibited higher AA than that of metronidazole at various concentrations were *Costus afer,*

Polysia fulva, Ficus thonningii, Olax subcorpioideae, Voacanga africana, Occimum gratissimum, Bidens pilosa.

The EC_{50} values of different plants (Table 3) show variation from the second to sixth day of incubation time. At the end of the test, we realised that only the extract of *C. variegatum* has a decrease EC_{50}. From fourth to sixth day, the EC_{50} was not evaluated for this extract due to the highest mortality (100%). In the normal case, when the mortality percentage increased, the EC_{50} value decreased.

Discussion

The results obtained in this study show other potential therapeutic effects of medicinal plants used by the Bamun against liver disorders. Many of the plant extract tested exhibited amoebicidal activity mainly *C. variegatum* and *V. africana.* In addition their *in vitro* activity were higher than that of metronidazole, the reference drug (EC_{50} respectively 10.74, 9.99, and 46.00 at the second day of incubation). For the other extracts tested, their activities were similar to what was observed by Tona et al. (1998) and may be due to their harmful effect on bacteria living in amoeba polyxenic culture (Rabe et al., 1975, Ibrahim and Osman, 1995, Nakamura et al., 1999). It is well documented that *E. histolytica* is more virulent in association with suitable bacterial cells (Wittner et al., 1970., Bracha et al., 1984). Furthermore the leave extract of *O. gratissimum, Psidium guyava* have antidiarrheal properties (IIori et al., 1996, Almeida et al., 1995, Offiah and Chikwendu, 1999). However, according to our study these plants did not have a pronounced antiamoebic effect.

In other respects, some of the plant extracts investigated in this study have been studied elsewhere and the results obtained are similar (*Psidium guyava, Mangifera indica* (Tona et al., 1998) and *Euphorbia hirta* (Murengezi, 1993).

At 10µg/ml and after six days of incubation the best antiamoebic activity was obtained with the extract of *Codiaeum variegatum.* This plant is a good potential candidate for future studies, mainly to confirm the true amoebicidal activity in axenical culture, and biochemical mechanism of antiamoebic inhibition.

Table 1 : Effect of extracts on *Entamoeba histolytica* in vitro after 48 hrs and 96 hrs of incubation at the dose of 100µg /ml

Species and voucher number	FAMILY	Part used	Incubation time	
			2 d.	4 d.
Eremomastase speciosa (Hochst.) Cufod (24165 YA)	ACANTHACEAE	Leaves	22.33	58.82
Draceana dusteliana Engl (27673 YA)	AGAVACEAE	Leave	0	21.73
Mangifera indica Lin (18646 YA)	ANACARDIACEAE	Root bark	88.23	88.24
Anona senegalensis Pers. (7783 YA)	ANNONACEAE	Leaves	0.43	0
Enantia chlorantha Oliv. (6420 YA)		Root bark	32.00	17.64
Voacanga africana Stapf (47215 YA)	APOCYNACEAE	Root bark	67.14	58.38
Xanthosoma sagittifoliom L .Schott (42355 YA)	ARACEAE	Tuber	22.33	35.29
Polysias fulva (Hiern.) Harms (2990 YA)	ARALIACEAE	Root bark	50	64.70
Agerantum conyzoïdes Lin. (6575 YA)	ASTERACEAE	Aera part	82.35	70.29
Bidens pilosa Lin. (6555 YA)		Aera part	64.51	68.38
*Chrysanthelium americanum (*Lin.*)* Vatke (7992 YA)		Whole plant	3.71	0
Dichrocephala integrifolia (Lin.F)O.Ktze (5603 YA)		Whole plant	46.74	39.67
Emilia coccina (Sims) G.Don (41003 YA)		Aera part	81.76	30.43
Sonchus oleraceus Lin. (37069 YA)		Leaves	27.35	52.94
Vernonia amygdalina Del. (3114 YA)		Leaves	40	56.52
*Dacryodes edulis (*G.Don)H.Lam (18258 YA)	BURSERACEAE	Leaves	0	21.73
Piliostigma thonningii (Schum.) (2689 YA)	CAESALPINIACEAE	Leaves	82.35	56.52
Piliotigma thonningii (Schum.)		Root bark	0	25.57
Senna alata (Lin.) Link (11002 YA)		Leaves	60.43	88.23
*Terminalia glaucescens*Planch.ex benth. (4861 YA)	COMBRETACEAE	Root bark	1.176	23.52
Ipomea batatas (Lin.) Lam. (18597 YA)	CONVOLVULACEAE	Leaves	1.176	35.52
Kalonchoe crenata (Andr.) Haw. (41870 YA)	CRASULACEAE	Leaves	23.35	76.17
Carica papaya Lin. (18647 YA)	CARICACEAE	Leaves	0	0
Alchornea laxiflora (Benth*)* Pax &K.H.(2039 YA)		Leaves	60.43	52.17
Codiaeum variegatum var *moluccanum*	EUPHORBIACEAE	Leaves	75.55	/
Euphorbia hirta		Aera part	93.04	91.30

