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Review

Antiplasmodial activity of various parts of *Phyllanthus niruri* according to its geographical distribution

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Extracts of *Phyllanthus niruri* L., collected from three different areas in the Congo (Kisantu, Kimwenza and University of Kinshasa), used for malaria treatment were tested *in vitro* in order to evaluate their antiplasmodial properties. Whereas the whole plant is traditionally used, aqueous extracts of the various parts of the *P. niruri* plant (stems, leaves and roots) tested on the chloroquine-resistant strain FcM29-Cameroon showed that only the leaves and the stems presented real *in vitro* antiplasmodial activity without any cytotoxicity. This information is particularly important because the leaves are affordable and their use is less damaging to plant stocks.

Key words: Ethno-pharmacology, *Plasmodium falciparum*, harvest areas, parts of plant.

INTRODUCTION

Malaria is a public health problem in tropical and subtropical regions. WHO estimates the number of clinical cases to be between 300 and 500 million, with more than 2 million deaths annually (WHO, 2003). The search for new antimalarial drugs with new modes of action is urgently needed and the ethno-pharmacological approach is a very interesting resource by which new therapies may be discovered.

The Democratic Republic of Congo (DRC), located in one of the richest floristic zones of Africa (Guineo-congolian zone) has an ancient cultural tradition of knowledge and use of medicinal plants. Among the plants used in this country for the treatment of malaria and its associated symptoms, we selected the plant *Phyllanthus niruri* for study, in collaboration with local traditional healers by ethnobotanical surveys. This plant is used for antimalarial treatment in all areas of the DRC and in other countries of Africa and Asia but its availability is drastically decreasing because of numerous harvests. In the DRC, local treatment against malaria or its associated symptoms consists of using a decoction of the whole plant with a mixture of roots, stems and leaves of *P. niruri*.

The objective of this study was to screen the *in vitro* antiplasmodial activity and the cytotoxicity of aqueous and ethanolic extracts of different parts (roots, stems and leaves) of *P. niruri* coming from 3 areas (Kisantu, Kimwenza and UNIKIN) of the DRC.

RESULTS AND DISCUSSION

Phyllanthus niruri L. (Syn. *P. fraternus* Webster), Euphorbiaceae, is a common weed found in both cultivated fields and wasteland. *P. niruri* is also a well-known medicinal herb that is traditionally and widely used in Asia, Africa and South America. This plant is

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Table 1. Antiplasmodial and cytotoxicity activities of the different parts of *Phyllanthus niruri* from three different areas.

Plant part tested (area) [a]	Extraction solvents [b]	Extraction yield (%)	IC_{50} (µg/ml) against *Plasmodium* [c]	IC_{50} (µg/ml) on cell lines: KB / Vero [d]
P. niruri stems (Kimwenza)	Ethanol	5.8	22 ± 4 [e]	100 / >100 [e]
	Water	10.3	14 ± 4	>100 / >100
P. niruri stems (Kisantu)	Ethanol	3.8	>50	100 / >100
	Water	6.5	11±2	>100 / >100
P. niruri stems (UNIKIN)	Ethanol	5.8	>50	100 / >100
	Water	8.9	16 ± 0	>100 / >100
P. niruri leaves (Kimwenza)	Ethanol	17.7	25 ± 4	85 / 100
	Water	16.7	19 ± 3	>100 / >100
P. niruri leaves (Kisantu)	Ethanol	6.5	19 ± 0	75 / 100
	Water	11.4	14 ± 1	>100 / >100
P. niruri leaves (UNIKIN)	Ethanol	13.9	22 ± 5	100 / >100
	Water	18.8	16 ± 1	>100 / >100
P. niruri roots (Kimwenza)	Ethanol	5	>50	100 / >100
	Water	6.3	>50	>100 / >100
P. niruri roots (Kisantu)	Ethanol	3	>50	>100 / >100
	Water	6.2	>50	>100 / >100
P. niruri roots (UNIKIN)	Ethanol	5.1	>50	100 / >100
	Water	4.3	>50	>100 / >100
P. niruri whole plant	Ethanol	ND [f]	26 ± 11	ND
Chloroquine (antimalarial drug control) [g]			290.10^{-3} µM	ND
Artemisinin (antimalarial drug control) [g]			7.10^{-3} µM	>300 µM
Taxotere (anticancer drug control) [g]			ND	$2.5.10^{-4}$ µM

traditionally used as an anti-hepatotoxic or anti-hypertensive, but it is more often used as anti-infective and principally against malaria. *In vitro* studies have confirmed these indications since *P. niruri* extracts showed therapeutic effects such as anti-hepatotoxic (Syamasundar et al., 1985), anti-HIV (Ogata et al., 1992), anti-hepatitis B (Venkateswaran et al., 1987) and antiplasmodial (Tona et al., 2004).

