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Screening of Rwandese medicinal plants for anti-trichomonas activity

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A series of 30 medicinal plants used in Rwandese traditional medicine has been screened for anti-trichomonas activity against the protozoan *Trichomonas vaginalis*. Seventeen plants showed anti-trichomonas activity.

Key words: anti-trichomonas activity; Rwandese traditional medicine.

Introduction

In the course of systematic studies on biologically active substances with anti-protozoal activity from medicinal plants, we have screened a series of 30 Rwandese medicinal plants against *Trichomonas vaginalis*. This parasite is quite important as its distribution in a randomly selected population is estimated to be 5–20% (Wéry, 1983) and that it is responsible for urogenital infections.

All 30 species studied are being used in Rwandese traditional medicine, based on our personal enquiries to traditional healers in Rwanda (Van Puyvelde et al., 1975, 1977, 1982); 21 of these are used in the treatment of protozoal diseases (Table 1), while nine were chosen arbitrarily among well-known medicinal plants. Eighteen of the plants that are used to treat malaria have previously been evaluated for their anti-malarial activity (Hakizamungu and Wéry, 1988). The anti-trichomonas activity of the plant extracts against *Trichomonas vaginalis* was determined, using the Kupferberg medium by counting the mobile parasites, after 48 h of incubation under a microscope in a Burker cell.

Material and Methods

Plant material

Plants were collected in the district of Butare (Southwestern Rwanda) at a mean altitude of 1700 m and identified by the authors. Voucher herbarium specimens (Van Puyvelde's collection) are preserved in the herbarium of the Institut de Recherche Scientifique et Technologique (IRST), Butare, Rwanda. Samples were dried in an oven at 40°C and powdered mechanically.

Preparation of extracts

Approximately 30 g of the plant powder was extracted in a percolator with methanol to exhaustion. The methanolic extract was evaporated under reduced pressure (40°C) and the residue suspended in distilled water to obtain a concentration of 50 mg/ml.

Parasite used

Trichomonas vaginalis was supplied by the Laboratory of Protozoology of the Institute of Tropical Medicine 'Prins Leopold' of Antwerp (Belgium).

Culture medium

The medium used was a Bacto Kupferberg Trichomonas broth (Difco) enriched with fetal calf serum. The ingredients of the 1-l medium consisted of Bacto Tryptose, 20 g; Bacto Maltose, 1 g; cysteine hydrochloride, 1.5 g; Bacto Agar, 1 g; Bacto Methylene Blue, 0.003 g; and chloramphenicol, 0.1 g.

To rehydrate the medium, 23.6 g was suspended in 950 ml of distilled water and warmed to boiling point, then sterilized in an autoclave for 10 min at

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TABLE 1

ANTI-TRICHOMONAS ACTIVITY OF RWANDESE MEDICINAL PLANTS

Botanical name (family) (voucher specimen)[a]	Folk name	Plant parts[b]	Use against protozoan diseases	Test result
Aspilia pluriseta Schweinf. (Asteraceae) (VP 52)	Icyumwa	LF	Malaria	+
Blumea alata (D.Don) DC. (Asteraceae) (VP 85)	Igitabi cy'impyisi	LF	Trypanosomiasis	+
Cajanus cajan (L.) Millsp. (Fabaceae) (VP 40)	Umukunde	LF	Malaria	+
Cissus petiolata Hook f. (Vitaceae) (VP 137)	Umuhororo	LF	Not used	–
Clematis hirsuta Perr. et Guill. var. *hirsuta* (Ranunculaceae) (VP 110)	Umunkamba	LF	Malaria	+
Conyza sumatrensis (Retz) E.K. Walker (Asteraceae) (VP 96)	Wambuba	LF	Malaria	–
Crassocephalum vitellinum (Benth.) S. Moore (Asteraceae) (VP 44)	Umusununu	LF	Malaria	–
Dalbergia lactea Vatke (Fabaceae) (VP 55)	Umuhashya	LF	Malaria	–
Gardenia ternifolia Schum et Thonn. subsp. *jovis-tonantis* (Welw.) Verdc. (Rubiaceae) (VP 320)	Umutarama	LF	Malaria	–
Gynura scandens O. Hoffm. (Asteraceae) (VP 191)	Ikizimyamuliro	LF	Malaria	–
Harungana madagascariensis (Lam.) ex Poir (Clusiaceae) (VP 79)	Umushayishayi	LF SB	Malaria Malaria	+ +
Lablab purpureus (L.) Sweet subsp. *uncinatus* Verdc. (Fabaceae) (VP 388)	Umuharakuku	LF	Not used	+
Lantana camara L. (Verbenaceae) (VP 389)	Maviyakuku	LF	Malaria	–
Ludwigia abyssinica A. Rich (Onagraceae) (VP 70)	Umuzigangore	ST	Malaria	–
Lysimachia ruhmeriana Vatke (Primulaceae) (VP 391)	Umuyobora	LF	Not used	+
Maesa lanceolata Forsskal (Myrsinaceae) (VP 304)	Umuhanga	LF	Not used	+
Markhamia lutea (Benth.) Schum. (Bignoniaceae) (VP 84)	Umusave	LF	Malaria	+
Momordica foetida Schum. (Cucurbitaceae) (VP 64)	Umwishwa	LF	Malaria	+
Monechma subsessile (Oliv.) C.B. Clarke (Acanthaceae) (VP 100)	Umubazi	LF	Not used	+

