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Cytotoxic Activity of Some Triterpenoid Saponins

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A crude extract of *Hedera helix* L. (Araliaceae) has been shown to exert a cytotoxic activity on Ehrlich tumor cells, both *in vitro* and *in vivo* (1). Furthermore, extensive studies performed in the Faculty of Pharmacy, Marseille, have indicated that saponins extracted from this plant collected in France exhibit antimutagenic and parasiticidal properties (2–4). The present note deals with the cytotoxic activity of these purified saponins and of saponins isolated from other plants (*Calendula arvensis*, *Calendula officinalis*, and *Sapindus mukurossi*), with a view to the detection of possible structure-activity relationships.

The possible cytotoxic effects were detected *in vitro* by the use of a semi-quantitative microtest previously applied by us for the study of the cytotoxicity of some alkaloids (5), one of which, strychnopentamine, was included in the present work as a reference compound. The biological test was carried out on four cell strains: mouse B16 melanoma cells, mouse 3T3 non cancer fibroblasts, Flow 2002 non-cancer human cells, and human HeLa tumour cells (5). For the 3T3 fibroblasts, 8,000 cells were plated in each well of a multiwell plate, in a culture medium containing 90% Gibco DMEM complemented with 10% foetal calf serum and 100 units/ml penicillin. The other experimental conditions for the other strains were as described previously (5).

Of the sixteen saponins tested here, the isolation of fourteen has already been reported (6–11), while β-glycyrrhetinic acid and the ammonium salt of glycyrrhizic acid were purchased from Aldrich. The sodium salts of the monodesmosides and genins were used in order to increase their solubility in water (3).

The results (Table 1) show that the saponins are at least five times less active than the reference compound (strychnopentamine) and that none of them seems to have any specific action on cancer cells. The most active compounds are the monodesmosides, which show some degree of cytotoxicity at concentrations of 10 μg/ml and above. Among them, α- and β-hederin are the most potent substances.

Table 1 *In vitro* cytotoxicity of saponins tested.

Substance (type)[a]	Source (Ref.)	Conc. µg/ml	Effect on cells[b] B16	3T3	HeLa	2002
Strychnopentamine	reference (5)	1	++	+++	+	++
		5	++++	++++	++++	++++
α-Hederin (M)	*Hedera helix* (6)	10	+	++	0	+++
		25	++++	++++	++++	++++
		50	++++	++++	++++	++++
Hederasaponin C (B)	*H. helix* (6)	50	0			
		100	0	0	0	0
		200	0	0	0	0
β-Hederin (M)	*H. helix* (6)	10	++	++	0	+++
		25	++++	++++	++++	++++
		50	++++	++++	++++	++++
Hederasapinin B (B)	*H. helix* (6)	50	0			
		100	0	0	0	0
		200	0	0	0	0
δ-Hederin (M)	*H. helix* (6)	10	0	0	0	0
		25	++++	++++	++++	++++
		50	++++	++++	++++	++++
Hederasaponin D (B)	*H. helix* (6)	50	0			
		100	0	0	0	0
		200	0	0	0	0
Hederagenin (G)	*H. helix* (6)	10	0	0	0	0
		25	0	0	0	0
		50	++	++++	0	0
		100	++++			
Arvensoside D (M)	*Calendula arvensis* (7)	50	++	0	0	+++
		100	++++	++++	+++	++++
		200	++++	++++	++++	++++
Arvensoside B (M)	*C. arvensis* (8)	10	0	+	0	++
		25	+++	+++	+	+++
		50	++++	++++	++++	++++
Saponin S_2 (M)	*Calendula officinalis* (9)	50	0	0	0	++
		100	++	++	0	++++
		200	++++	++++	++++	++++
Sapindoside B (M)	*Sapindus mukurossi* (10)	10	0	+	0	+
		25	++	+++	+	+++
		50	++++	++++	++++	++++
Sapindoside A (M)	*S. mukurossi* (10)	10	0	+	0	+
		25	++	++	0	++
		50	++++	++++	++++	++++
Sapindoside C (M)	*S. mukurossi* (10)	10	0	+	0	++
		25	++++	+++	++	++++
		50	++++	++++	++++	++++
Sapindoside D (M)	*S. mukurossi* (11)	10	0	+	0	++
		25	++++	++++	++	++++
		50	++++	++++	++++	++++
Glycyrrhizic acid (M) monoammonium salt	Aldrich	100	0	0	0	0
		200	+	0	0	0
18β-Glycyrrhetinic acid (G)	Aldrich	10	0	0	0	0
		25	0	0	0	0
		50	0	0	0	0
		100	+++			