The local practice for malaria treatment by the population of the DRC with *P. niruri* consists essentially in using an extract of the whole plant (mixture of leaves, stem and root). The antiplasmodial activity assays (Table 1) showed that the ethanolic extract of the whole plant gave an IC_{50} of 26 µg/ml on the reference *Plasmodium* strain FcM29-Cameroon. This value is higher than that one already reported (Tona et al., 2004) who presented an IC_{50} value of 2.5 µg/ml. The difference between both IC_{50} values could be explained by the fact that we carried out the antiplasmodial tests on continuous laboratory cultures whereas Tona's team used isolates, directly obtained from the blood of adult subjects with acquired *P. falciparum* infection in endemic areas. Our value of 26 µg/ml is in the same range as crude extracts from other plants traditionally used in this area against malaria and also tested on continuous laboratory cultures such as *Momordica balsamina*, *Cognauxia podoloena*, *Uapaca paludosa, Vernonia brazzavillensis* (Benoit-Vical et al., 2006; Mbatchi et al., 2006; Zirihi et al., 2005; Menan et al., 2006).

The most interesting result was to prove that whatever the cultivation area and for both solvent of extractions, the leaves of *P. niruri* were effective *in vitro* against *Plasmodium* with IC_{50} ranging from 14 - 19 µg/ml for the aqueous extract and from 19 - 25 µg/ml for the ethanolic extract. Close results have been obtained, concerning the antiplasmodial activity of stems and leaves of the species *P. reticulatus* against different strains of *P. falciparum* (1 µg/ml < IC_{50} < 25 µg/ml) (Omulokoli et al., 1997). In parallel, all the aqueous extracts of stems, regardless of the source area showed interesting *in vitro* antiplasmodial efficacy (IC_{50} ranging from 11 - 16 µg/ml). On the contrary, our results showed that the ethanolic extract of only stems harvested in the Kimwenza zone (IC_{50} = 22 µg/ml) were effective whereas those from the stems harvested in both Kisantu and UNIKIN were devoid of *in vitro* antiplasmodial activity (CI_{50} > 50 µg/ml). The differences in antiplasmodial activities of the same plant extracts but harvested in different zones have already been demonstrated (Benoit-Vical et al., 1998). It seems that the properties of the soil and climatic conditions could be responsible. Here, we can note that the ground of kimwenza was sandy whereas ground of kisantu and UNIKIN was clayey that it could explain these differences of activity according to the source area.

Finally, all the extracts from *P. niruri* roots, whatever the source area or the extraction solvents, were not effective *in vitro* against *Plasmodium falciparum* (IC_{50} > 50 µg/ml). Omulokoli et al. have already shown that aqueous

extracts of the root of *P. reticulates,* another species, from the western province of Kenya were also inactive (IC_{50} > 100 µg/ml) against chloroquine-sensitive and chloroquine-resistant strains of *P. falciparum* (Omulokoli et al., 1997).

Out of all the extracts tested in the present study, the water extraction showed better results than ethanolic extraction. This information is particularly important because water is the extraction solvent used the most (and often the only one available) in traditional medicine in endemic areas. It is important to note that the water extracts that had the highest antiplasmodial activities also gave the best extraction yields (Table 1).

The fact that the leaves alone, but also some stem extracts, were active *in vitro*, means that it is possible to harvest only these plant parts for malaria treatment without destroying the whole plant (in particular the roots).

However, even if only the leaves and stems are active *in vitro* against *Plasmodium*, it would be very interesting to carry out further investigations on the pharmacological effects of roots during antimalarial treatment. Indeed, some plants (or parts of plants), without direct activity against the parasite, can show properties that could be acting on the symptoms of malaria (fever, anemia, hypoglycemia, etc.), and/or increase the bio-availability and/or enhance immunological stimulation *in vivo* (Benoit-Vical, 2005).

We have found in our study a weak *in vitro* cytotoxicity on 2 reference cell-lines (KB and Vero) of these extracts with IC_{50} values ranging from 75 µg/ml to largely superior to 100 µg/ml leading to promising security indexes. Furthermore, this low *in vitro* cytotoxicity is confirmed by the frequent use of *P. niruri* extracts in indigenous medical systems for a considerable time in the DRC with no evidence of clinical toxicity while ethnobotany surveys also exclude pronounced human toxicity.

