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Ethnopharmacological Investigation and *In vitro* Anti-giardial Activity of Some Cameroonian Medicinal Plants

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Abstract: Background: Infection caused by *Giardia* is a major worldwide health problem with high endemicity in developing countries. The number of persons afflicted by parasites runs into many millions. Antimicrobial drugs and vaccines that have made possible the effective control of most bacterial and viral diseases have not yet been successful against parasitic infections. Thus, it is necessary to develop novel more effective and cheaper drugs for the management of these diseases. **Objective:** An ethnopharmacological survey was done in Fongo-Ndeng (one of the villages of West Cameroon) to identify some plants used in that village for the treatment of certain parasitic diseases. These plants were collected, identified and the crude extract prepared by maceration in the mixture of methylene chloride and methyl alcohol (v/v). **Results:** The obtained crude extracts were then assessed for their anti-giardial activity. *Giardia lamblia* was incubated with increased concentrations of different plant extracts ranging from 12.5 to 1600 μg mL^{-1} . After 24, 48 and 72 h of incubation, the effects of these plant extracts on the viability of the parasites were evaluated. For each plant extract, the Minimal Effective Concentration (MEC) was determined. The reference drug used in this study was Tinidazole at the final concentration of 0.3 μg mL^{-1}. The results revealed that after 72 h of incubation, *Phoenix reclinata* Jacq (4) and *Solanocia mannii* Hook. F. (7) were the best candidates with MEC of 100 μg mL^{-1} for both plants. **Conclusion:** These results confirm and validate the traditional use of these plants which could be a good sources and alternatives of anti-giardial drugs.

Key words: *Giardia lamblia*, ethnopharmacology, *Phoenix reclinata*, *Solanocia mannii*, parasitic disease

INTRODUCTION

Infection caused by *Giardia* is a major worldwide health problem with high endemicity in developing countries. The number of persons afflicted by parasites runs into many millions. Antimicrobial drugs and vaccines that have made possible the effective control of most bacterial and viral diseases have not yet been successful against parasitic infections (Paniker, 2007). Although major advances have been made in the field of treatment and prevention of these diseases, they are far from total satisfaction. In some cases of parasitic infections, no effective specific modern treatment is available. Moreover, these modern treatments are of long-term and are expensive for developing countries, therefore limiting their availability to populations with low incomes (Balana-Fouce *et al*., 1998; Mendonca-Filho *et al*., 2004). Thus, it is necessary to develop novel more effective and cheaper drugs for the management of these diseases.

Plant-based systems continue to play an essential role in health care. It has been estimated that approximately 80% of the population in developing countries depends on traditional medicine for their primary health care (Kirby 1996; Hostettmann and

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Marston, 2002; WHO 2003). In industrialized countries, adaptations of traditional medicine often termed Complementary or Alternative Medicine (CAM), also play an important role in the health care system of 20% of the population (WHO, 2003). In fact, plants have been the basis of many traditional medicine systems throughout the world for thousands of years and continue to provide mankind with new remedies (Jachak and Saklani, 2007). It is estimated that around 250,000 flowering plant species exist globally. Approximately half (125,000) of these are found in the tropical forests. They continue to provide natural product to chemists with invaluable compounds for new drugs development. The potential for finding new compounds is enormous, however only about 1% of tropical species have been studied for their pharmaceutical potential (Jachak and Saklani, 2007).

As a source of new drugs, African medicinal plants are understudied, considering the high percentage of plants not yet screened for their chemical composition or for their biological properties. One of the exceptions though is *Ancistrocladus Korupensis*, a plant found in Korup forest in Cameroon which contains an alkaloid-Michellamine B, efficient against HIV/AIDS and under study presently. Because of its importance in AIDS research, scientists in the country have started a program to conserve this rare plant. In addition to the unique oldest, Korup forest, Cameroon is the host of many more forest reserves as well as sacred untouched forests with unexplored treasures (medicinal plants). For example the Mount Cameroon's forest reserve is host to more than 40 unique plant species (Cheek *et al*., 1996). *Prunus Africana,* a montane wild tree species found in this forest is used as a drug for the treatment of prostate disorder. In general, Cameroon, known as Africa in miniature is a playground for scientists in the quest of unique species or molecules from its renowned biodiversity. An ethnopharmacological survey was conducted among some traditional healers of Fongo-Ndeng (one of the villages of West Cameroon). Some of these traditional healers have accepted to let out the secrets of some plants that they used since decades, to treat several infectious diseases. This article presents the results of this survey and the preliminary anti-giardial tests carried out *in-vitro* on crude extracts of these plants.