[a] (M) = monodesmoside; (B) = bisdesmoside; (G) = sapogenin.

[b] 0 = no effect; + = decrease in the number of cells as compared to the controls, after 72 h; ++ = decrease in the number of cells, as compared to the controls, after 24 h; +++ = considerable decrease of the number of cells, as compared to the controls, after 24 h; ++++ = all cells dead after 24 h.

We also find that the bisdesmosides are inactive at concentrations up to 200 µg/ml. This agrees with the results obtained from the analyses of other biological properties (antiparasitic and antimutagenic) (2–4). The bisdesmosides were inactive *in vitro*, but more active than monodesmosides *in vivo*. It would therefore be of interest to test both types of saponins *in vivo* and to compare their effects.

Our results show that hederagenin is less active than its corresponding monodesmosides, while β-glycyrrhetinic acid is more potent than glycyrrhizic acid. The activities exerted by these last two compounds are also in agreement with the conclusions of a recent paper reporting the inhibition of protein synthesis by some saponins (12). These authors explain the cytotoxicity of the saponins by a direct inhibition of ribosomal activity and an indirect inhibition of elongation factors EF-1 and EF-2. Nevertheless, α- and β-hederin are about ten times more active than the saponins tested in this paper and it is possible that they have another mode of action. The fact that α- and β-hederin are cytotoxic but also antimutagenic is of interest because many substances used in cancer chemotherapy are, on the contrary, mutagenic. We plan now to perform other experi-

ments, both *in vitro* and *in vivo*, in order to analyse more precisely their mode of action and to try to establish structure-activity relationships.

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Inhibitory Effect of Piquerol A on the Growth of Epimastigotes of *Trypanosoma cruzi*

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Piqueria trinervia Cav. (Asteraceae) is a herbaceous perennial shrub that grows in tropical and temperate zones in Mexico. It is used in popular medicine because it is believed that this plant has antipyretic, antimalarial, and antirheumatic properties (1).

Piquerol A, a monoterpene, was isolated from this plant (2, 3). Piquerol A has been found to be a biologically active substance in several *in vitro* studies, where it has been reported to inhibit the germination and growth of plants (4), to have molluscicidal activity on snails (5), and acaricidal potential on *Boophilus microplus* (M. Gonzalez de la Parra, et al., accepted for publication in Pesticide Science).

The broad spectrum of biological activity shown by this compound and its moderate toxicity to mammals [the department of Pharmacology of the Universidad Nacional Autónoma de México determined the acute toxicity of piquerol A, LD_{50} (*i.p.*), to rats to be 600 mg per kg] encouraged further experiments on *Trypanosoma cruzi*. *T. cruzi* is a flagellate protozoan which is the causal agent of American trypanosomiasis, or Chagas' disease, which afflicts millions of people in Latin America. Few drugs are available for the treatment of *T. cruzi* infections; although drugs such as nifurtimox, benznidazole, and ketoconazole can ameliorate Chagas' disease (6) they have little or no effect on the chronic phase of the disease (7).

The main objective of this project was to carry out an *in vitro* study to observe possible inhibitory effects of piquerol A on the growth of epimastigotes of *T. cruzi*. This is the first step in assessing the potential use of this compound in the treatment of Chagas' disease.

The isolation and purification of Piquerol A from *P. trinervia* have been described elsewhere (2).

A Mexican strain of *T. cruzi* (approximately 99 % of epimastigote forms) used in this study was isolated from insects of the *Triatoma* genus from La Cruz, Jalisco. It has been maintained in this form by *in vitro* cultivation.