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Full Length Research Paper

In vitro cytotoxicity of crude alkaloidal extracts of South African Menispermaceae against three cancer cell lines

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The cytotoxicity of crude alkaloid extracts obtained from the leaves and rhizomes of all the South African members of the family Menispermaceae (seven genera and 13 species) was tested against MCF7 (breast), UACC62 (melanoma) and TK10 (renal) cancer cell lines. Extracts of ten of the thirteen species showed positive activity against all three cancer cell lines with significant inhibition of cellular growth at TGI (total growth inhibition) values lower than 6.25 µg/ml for *Albertisia delagoensis* and *Antizoma angustifolia* leaves.

Key words: Cytotoxicity, alkaloids, menispermaceae, South Africa.

INTRODUCTION

Hartwell (1982) lists more than 3 000 plant species that reportedly has been used in the treatment of cancer and Graham et al. (2000) had added another 350 species to Hartwell's list. Plants have been a major source of highly effective conventional drugs for the treatment of many forms of cancer and while the actual compounds isolated from the plant frequently may not serve as the drug, they provide leads for the development of potential novel agents like, paclitaxel, vinblastine and vincristine (Gragg and Newman, 2005). There are not many documented plants species in South African which are used to treat cancer. As a result, a collaborative research programme was initiated between the Council for Scientific and Industrial research (CSIR) in South Africa and the NCI (U.S. National Cancer Institute) during the period of 1999 and 2006. They had screened 7 500 randomly selected plant extracts (representing 700 plant species) against the three-cell lines MCF7 (breast), UACC62 (melanoma) and TK10 (renal) (Fouche et al., 2008). This research did not include any of the South African Menispermaceae species, although species from five genera in this family are used in cancer treatment (De Wet, 2006) in other parts of the world. These genera are *Cissampelos*, *Sphenocentrum*, *Menispermum*, *Stephania* and *Tinospora*, and were used in treating stomach, skin, breast, cervix and throat cancers. The thirteen South African Menispermaceae species are well known for their traditional uses in treating various ailments (De Wet and Van Wyk, 2008). *Cissampelos capensis* is the only species which has a recorded (published) history of use against stomach and skin cancer in South Africa (Van Wyk and Gericke, 2000), but no screening for cytotoxicity against cancer cell lines has been reported on any of the 13 South African species.

Most of the medicinal uses of the South African Menispermaceae can be attributed to its rich variation in alkaloids. Previously isolated classes of alkaloids from extracts of the 13 Menispermaceae species were as follows. *Albertisia delagoensis*: bisbenzyltetra-hydroisoquinoline alkaloids (four), aporphine alkaloid (one) (De Wet et al., 2007). *Antizoma angustifolia*: proaporphine alkaloids (three), aporphine alkaloid (one), morphinane alkaloid (one), bisbenzyltetrahydroisoquinoline alkaloids (two) (De Wet et al., 2004). *A. miersiana*: proaporphine alkaloid (one), aporphine alkaloids (two), bisbenzyltetra-hydroiso-

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Table 1. Localities and voucher specimens for the 13 South African Menispermaceae species.

Species	Voucher specimen	Locality
Albertisia delagoensis (N.E. Br.) Forman	Van Wyk and De Wet 4075 (ZULU)	Tembe Elephant Park [2632 CD]
Antizoma angustifolia (Burch.) Miers ex Harv.	Van Wyk and De Wet 4059 (ZULU)	6 km south of Pienaars River [2528 AD]
Antizoma miersiana Harv.	Van Wyk and De Wet 4067 (ZULU)	Botterkloof Pass [3119 CD]
Cissampelos capensis (L.f.) Diels	De Wet 2 (ZULU)	15 km east from Ladismith [3321 AD]
Cissampelos hirta Klotzsch	De Wet 12 (ZULU)	Mabibi [2632 DD]
Cissampelos mucronata Rich.	De Wet 33 (ZULU)	5 km east of Coastal Cashew A. Farm [2732 BC]
Cissampelos torulosa E. Mey. ex Harv.	De Wet 42 (ZULU)	Owen Sithole Agriculture College [2831 DB]
Cocculus hirsutus (L.) Diels	Van Wyk and De Wet 4062 (ZULU)	Letsitele [2330 CD]
Stephania abyssinica (Dill. & A. Rich.) Walp.	Van Wyk and De Wet 4061 (ZULU)	Ebenezer Dam, Tzaneen [2329 DD]
Tiliacora funifera (Miers) Oliver	Van Wyk and De Wet 4063 (ZULU)	Stentor Farm, Kaapmuiden [2531 CB]
Tinospora caffra (Miers) Troupin	Van Wyk and De Wet 4074 (ZULU)	Pongola Dam, Jozini [2732 AA]
Tinospora fragosa Troupin	Van Wyk and De Wet 4060 (ZULU)	Zeekoegat, Sefaten Verdoorn and mine [2429 BD] and Atok (Verdoorn)
Tinospora tenera	Van Wyk and De Wet 4064 (ZULU)	Crocodile Gorge, Miers Nelspruit [2531 CC]