Phyllanthus niruri pharmacognosy researches showed the presence of alkaloids (Joshi et al., 1986), terpenoids (Singh et al., 1991), and lignans (Huang et al., 1992). Some of these compounds, such as phyllanthin, and hypophyllanthin, have been reported to be hepatoprotective (Syamasundar et al., 1985) whereas niruriside appears to be a specific inhibitor of HIV replication (Qian-Cutrone et al., 1996). Lastly, four lignans (phyllanthin, hypophyllanthin, phyltetralin and niranthin) were found in these plant samples, with the highest amount of lignans found in the leaves and the least amount in the roots (Murugaiyaha and Chan, 2007). This confirms the importance of the leaves in future studies of this plant in the search for the molecules responsible for the antimalarial activity.

Conclusion

The aim of this manuscript was to make a transverse study on the relation antiplasmodial activity / biotope for *P. niruri* extracts. The low cytotoxicity and the antiplasmodial efficacy of *P. niruri*, principally the aqueous extracts, against *Plasmodium in vitro* validates the wide use of this plant in traditional medicine against malaria in the DRC, and other countries of sub-Saharan Africa where malaria is endemic. The biological activity of the leaves is particularly important for the biodiversity of this plant because leaves are affordable and their use does not damage the plants and limit the supply as the use of the roots would.

Moreover, the promising results of the aqueous extracts of leaves of *P. niruri* whatever the geographical region that was harvested indicate that the chemical fingerprints seem to be reproducible for this part of this plant. This justifies our continuing research to firstly fractionate leaf extracts to determine the active principles responsible of this antiplasmodial activity.

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Footnotes

a: The various parts of *Phyllanthus niruri* were harvested from January to April in the rainy season respectively by Nzeza, A. Carlier and Mukendi in 3 different areas of the Democratic Republic of Congo (Kisantu, UNIKIN, Kimwenza) where this plant is used for its antiplasmodial properties. The locality UNIKIN corresponds to the neighborhood of the University of Kinshasa. The distances between Kisantu and Kimwenza, between UNIKIN and Kimwenza, and between Kisantu and UNIKIN are 116, 4 and 120 km respectively. The botanical identification was assured by Mr Nlandu of the INERA (Institut National pour l'Etude et la Recherche Agronomiques) herbarium - University of Kinshasa. The herbarium samples were deposited at the herbarium under the numbers respectively 72 bis (Kisantu), 66 (Kimwenza) and 83 (UNIKIN). The fresh plant material was dried on mats on the floor at ambient temperature, avoiding direct sunlight. Once dried, the plants were crushed and packaged before being sent to France for extraction and biological testing.

b: The various parts of the plant were extracted in water and ethanol according to the traditional methods of pre-

paration. Ethanol extracts were obtained by simple maceration of 30g of powder in 300 ml of ethanol over 24 h. The operation was repeated twice on the residues. The three successive extracts were mixed together and then concentrated at reduced pressure at 35°C until a syrupy liquid was obtained. This liquid was taken up in 20 ml of distilled water and then freeze-dried to obtain a homogeneous dry extract. Aqueous extracts were prepared by simple decoction of 5 g of plant powder in 50 ml of boiling distilled water. The mixture was boiled for 10 min before being filtered through filter paper, and then centrifuged at 3000 rpm for 20 min. Each aqueous extract thus obtained was freeze-dried and stored at -20°C before the pharmacological tests.

c: The antiplasmodial activity was evaluated on the chloroquine-resistant (IC_{50} for chloroquine of 400 nM) strain FcM29-Cameroon (Soh et al., 2009). Parasites were cultured according to the procedure of Trager and Jensen (Trager and Jensen, 1976) with modifications (Benoit et al., 1995). The antiplasmodial activity of the *P. niruri* extracts was evaluated three times in triplicate by the radioactive microdilution method described by Desjardins et al (Desjardins et al., 1979) and modifications (Benoit-Vical et al., 2007).

d: The cytotoxicity of *P. niruri* extracts was assayed against KB (human epidermoid carcinoma) and Vero (monkey African green kidney) cells according to the methodology of Mbatchi et al. (2006).

e: IC_{50} in µg/ml $\pm$ sd (standard deviation) obtained from at least 3 independent experiments.

f: not determined.

g: molecules routinely tested as control.

REFERENCES

Benoit-Vical F (2005). Ethnomedicine in malaria treatment. IDrugs 8: 45-52.

Benoit-Vical F, Grellier P, Abdoulaye A, Moussa I, Ousmane A, Berry A, Ikhiri K, Poupat C (2006). *In vitro* and *in vivo* antiplasmodial activity of *Momordica balsamina* alone or in a traditional mixture. Chemother. 52: 288-292.

Benoit-Vical F, Lelievre J, Berry A, Deymier C, Dechy-Cabaret O, Cazelles J, Loup C, Robert A, Magnaval J, Meunier B (2007). Trioxaquines: new antimalarial agents active on all erythrocytic forms including gametocytes. Antimicrob. Agents Chemother. 51: 1463-1472.

