CHAPTER 5

CYTOTOXICITY OF CAESPITATE, A PHLOROGLUCINOL ISOLATED FROM *HELICHRYSUM CAESPITITIUM*

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5.1 Introduction

Traditional medicine is widely used in South Africa with traditional healers treating over 75% of patients (Hutchings *et al.*, 1996). A decoction of *Helichrysum caespititium* (DC.) Harv., (*impepo* (Zulu), *seledu-sa-phooko* (South Sotho), *moriri-wa-naha* (Kwena), and *sephanyane* (Kgatla), is drunk in the treatment of, broncho-pneumonial diseases, sexually transmitted diseases, tuberculosis, ulceration and is used as a styptic wound dressing (Phillips, 1917; Watt and Breyer-Brandwijk, 1962). The importance of this indigenous herbal knowledge is therefore recognized and the use of the plant has been well documented (Watt and Breyer-Brandwijk, 1962; Hutchings *et al.*, 1996), however, caespitate, a new phloroglucinol (2-methyl-4-[2',4',6'-trihydroxy-3'-(2-methylpropanoyl) phenyl] but-2-enyl acetate) isolated from *H. caespititium* has not been analysed for cytotoxicity (Mathekga *et al.*, 2000). We demonstrated earlier that this plant exhibits significant potency against human bacterial and fungal pathogens (Chapters 2 and 3). Its antimicrobial spectrum is comparatively limited but its potency is reasonable.

It is interesting to note that *H. caespititium* contains more than one highly potent antimicrobial agent (Dekker *et al.*, 1983 and Mathekga *et al.*, 2000). The antimicrobial spectrum of caespitate (Table 5.1) seems to be limited to Gram-positive bacteria only but also, showed activity against fungi (Table 5.2) tested in this study. The information gained from studying caespitate might lead to the development and understanding of new molecular interactions, which in turn may lead to the development of new classes of therapeutic agents. With the rapid explosion of new molecular targets available for drug discovery and advances in automated high throughput screening technologies, there has been a dramatic increase in interest by the pharmaceutical and biotechnology industries in

sources of molecular diversity. The resources of the genus *Helichrysum* might play an important role in the discovery of novel lead structures for many of these new targets. The purpose of this study was to investigate the cytotoxicity of caespitate and to relate it to its folkloric use. The cytotoxicity and efficacy of caespitate was determined microscopically on vervet monkey kidney cells.

5.2. Materials and Methods

5.2.1 Plant material

Shoots of *H. caespititium* were collected from the Drakensberg in the Mont-aux-Sources area in QwaQwa, South Africa during August 1998. A voucher specimen (AM11) of the species was deposited in the herbarium of the National Botanical Institute of South Africa in Pretoria.

5.2.2 Preparation of extract

Air dried (80 g) plant material was immersed in acetone and shaken on a rotary shaker for 5 minutes without homogenizing it. The extract was filtered and concentrated to dryness under reduced pressure at 40 ^{0}C with a rotary evaporator. After determining the yield (8.4 g (w/w)), the extract was stored at 4 ^{0}C.

5.2.3 Preparation of caespitate

The antimicrobial activity guided fractionation of the acetone extract (Chapter 4) of the aerial parts of *H. caespititium* led to the isolation of the new phloroglucinol derivative, caespitate (2-methyl-4-[2',4',6'-trihydroxy-3'-(2-methylpropanoyl)-phenyl]but-2-enyl acetate). Caespitate was serially diluted in acetone to obtain a concentration range of 100.0 to 0.5 µg/ml.

5.2.4 Cytotoxicity

5.2.4.1 Stock solution

A stock solution of caespitate (60 mg/ml) was prepared in cell culture tested dimethyl sulfoxide (DMSO) purchased from Sigma.

5.2.4.2 Cell culture

Microtitres with vervet monkey kidney cells were prepared for testing caespitate cytotoxicity and cells were examined microscopically for pre-experimental infection and vitality. The multilayer cells in the tissue were rinsed three times with phosphate buffer saline (PBS) followed by 3 ml Trypsin EDTA. This facilitates dislodging cells adhering to the plate's bottom surface. The cell plates were incubated for 5 minutes at 37^{o}C. Eight ml of fresh maintenance medium (MM) were added to the tissue culture.