(ZULU) = Botany Herbarium at the University of Zululand.

quinoline alkaloids (five) (De Wet et al., 2005). *Cissampelos mucronata*: bisbenzyltetrahydroisoquinoline alkaloids (Ferreira et al., 1965). *Cocculus hirsutus*: aporphine alkaloid (one), benzyltetrahydroisoquinoline alkaloids (two), bisbenzyltetrahydroisoquinoline alkaloids (eight), cohirsine alkaloids (four), hirsutine alkaloids (five) (Barbosa-Filho et al., 2000). *Tiliacora funifera*: benzyltetrahydroisoquinoline alkaloid (one), bisbenzyltetrahydroisoquinoline alkaloids (ten) (Barbosa-Filho et al., 2000).

In this paper we evaluate *in vitro* cytotoxicity of crude alkaloidal extracts of the South African Menispermaceae against the same three cancer cell lines used by Fouche et al. (2008).

MATERIALS AND METHODS

Plant materials

All the South African members of the family Menispermaceae (Table 1) were collected from different sites in South Africa during 2001. The identity of the materials was authenticated by Dr. Helene de Wet and Prof. Ben-Erik van Wyk. Voucher specimens were deposited in the Herbarium of the Department of Botany, University of Zululand (ZULU) (Table 1).

Extractions

The samples used for testing are listed in Table 2. Plant materials (1 g, air-dried at maximum 40°C) were finely ground with a pestle and mortar. It was then mixed with 15 ml 0.05 M H_2SO_4 and left standing at room temperature for 30 min. A cylindrical glass column (27 cm x 2.5 cm) was plugged with cotton wool and 2.5 g anhydrous sodium sulfate and 24 g of coarse grade celite-577 was added consecutively to the column. Finally, filter paper (Whatman no. 4) was placed on the celite. The acid plant extract (water phase) was then poured onto the filter paper and allowed to soak into the column. After the acidic filtrate has soaked into the column, approximately 4 ml of 25% ammonia was added to the column. The column was then eluted with 100 ml of CH_2Cl_2 and the solvent collected in a flat-bottomed flask placed below the column. Evaporation under vacuum in a rotary evaporator operating at a temperature of 60°C yielded the alkaloid extract.

Table 2. In vitro cytotoxicity activity of crude alkaloidal extracts of South African Menispermaceae against three cancer cell lines.

Plant species (UACC62)	Breast (MCF7)		Melanoma		Renal (TK10)	
	GI_{50}	TGI	GI_{50}	TGI	GI_{50}	TGI
A. delagoensis leaves	< 6.25	< 6.25	< 6.25	< 6.25	< 6.25	< 6.25
A. angustifolia leaves	< 6.25	7.00	< 6.25	< 6.25	< 6.25	< 6.25
rhizome	19.00	50.00	15.00	25.00	13.00	30.00
A. miersiana leaves	10.00	38.00	12.50	25.00	12.50	52.00
rhizome	12.50	27.00	12.25	19.00	12.50	23.00
C. capensis leaves	16.00	37.50	19.00	37.50	16.00	37.50
rhizome	25.00	50.00	18.75	27.00	12.50	22.00
C. hirta rhizome	6.25	25.00	12.50	18.00	12.50	25.00
C. mucronata rhizome	< 6.25	18.00	6.25	15.00	9.00	24.00
C. torulosa rhizome	12.50	37.50	12.50	28.00	9.00	50.00
C. hirsutus rhizome	< 6.25	25.00	< 6.25	17.00	6.25	30.00
T. funifera leaves	7.50	10.00	7.00	8.00	7.00	8.00
rhizome	7.50	9.00	7.00	8.50	6.50	8.5
T. fragosa leaves	10.00	50.00	12.50	50.00	23.00	33.00

Potent activity: TGI < 6.25 µg/ml for 2 to 3 cell lines (Fouche et al, 2006).
Moderate activity: 6.25 µg/ml < TGI < 15 µg/ml for 2 to 3 cell lines.
Weak activity: 15 µg/ml < TGI < 50 µg/ml for 2 to 3 cell lines.