MATERIALS AND METHODS

Ethnopharmacological survey: An ethnopharmacological survey was done at Fongo-Ndeng, village situated at about 13 km from Dschang. Dschang is a city located in the Western Region of Cameroon and is well known for its biodiversity. Most people of this part of Cameroon relied on traditional healers for their primary health care. These traditional healers know plants that are used in the treatment of certain diseases. They have inherited this knowledge from their parents, grandparents and ancestors. To investigate the plants used by these traditional healers to treat many parasitic diseases, consultations were made through the representatives of these traditional healers or through the president of the association they belong to, or individually. They were informed about the purpose and the implications of this study. Those who agreed to share their knowledge signed a consent form. These consented traditional healers cooperated in the collection of plant parts they have been using to treat diarrhea, fever and colic pains. Each of these plants was identified from its leaves, flowers and fruits. The identification was made at the National Herbarium of Cameroon where voucher specimen numbers were obtained.

Preparation of the plant extracts: Following instructions of the traditional healer, different parts of collected plants were cleaned and cut into small pieces. These samples were then dried at room temperature, powdered and weighed for the preparation of crude extracts. The preparation of crude extracts was made using a mixture of solvents consisting of methanol and methylene chloride (v/v). Briefly, 100 g of each plant powder were macerated in the solvent mixture for 48 h, filtered and the filtrate concentrated using a rotary evaporator. The obtained crude extracts were thus stored at 4°C for subsequent works.

***In vitro* anti-giardial screening:** The strain of *Giardia lamblia* used in this study was obtained from the Department of Parasitology, Postgraduate Institute of Medical Education and Research (India). The trophozoites were axenically maintained. They were used at their log phase of growth. Briefly, about 10^6 parasites mL^{-1} were maintained in a logarithmic growth phase in a 96 well plate for 24 h before the treatment with plant extract or reference drug. The plant extract was tested at the dosages of 1600, 800, 400 and 200 μg mL^{-1} for 24, 48 and 72 h. The most potent plant extracts were then diluted again and tested at the concentrations of 100, 50, 25 and 12.5 μg mL^{-1}. After appropriate time of incubation (24, 48 or 72 h), living cells were counted by trypan blue exclusion technique, using haemocytometer chamber. The concentration of the plants at or above the optimal trophozoite killing concentration was defined as the Minimal Effective Concentration (MEC) for the selected plants. The experiment was carried

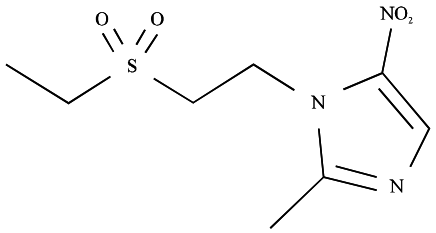

Fig. 1: Chemical structure of tinidazole

out in triplicate and repeated 3 times. The reference drug used in this study was tinidazole (Fig. 1) at the final concentration of 0.3 μg mL^{-1}.

This molecule is an anti-parasitic drug used against protozoan infections. It is widely known throughout Europe and the developing world as treatment for a variety of amoebic and parasitic infections. It was developed in 1972. The most common side effects reported with tinidazole are upset stomach, bitter taste, diarrhea and itchiness. Other side effects which occur are headache, physical fatigue and dizziness. Anecdotally, people who have taken both metronidazole and tinidazole report toxicity is much the same except the side effects don't last as long with the latter.