Manihot esculenta Crantz (42571 YA)		Leaves	30.43	56.82
Crotalaria lachnophora Hochst. Ex A.R (39337 YA)	FABACEAE	Leaves	0.58	0
Erythina senegalensis D.C. (35259 YA)	FABACEAE	Root bark	41.17	17.64
Harungana madagascariencis Lam. (4224 YA)	HYPERICACEAE	Leaves	67.74	62.90
Gladiolus dalenii (bulb) Van Geel. (17260 YA)	IRIDACEAE	Bulb	19.35	58.38
Ocinum gratissinum Lin. (42852 YA)	LABICEAE	Leaves	40.32	62.10
Persea americana Mill. (18604 YA)	LAURACEAE	Root bark	0	42.10
Persea americana Mill.		Leaves	29.56	42.10
Anthocleista schweinfurthic Gil. (2281 YA)	LOGANIACEAE	Root bark	0	0
Gossypium barbadense) (Mac fedyen) (25771 YA)	MALVACEAE	Leaves	41.17	41.17
Khaya grandifoliola D.C (52661 YA)	MELIACEAE	Root bark	0.588	11.72
Entada africana (Guill. Et Pers.*)* (2334 YA)	MIMOSACEAE	Root bark	58.82	34.78
Ficus exasperata Vahl. (43999 YA)	MORACEAE	Root bark	23.52	0
Ficus thonningii Bl. (50164 YA)		Root bark	0.55	3
Musa sapientum Lin.	MUSACEAE	Leaves	23.52	35.29
Psidium guyava Lin. (2885 YA)	MYRTACEAE	Leaves	69.41	64.70
Olax subscorpioideae Oliv. (3528 YA)	OLACACEAE	Leaves	62.5	56.52
Cymbopogon citratus (D.C) Stapf (18628)	POACEAE	Leaves	22.35	41.17
Melinis minutiflora P. Bearw. (5008 YA)		Aera part	51.6	72.58
Coffea arabica Lin. (58228 YA)	RUBIACEAE	Leaves	81.73	47.82
Coffea robusta Lin.		Leaves	0.43	0
Nauclea latifolia Sm. (7257 YA)		Root bark	48.38	51.81
Citrus aurantifolia Swingle	RUTACEAE	Leaves	22.35	5.88
Citrus cinensis L. (Osbeck)		Leaves)	34.23	82.35
Solanum acaleastrum Dunal. (35680 YA)	SOLANACEAE	Leaves	16.95	0
Trema orientalis Lour. (1917 YA)	ULMACEAE	Leaves	0	1.16
Costus afer Ker. Gwl. (23056 YA)	ZINGIBERACEAE	Leaves	29.41	83.33
Curcuma longa (rhyzomzq) Lin. (38292 YA)		Leaves	44.11	36.24
Control Metronidazole			54.08	73.4

Table 2 : *In vitro* amoebicidal activity (%mortality) of different extracts on *Entamoeba histolytica* after 48hrs, 96hs and 144hrs of incubation at different doses