Cytotoxic assay

Crude alkaloidal extracts were tested for cytotoxicity against three cancer cell lines at a single concentration of 100 µg/ml. The extracts which reduced the growth of two of the cell lines by 75% or more, were further tested at five serial dilution concentrations ranging from 6.25 - 100 µg/ml. The results of the five dose assays were reported as TGI (total growth inhibition) and GI_{50} (concentration required for 50% inhibition of cell growth). The two standards used as controls were Adriamycin and 5-fluorouracil (Monks et al., 1991). The cancer cell lines used were MCF7 (breast), UACC62 (melanoma) and TK10 (renal).

RESULTS AND DISCUSSION

In Table 2, the extracts of the leaves and/or rhizomes of ten of the thirteen South African Menispermaceae species showed positive activity against all three cancer cell lines. Crude extracts with TGI values lower than 6.25 µg/ml are usually considered to have potent activity (Fouche et al., 2006). The low GI_{50} and TGI values for *A. delagoensis* leaves (< 6.25 µg/ml) against all three cancer cell lines may be related to the presence of dicentrine and cycleanine which have known anticancer activities (Schiff, 1991). The leaves extract of *Antizoma angustifolia* also have values of GI_{50} and TGI less than 6.25 µg/ml. Crotsparine was shown to be the main alkaloid in *A. angustifolia* leaves (De Wet et al., 2004) and may prove to be the active constituent. Both leaf and rhizome alkaloid extracts of *Tiliacora funifera* showed moderate activity (TGI < 10 µg/ml) values for all three cancer cell lines. This is supported by the presence of the alkaloid isotetrandrine which is known for its antitumour activity (Schiff, 1987). *Cissampelos capensis* rhizomes that have been used to treat stomach and skin cancer have surprisingly high GI_{50} and TGI values against all three cell lines. It appears that unrelated medicinal uses of the source plants may serve as an initial guide to selection of taxa for anticancer screening.

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REFERENCES

Barbosa-Filho JM, Ca-Cunha EVL, Gray AI (2000). Alkaloids of the Menispermaceae. In: Cordell GA (eds.). The Alkaloids (54), Academic Press, Illinois, pp. 1-190.

De Wet H (2006). An ethnobotanical and chemotaxonomic study of South African Menispermaceae. PhD thesis, Department of Botany and Plant Biotechnology, University of Johannesburg, South Africa.

De Wet H, Van Heerden FR, Van Wyk BE (2004). Alkaloids of *Antizoma angustifolia* (Menispermaceae). Biochem. Syst. Ecol. 32: 1145-1152.

De Wet H, Van Heerden FR, Van Wyk BE (2005). Alkaloids of *Antizoma miersiana* (Menispermaceae). Biochem. Syst. Ecol. 33: 799-807.

De Wet H, Van Heerden FR, Van Wyk BE, Van Zyl RL (2007). Anitplasmodial activity and cytotoxicity of *Albertisia delagoensis*. Fitoterapia, 78: 420-422.

De Wet H, Van Wyk BE (2008). An ethnobotanical survey of southern African Menispermaceae. SA J. Bot. 74: 2-9.
Ferreira MA, Prista LN, Alves AC, Roque AS (1965). Estudo QuÚmico De *Cissampelos mucronata* A. Rich 1. Isolamento de *d*-isochondoden-drina. Garcia de Orta, 13: 395-405.
Fouche G, Cragg GM, Pillay P, Kolesnikova N, Maharaj VJ, Senabe J (2008). *In vitro* anticancer screening of South African plants. J. Ethnopharmacol. 119: 455-461.
Fouche G, Khorombi E, Kolesnikova N, Maharaj VJ, Nthambeleni R, Vander Merwe M (2006). Investigation of South African Plants for anticancer properties. Pharmacologyonline, 3: 494-500.
Gragg GM, Newman DJ (2005). Plants as a source of anticancer agent. J. Ethnopharmacol. 100: 72-79.
Graham JG, Quinn ML, Fabricant DS, Farnsworth NR (2000). Plants used against cancer, an extension of the work of Jonathan Hartwell. J. Ethnopharmacol. 73: 347-377.
Hartwell JL (1982). Plants used against cancer. Lawrence MA, Quarterman Publications, pp. 1-710.
Monks A, Scudiero D, Skehan P, Shoemaker R, Paull K, Vistica D, Hose C, Langley J, Cronise P, Vaigro-Wolff A, Gray-Goodrich M, Campbell H, Mayo J, Boyd M (1991). Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. J. Nat. Cancer Inst. 83: 757-766.
Schiff PL (1987). Bisbenzylisoquinoline alkaloids. J. Nat. Prod. 50: 529-599.
Schiff PL (1991). Bisbenzylisoquinoline alkaloids. J. Nat. Prod. 50: 529-599.
Van Wyk BE, Gericke N (2000). People's plants. A guide to useful plants of southern Africa, Briza Publications, Pretoria, p. 124.