Benoit-Vical F, Valentin A, Cournac V, Pelissier Y, Mallie M, Bastide JM (1998). *In vitro* antiplasmodial activity of stem and root extracts of *Nauclea latifolia* S.M. (Rubiaceae). J. Ethnopharmacol. 61: 173-178.

Benoit F, Valentin A, Pelissier Y, Marion C, Dakuyo Z, Mallie M, Bastide JM (1995). Antimalarial activity *in vitro* of *Cochlospermum tinctorium* tubercle extracts. Trans. Roy. Soc. Trop. Med. Hyg. 89: 217-218.

Desjardins RE, Canfield CJ, Haynes JD, Chulay JD (1979). Quantitative assessment of antimalarial activity *in vitro* by a semiautomated microdilution technique. Antimicrob. Agents Chemother. 16: 710-718.

Huang YL, Chen CC, Ou JC (1992). Isolintetralin: A New Lignan from *Phyllanthus niruri*. Planta Med. 58: 473-474.

Joshi BS, Gawad DH, Pelletier SW, Kartha G, Bhandary K (1986). Isolation and structure (X-ray analysis) of ent-norsecurinine, an alkaloid from *Phyllanthus niruri*. J. Nat. Prod. 49: 614-620.

Mbatchi SF, Mbatchi B, Banzouzi JT, Bansimba T, Nsonde-Ntandou GF, Ouamba JM, Berry A, Benoit-Vical F (2006). *In vitro* antiplasmodial activity of 18 plants used in Congo Brazzaville traditional medicine. J. Ethnopharmacol. 104: 168-174.

Menan H, Banzouzi JT, Hocquette A, Pelissier Y, Blache Y, Kone M, Mallie M, Assi LA, Valentin A (2006). Antiplasmodial activity and cytotoxicity of plants used in West African traditional medicine for the treatment of malaria. J. Ethnopharmacol. 105: 131-136.

Murugaiyaha V, Chan KL (2007). Determination of four lignans in *Phyllanthus niruri* L. by a simple high-performance liquid chromatography method with fluorescence detection. J. Chromatogr. A. 1154: 198-204.

Ogata T, Higuchi H, Mochida S, Matsumoto H, Kato A, Endo T, Kaji A, Kaji H (1992). HIV-1 reverse transcriptase inhibitor from *Phyllanthus niruri*. AIDS Research and Human Retroviruses 8: 1937-1944.

Omulokoli E, Khan B, Chhabra SC (1997). Antiplasmodial activity of four Kenyan medicinal plants. J. Ethnopharmacol. 56: 133-137.

Qian-Cutrone J, Huang S, Trimble J, Li H, Lin PF, Alam M, Klohr SE, Kadow KF (1996). Niruriside, a new HIV REV/RRE binding inhibitor from *Phyllanthus niruri*. J. Nat. Prod. 59: 196-199.

Singh B, Agrawal PK, Thakur RS (1991). Isolation of trans-Phytol from *Phyllanthus niruri*. Planta Med. 57: 98.

Soh PN, Witkowski B, Olagnier D, Nicolau ML, Garcia-Alvarez MC, Berry A, Benoit-Vical F (2009). *In vitro* and *in vivo* properties of ellagic acid in malaria treatment. Antimicrob. Agents Chemother. 53: 1100-1106.

Syamasundar KV, Singh B, Thakur RS, Husain A, Kiso Y, Hikino H (1985). Antihepatotoxic principles of *Phyllanthus niruri* herbs. J. Ethnopharmacol. 14: 41-44.

Tona L, Cimanga RK, Mesia K, Musuamba CT, Bruyne TD, Apers S, Hernans N, Van Miert S, Pieters L, Totte J, Vlietinck AJ (2004). *In vitro* antiplasmodial activity of extracts and fractions from seven medicinal plants used in the Democratic Republic of Congo. J. Ethnopharmacol. 93: 27-32.

Trager W, Jensen JB (1976). Human malaria parasites in continuous culture. Science 193: 673-675.

Venkateswaran PS, Millman I, Blumberg BS (1987). Effects of an extract from *Phyllanthus niruri* on hepatitis B and woodchuck hepatitis viruses: *in vitro* and *in vivo* studies. PNAS 84: 274-278.

WHO (2003). Traditional medicine. WHO (ed). Geneva pp. 1-334.

Zirihi GN, Mambu L, Guede-Guina F, Bodo B, Grellier P (2005). *In vitro* antiplasmodial activity and cytotoxicity of 33 West African plants used for treatment of malaria. J. Ethnopharmacol. 98: 281-285.