RESULTS AND DISCUSSION

Dynamics of ethnopharmacology in Western Cameroon: In Cameroon, the use of medicinal plants to cure diseases dates back several decades. Some people derived their livelihood from selling these medicinal plants. Thus, in each market for example, there is always a reserved space to sale natural products and their derivatives for the relief of illness of the population. These natural products are generally made of bark, roots, leaves or fruits of certain medicinal plants with various virtues. Some of these sellers of natural products and their derivatives have very good knowledge about the value and properties of the plant parts they sell and at times they try to process and package these products. But in most cases, the buyers of these products are not able to recognize or identify the plants or part of the plants used for the preparation of these products. Indeed, in most cases, the products they buy are generally powders, decoction, infusion or maceration of the medicinal plants. Apart from these vendors of plant and derived products to heal, there is another category of traditional healers, well established and have "their consultation offices" where patients come for help. Most of them are usually not willing to share the knowledge they have inherited from their ancestors. After persistent negotiations, some of these traditional healers may nonetheless agree to reveal the identification of the plants they use. So, the ethno-pharmacological survey conducted in a Fongo-Ndeng village (by Western-Cameroon) among some traditional healers revealed that some residents in the village depend mainly on medicinal plants for treating certain parasitic diseases. The results of this survey are presented in Table 1. This investigation allowed us to collect a total of 12 plants that are used by traditional healers in this village to treat, diarrhea, colic pains and fever (as they said). The fact that these symptoms can be due to some parasites prompted us to evaluate the anti-giardial activity of these collected plants.

Anti-giardial activity of collected medicinal plants: *Giardia lamblia* is the protozoa that cause giardiasis which is characterized by several symptoms including: diarrhea or dysentery, stomachache, cramps or bloating. The classical and effective treatment for this disease is metronidazole/tinidazole. However, besides other side effects, drug resistance as well as risk of potential mutagenicity and carcinogenicity have been described. Additionally, lengthy treatment or high doses often cause side effects such as headache, dry mouth, metallic taste, glossitis and urticaria (Aguirre-Cruz and Munoz, 1990; Oxberry *et al*., 1994; Kapoor *et al*., 1999, Calzada *et al*., 2005). The search for new drugs for the treatment of giardiasis is therefore necessary. According to our ethnopharmacological survey, different parts of the plants specified by the traditional healers were collected and crude extracts were prepared for the anti-giardial tests. The yields of these extractions from different plants are represented in Table 2. The results of the anti-giardial activity of these crude extracts are presented in Table 3. These results show that, all these plant extracts inhibit the growth of *G. lamblia* in a dose-dependent manner. Otherwise, 48 h after the incubation of the parasites with plant extracts, crude extracts of plants 2, 3, 4, 5, 7, 8 and 10 at the dose of 1600 μg mL^{-1} completely inhibits the growth of the parasites. These plants were considered the most potent and were then diluted for subsequent anti-giardial test. These potent plant extracts were submitted to a serial dilution and tested at the final concentration of 100, 50, 25 and 12.5 μg mL^{-1}. Table 4 presents the results of this analysis. These results clearly showed that after dilution, the crude extracts from *Phoenix reclinata* (4) and *Solanocia mannii* (7) completely inhibit the growth of the parasites with a MEC of 100 μg mL^{-1} after 72 h. In our study, tinidazole (taking as refecence) had a MEC of 0.3 μg mL^{-1} after 24 h. This great difference between the MEC of the plant extract and the MEC of the reference compound can be explained by the fact that, tinidazole is

Table 1: Some plants collected during the ethnopharmacological survey and their local use

Plan No.	Local names (in Fongo-ndeng)	Scientific names	Families	Specimen voucher numbers	Plant part with traditional use	Tradition method for extract preparation with water	Traditional use
1	Nveng lapin	*Emilia coccinea* (Sinus) G. Don.	Asteraceae	22066/SRFCam	Hole plant	Trituration	Diarrhea, stool with blood
2	Nduét nteuzan	*Chromolaena odoratum* L.	Asteraceae	9520/SRCam	Aerial part	Maceration	Diarrhea, fever and palpitation
3	Banhàhcoup or mgugueup	*Ficus exasperata* yaht	Moraceae	152441/HNC	barck	Decoction	Diarrhea and fever
4	Ngang lénàh	*Phoenix reclinata* Jacq	Arecaceae	12872/SRFCam	roots	Trituration	Diarrhea and stool with blood
5	Techieu fòn	*Solanum torvum* SW	Solanaceae	21104/SRFCam	leaves	Maceration	Diarrhea, fever and hepatosplenomegalia
6	Ntsapnè	*Hibiscus noldeae* Bak. F.	Malvaceae	49142/HNC	Aerial plant	Maceration	Digestive troubles, fever and diabetes
7	Abieu	*Solanocia mannii* Hook. F.	Asteraceae	7623 SRF Cam	Leave	Maceration	Diarrhea, fever and vomiting of bile
8	Banhàhcoup or mgugueup	*Ficus exasperata* yaht	Moraceae	152441/HNC	Leaves	Decoction	Diarrhea and fever
9	Bangàa toue	*Spilanthes africana* D.C.	Asteraceae	33075/HNC	Aerial part	Maceration	Digestive troubles, stool with blood, fracture, teeth pains
10	Nkieukieu	*Euphorbiaceae cordifolia* Elliot	Euphorbiaceae	20631 SRF Cam	Aerial part	Maceration	Digestive troubles, stool with blood and fever
11	Ajoujouh	*Kalanchoe crenata Haw*	Crassulaceae	33399/HNC	Aerial part	Maceration	Fever, fracture and ear pain
12	A foueu tsoung	*Entendophragma angolense* (Welw.) C.DC.	Meliaceae	29031/SRFCam	Bark	Decoction	Digestive problems, fever and rhumatism