TABLE 1 (*Cont.*)

Botanical name (family) (voucher specimen)[a]	Folk name	Plant parts[b]	Use against protozoan diseases	Test result
Myrica kandtiana Engl. (Myricaceae) (VP 131)	Isubuyo	LF	Not used	+
		ST	Not used	+
Orthosiphon suffrutescens (Thonn.) J.K. Morton (Lamiaceae) (VP 106)	Ubushohera	PL	Malaria	+
Pavetta ternifolia (Oliver) Hiern (Rubiaceae) (VP 20)	Umumenamabuye	LF	Malaria	−
Polygala luteo-viridis Chodat (Polygalaceae) (VP 124)	Kamenamaseka	PL	Malaria	+
Rumex bequaertii De Wild. (Polygonaceae) (VP 24)	Nyiramuko	RT	Malaria	−
Rytigynia beniensis (De Wild.) Robyns (Rubiaceae) (VP 390)	Umusugi	LF	Not used	−
Senecio mannii Hook. f. (Asteraceae) (VP 102)	Umutagara	LF	Malaria	−
Sesamum angolense Welw. (Pedaliaceae) (VP 23)	Igonde	LF	Not used	+
Tetradenia riparia (Hochst.) Codd. (Lamiaceae) (VP 1)	Umuravumba	LF	Malaria	+
Triumfetta cordifolia A. Rich. (Tiliaceae) (VP 255)	Umushyigura	LF	Not used	+
Vernonia amygdalina Del. (Asteraceae) (VP 39)	Umubilizi	LF	Malaria	+

[a]VP, Van Puyvelde collection.
[b]LF, leaf; PL, whole plant; RT, root; SB, stembark; ST, stem.

15 pounds pressure (121°C). After cooling to 50°C in a water bath, 50 ml of sterile calf serum were aseptically added and mixed gently to obtain a uniform solution.

Inoculum

T. vaginalis was inoculated in the Kupferberg medium and incubated at 37°C for 48 h. The inoculum was standardized to obtain 1×10^4 parasites/ml; parasites were counted under the microscope in a Burker cell.

Determination of anti-trichomonas activity

Plant extract diluted in distilled water (0.2 ml) was added to each of the test tubes containing 9.8 ml of the medium, to obtain a concentration of 1 mg/ml. Then, 0.5 ml of the standardized inoculum was added to the test tubes. The samples were incubated as static cultures for 48 h at 37°C. The anti-trichomonas activity was measured by counting the mobile parasites under the microscope in a Burker cell.

To confirm the results, subcultures were made and evaluated for anti-parasitic activity after 48 and 72 h of incubation at 37°C. Metronidazole [(1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole], a trichomonacide, was used as a positive control (25 μg/ml), while distilled water was used as a negative control. Three series of determinations were run for each plant extract.