Doses	10µg/ml			100µg/ml			500µg/ml		
Incubation times / Specis	48h	96h	144h	48h	96h	144h	48h	96h	144h
Control Metronidazole	18.75±6.84	63.09±1.686	72.55±0.520	58.82±4.023	77.586±12.04	71.42±1.08	74.37±4.09	59.35±7.66	91.66±11.11
Bidens pilosa	27.33±15.06	16.66±8.57	60.00±0.00*	64.51±2.82*	68.38±8.39*	74.19±10.57*	27.94±6.31	42.64±6.98	14.70±3.14*
Senna alata	15.00±1.37	42.5±5.33	62.30±2.524*	1.83±0.00	43.54±4.18	64.51±8.71*	29.16±0.00	52.94±2.47	87.82±3.37
Codiaeum variegatum	82.25±0.86 *	91.17±3.090*	*	75.82±0.69*	/*	/*	75.00±0.00*	/ *	/*
Costus afer	15.00±0.00	26.65±1.14	60.00±13.46*	67.74±0.116*	68.88±10.76*	70.96±0.59*	45.58±9.32	41.12±5.080	85.29±5.66*
Dichrocephala integrifolia	26.66±16.274	27.33±20.39	16.66±1.125	46.74±0.010	39.67±10.73	72.58±7.29*	41.66±8.290	40.27±8.857	77.77±1.82*
Ficus thonningii	15.00±0.000	11.25±13.17	36.75±1.004	80.88±2.20*	67.74±5.83*	54.83±3.169*	31.94±2.99	45.83±0.58	80.00±5.893*
Gladiolus dalenii	26.66±5.19	17.5±7.162	12.5±1.178	19.35±19.198	58.38±5.892*	51.61±1.32*	16.66±1.85	33.33±14.14	76.88±3.93*
Harungana madagascariencis	15.25±1.55	12.50±11.2	10.00±3.63	67.74±0.02*	62.90±0.00*	68.06±3.36*	67.64±10.62*	64.70±4.091*	79.41±0.004*
Melinis minutiflora	27.33±5.05	14.00±2.706	23.33±14.14	72.58±9.085*	51.6±9.08*	70.96±1.97*	26.385.656±	11.11±0.18	12.5±3.92
Nauclea latifolia	3.75±4.12	16.66±18.25	35.00±2.472	48.38±10.49	51.81±3.09*	80.64±2.71*	46.17±7.33	44.11±0.00	16.66±3.252
Ocinum gratissinum	53.33±4.395	37.6±0.654	37.5±0.00	40.32±0.054	62.10±2.17*	58.06±5.84*	42.64±6.80	48.52±4.46	85.29±7.77*
Olax subscorpioideae	30.00±15.20	12.5±5.672	23.33±14.12	62.5±0.013*	56.52±9.07*	58.06±1.60*	48.61±0.90	26.38±5.37	16.66±10.1
Polysias fulva	35.00±2.011	12.50±5.00	5.00±9.64	95.58±0.054*	48.38±4.95	48.38±10.61	27.77±5.89	50.00±4.16*	26.38±13.46
Voacanga africana	30.00±16.60	26.66±19.177	60.00±5.893*	67.74±2.84*	58.38±18.39*	79.03±3.34*	33.33±5.520	81.94±12.046*	76.38±1.8213*

Values are means ± SD of 2 different sets of experiments

* Plants with high antiamoebic activity

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Table 3 : Coomputed EC_{50} values according to incubation time

	EC_{50} (µg/ml)		
Species	2 days	4 days	6 days
Control Métronidazole	46.08	486.30	436.20
C. variegatum	10.74	-	-
S. alata	434.70	400.50	*
O subcorpioideae	11.48	10.15	477.40
M. minutiflora	-NC *	487.30	492.70
N. latifolia	13.02	9.87	30.67
B. spilosa	494.20	11.55	429.30
O. gratissimum	28.28	11.42	164.30
P. fulva	10.31	22.03	11.55
F. thonningii	12.71	35.52	-NC *
C. afer	11.43	11.02	191.10
G. dalenii	4.55	11.60	64.37
D integrifola	10.01	34.79	22.88
V. africana	9.99	79.57	10.84

-NC Not computed

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