Table 2: Yield of extraction of the crude extract from the collected plants

Plants No.	Name	Part used from the plant	Solvent of extraction	Yields of extraction (%)
1	*Emilia coccinea*	Whole plant	CH_2Cl_2/CH_3OH	7.89
2	*Chromolaena odoratum*	Roots	CH_2Cl_2/CH_3OH	4.80
3	*Ficus exasperata*	Barck	CH_2Cl_2/CH_3OH	10.48
4	*Phoenix reclinata*	Whole plant	CH_2Cl_2/CH_3OH	6.96
5	*Solanum torvum*	Leaves	CH_2Cl_2/CH_3OH	6.78
6	*Hibiscus noldeae*	Aerial plant	CH_2Cl_2/CH_3OH	6.48
7	*Solanocia mannii*	Leave	CH_2Cl_2/CH_3OH	11.07
8	*Ficus exasperata*	Leaves	CH_2Cl_2/CH_3OH	4.67
9	*Spilanthes africana*	Aerial part	CH_2Cl_2/CH_3OH	4.98
10	*Euphorbiaceae cordifolia*	Aerial part	CH_2Cl_2/CH_3OH	11.58
11	*Kalanchoe crenata*	Aerial part	CH_2Cl_2/CH_3OH	5.38
12	*Entendophragma angolense*	Bark	CH_2Cl_2/CH_3OH	4.05

Table 3: Anti-giardial activity of some Cameroonian medicinal plants extracts

Plants	Name	Doses (μg mL^{-1})	Cell viability after 24 h of treatment (%)	Cell viability after 48 h of treatment (%)	Cell viability after 72 h of treatment (%)
1	*Emilia coccinea*	1600	88.76±3.78	61.84±4.54	30.56±4.89
		800	90.86±2.80	68.94±5.73	45.84±6.44
		400	94.56±4.97	76.84±6.04	62.78±5.77
		200	95.89 ±3.09	80.84±4.90	73.05±6.30
2	*Chromolaena odoratum*	1600	0	0	0
		800	0	0	0
		400	17.98±4.78	0	0
		200	28.90±3.87	0	0
3	*Ficus exasperata*	1600	0	0	0
		800	0	0	0
		400	31.89±5.78	15.84±4.64	0
		200	47.43±3.73	24.84±5.63	0
4	*Phoenix reclinata*	1600	0	0	0
		800	0	0	0
		400	12.67±3.67	0	0
		200	21.78±7.67	0	0
5	*Solanum torvum*	1600	0	0	0
		800	21.09±7.90	0	0
		400	30.06±5.21	0	0
		200	37.92±5.81	10.04±4.76	0
6	*Hibiscus noldeae*	1600	80.04±6.83	50.77±6.92	36.02±5.03
		800	87.37±7.51	56.44±7.35	43.04±2.88
		400	92.04±5.18	61.90±4.84	54.39±5.81
		200	94.56±3.89	70.66±5.78	65.66±2.78
7	*Solanocia mannii*	1600	0	0	0
		800	0	0	0
		400	10.09±4.78	0	0
		200	15.90±3.78	0	0
8	*Ficus exasperata*	1600	21.84±6.73	0	0
		800	31.90±3.89	0	0
		400	36.90±5.78	0	0
		200	43.84±8.30	17.98±4.81	0
9	*Spilanthes africana*	1600	33.08±4.23	45.94±5.07	52.98±4.85
		800	37.82±4.82	47.56±4.65	61.40±4.84
		400	54.89±5.66	65.45±7.33	67.05±5.32
		200	70.84±4.39	73.83±6.54	77.04±3.89
10	*Euphorbiaceae cordifolia*	1600	0	0	0
		800	0	0	0
		400	12.78±6.83	0	0
		200	26.73±5.63	0	0
11	*Kalanchoe crenata*	1600	25.75±7.40	10.94±4.56	0
		800	33.83±3.18	15.78±3.78	0
		400	41.03±4.84	37.89±4.77	13.83±3.67
		200	53.83±3.89	46.90±5.89	26.84±4.78
12	*Entendophragma angolense*	1600	58.09±6.84	40.90±5.66	28.98±4.78
		800	71.56±5.05	56.78±2.90	46.90±5.88
		400	81.08±7.89	65.78±4.89	56.94±4.77
		200	92.89±4.89	70.94±6.67	68.96±5.78
Tinidazole*		0.3	24.89±4.82	0	0