Only 100% inhibition of the parasites (namely, when there were no mobile parasites observed) was taken as a positive (+) result.

Results and Discussion

Of 30 species tested (32 plant samples), 17 showed an anti-trichomonas activity (showing 100% inhibition) (Table 1). The control with metronidazole showed 100% inhibition of the parasites, while

in the control with distilled water no inhibition was observed.

No pronounced correlation was observed between the plants used to treat antiprotozoal diseases and the anti-trichomonas activity. Of the 21 species used to treat malaria or trypanosomiasis, only 10 showed anti-trichomonas activity in this test, while 7 of the 9 chosen at random, but used as medicinal plants, were active. However, it has to be noted that the Rwandese traditional healer often combines several plants in the preparation of his drug (Van Puyvelde and Kayonga, 1977), as exemplified by the following anti-malarial remedies:

(a) A water macerate of the leaves of *Markhamia lutea*, *Tetradenia riparia* and *Vernonia amygdalina*: these three plants showed activity against *T. vaginalis*.

(b) Juice of a mixture of the leaves of *Aspilia pluriseta*, *Crassocephalum vitellinum* and *Gardenia ternifolia* subsp. *jovis-tonantis*. Only *A. pluriseta* showed activity against *T. vaginalis*.

(c) Juice of a mixture of the leaves of *Conyza sumatrensis*, the whole plant of *Polygala luteo-viridis* and the leaves of *Rumex bequaertii*. Only *P. luteo-viridis* showed activity against *T. vaginalis*.

It has also to be noted that the methanol extracts of the plants were suspended in water and thus not totally dissolved, which could influence the activity of the extract. The evaluation of a possible correlation between anti-trichomonas activity and specific plant families is difficult to make as only 30 species belonging to 19 families were studied.

Further phytochemical and biological investigations with the active plants are in progress in order to identify the active principles. In the meantime, the first anti-trichomonas principle, namely 8(14), 15-sandaracopimaradiene-7α,18-diol, has been isolated from the leaves of *Tetradenia riparia* (De Kimpe et al., 1982; Hakizamungu et al., 1988).

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References

De Kimpe, N., Schamp, N., Van Puyvelde, L., Dubé, S., Chagnon-Dubé, M., Borremans, F., Anteunis, M.J.O., Declercq, J.P., Germain G. and Van Meerssche, M. (1982) Isolation and structural identification of 8(14),15-sandaracopimaradiene-7α,18-diol from *Iboza riparia*. *Journal of Organic Chemistry* 40, 3628–3630.

Hakizamungu, E. and Wéry, M. (1988) L'usage de plantes médicinales dans le traitement du paludisme en médecine traditionnelle rwandaise. *Bulletin de Liaison ACCT: Médecine Traditionnelle et Pharmacopée* 2, 11–17.

Hakizamungu, E., Van Puyvelde, L., Wéry, M., De Kimpe, N. and Schamp, N. (1988) Active principles of *Tetradenia riparia* III. Anti-trichomonas activity of 8(14),15-sandaracopimaradiene-7α,18-diol. *Phytotherapy Research* 2, 207–208.

Van Puyvelde, L. and Kayonga, A. (1977) Préparation des médicaments traditionnels par le guérisseur. *Etudes Rwandaises* (U.N.R., Butare, Rwanda) 10, 9–10.

Van Puyvelde, L., Pagezy, H. and Kayonga, A. (1975) Plantes médicinales et toxiques du Rwanda (I). *Afrique Médicale* 14, 925–930.

Van Puyvelde, L., Mukarugambwa, S., Rwangabo, P.C., Nyaboyisonga, M. and Runyinya-Bwarabwiliza (1977) Plantes médicinales et toxiques du Rwanda (II). *Afrique Médicale* 16, 531–534.

Van Puyvelde, L., Rwangabo, P.C., Runyinya-Bwarabwiliza, Ayobangira, F.X. and Mungarulire, J. (1982) Plantes médicinales et toxiques du Rwanda (III). *Afrique Médicale* 21, 401–404.

Wéry, M. (1983), *Notes de Protozoologie Médicale*. Institute of Tropical Medecine, Antwerpen, Belgium, p. 84.