The percentages of viability were calculated from the negative control (without any treatmnent), *Tinidazole, taken as reference compound, was tested at the dose of 0.3 μg mL^{-1}

Table 4: Anti-giardial activity of the most potent plants after dilution

Plants No.	Name	Doses (μg mL^{-1})	% of cell viability of 3 experiments after 24 h	% of cell viability of 3 experiments after 48 h	% of cell viability of 3 experiments after 72 h
2	*Chromolaena odoratum*	100.0	30.08±4.80	20.94±4.43	05.40±5.03
		50.0	44.56±5.49	37.04±6.73	30.94±6.99
		25.0	49.03±2.03	45.50±3.56	36.22±4.10
		12.5	56.92±4.89	48.39±4.82	40.05±4.62
3	*Ficus exasperata*	100.0	36.04±5.78	30.05±4.84	18.94±4.82
		50.0	49.06±6.90	36.95±3.33	24.94±5.44
		25.0	58.89±4.88	42.78±6.77	33.33±4.75
		12.5	67.04±4.89	58.94±6.54	49.84±9.40
4	*Phoenix reclinata*	100.0	16.94±5.94	05.94±4.94	0
		50.0	20.89±6.89	16.95±6.30	08.90±4.72
		25.0	35.98±7.02	23.03±4.37	28.09±6.50
		12.5	54.98±5.30	47.84±5.84	53.85±7.50
5	*Solanum torvum*	100.0	45.89±5.78	33.09±4.80	21.05±3.90
		50.0	50.05±4.78	41.89±5.78	36.90±4.78
		25.0	67.90±3.56	56.89±4.37	44.04±3.04
		12.5	70.04±5.05	67.94±5.74	58.94±5.47
7	*Solanocia mannii*	100.0	21.74±4.67	14.56±5.64	0
		50.0	29.85±6.72	22.95±5.73	10.04±4.54
		25.0	41.84±7.89	27.97±5.38	16.94±6.94
		12.5	51.89±4.82	40.94±5.94	34.99±5.73
8	*Ficus exasperata*	100.0	50.74±5.63	43.84±5.89	36.90±5.52
		50.0	57.85±2.89	41.94±6.38	35.05±6.93
		25.0	64.96±5.89	53.84±4.84	41.90±5.95
		12.5	71.90±5.06	62.05±7.84	49.95±6.95
10	*Euphorbiaceae cordifolia*	100.0	37.04±5.92	25.04±5.84	12.94±6.83
		50.0	44.84±7.41	30.04±4.85	25.94±3.04
		25.0	53.89±7.94	41.04±5.84	35.95±5.40
		12.5	61.90±4.78	49.86±5.78	43.06±4.89

Anti-giardial activity of the most potent plants after dilution. The percentages of viability were calculated from the negative control (parasites without any treatmnent)

already a pure molecule compared to the plant extracts which are the mixture of several compounds including the one responsible for the anti-giardial activity. This study constitute the groundstand for the identification of active molecule from this plant.

CONCLUSION

These results obtained confirm and validate the traditional use of some of these plants which could be a good sources and alternatives of anti-giardial drugs. Further phytochemical and pharmacological studies of these plants are evidently worthwhile and our group is focusing on this effort.

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