5.2.4.3 *In vitro* cytotoxicity assay

Determination of the ID_{50} of caespitate was carried out according to Geran *et al.,* (1972). Cell survival was measured microscopically (Grist *et al*., 1979) instead of using the methyl tetrazolium bromide (MTT) method described by Mosmann (1983) and Scudiero *et al.,* (1988). Briefly stated, cells in the exponential growth phase were harvested and centrifuged at 3000 x g for 5 minutes, re-suspended in Eagle's minimum essential medium (MEM) to 1.0 x 10^5 cells/ml and 180 ml of the cell culture was added to each well of a flat bottom 96 well plate with a multichannel pipette. After 24 hours incubation in a 5 % CO_2 humidified incubator at 37^{o}C, 20 ml of the test agent was added in 6 replicates to give final concentrations of 100.0, 50.0, 25.0, 12.5, 6.0, 3.0, 1.5, 0.7, 0.3, 0.1 and 0.05 mg/ml. The concentration of DMSO used to dissolve the compound was adjusted to 100.0 mg/ml and this concentration of solvent was used in control wells. The compound was tested for cytotoxicity by exposing the mono layers to the compound in MM at 37^{o}C. The cells were monitored over a period of six days for cytotoxicity effects. Mono layers of cells exposed to MM without the addition of the compound were used as controls. Cells were examined daily by light microscopy for the appearance of cytotoxicity. The ID_{50} was expressed as the compound concentration in mg/ml that caused a 50 % inhibition of growth compared with controls.

5.3 Results

The maximum non-toxic concentration of caespitate on the vervet kidney monkey cell cultures was 50 mg/ml. At this concentration, the cells did not exhibit altered morphology or growth characteristics indicative of cytotoxic effects. The cytotoxicity results from caespitate are shown in Table 5.1

5.4 Discussion

H. caespititium has long been used as a food spice and medicine by the Free State Basothos and other indigenous people and is therefore, probably not toxic to humans. This probably explains the continued use of the extract from this plant by the indigenous people of South Africa against a number of infections for generations.

Table 5.1 Cytotoxicity effects of caespitate on vervet monkey kidney cells. Each value represents the mean of six replicates.

TREATMENT (g/ml)	**TOXICITY**		
	DAY 3	**DAY 4**	**DAY 6**
Control (MEM medium)	100% growth	100% growth	100% growth
100.0 g/ml DMSO	100% growth	100% growth	100% growth
100.0 g/ml caespitate	100% toxic	100% toxic	100% toxic
50.0 g/ml caespitate	No toxicity	No toxicity	No toxicity
25.0 g/ml caespitate	No toxicity	No toxicity	No toxicity
12.5 g/ml caespitate	No toxicity	No toxicity	No toxicity
6.0 g/ml caespitate	No toxicity	No toxicity	No toxicity
3.0 g/ml caespitate	No toxicity	No toxicity	No toxicity
1.5 g/ml caespitate	No toxicity	No toxicity	No toxicity
0.7 g/ml caespitate	No toxicity	No toxicity	No toxicity
0.3 g/ml caespitate	No toxicity	No toxicity	No toxicity
0,1 g/ml caespitate	No toxicity	No toxicity	No toxicity

5.5 Conclusion

The cytotoxicity results obtained in these tests suggest that further studies to investigate the potential for anti-cancer activity of *H. caespititium* may be useful as antimicrobial compounds which exhibit non-toxicity at concentrations below 8.0 µg/ml have potential as anti-cancer agents (Balick ,1990). The antimicrobial and non-toxicity properties of *H. caespititium* as detected in this *in vitro* study may partly explain the popularity of this plant in folk medicine as a remedy for many diseases and skin infections.

Traditional medicine potions are mostly obtained from natural products. The advantage in some cases is that the concentration of active principles in the plant is usually small and it is further diluted when a decoction for traditional medicine is prepared. As a result, it is concluded that such work generates a gratifying promise of novel lead structures and the possibility of finding additional agents for human or agricultural use based upon the antimicrobial and cytotoxicity of caespitate. Additional scientific investigation in this field awaiting discovery, is recommended.

REFERENCES.

BALICK, M.J. 1990. Ethnobotany and the identification of therapeutic agents from the rainforest. In: Bioactive compounds from plants. CIBA Foundation Symposium 154D.J. Chadwicks and J. Marsh, eds. John Wiley and Sons. New York. pp. 22-39.

DEKKER, T. G., FOURIE, T. G., SNYCKERS, F. D. and VAN DER SCKYF, C. F. 1983. Stidies of South African medicinal plants. Part 2. Caespitin, a new phloroglucinol derivative with antimicrobial properties from *Helichrysum caespititium. South African Journal of Chemistry* 36(4): 114-117.

GERAN, R. I., GREENBERG, N. H., MACDONALD, M. M., SCHUMACHER, A.M. and ABBOTT, B. D. 1972. Protocols for screening chemical agents and natural

products against animal tumors and other biological systems. *Cancer Chemotherapy Reports*. Part 3. 3 (2): 1-17, 59-61.

GRIST, N.R., BELL, F. J., FOLLETTE, E. A.C. and URQUHART, G.E.D. 1979. Diagonistic methods in clinical virology, 3rd edn. Oxford, Blackwell Scientific Publications. pp. 60-79.

HUTCHINGS, A., SCOTT, A. LEWIS, G. and CUNNINGHAM, A.B. 1996. Zulu Medicinal Plants. An inventory. University of Natal pp. 266-271.

MATHEKGA, A. D. M. and MEYER, J. J. M. (1998). Antimicrobial activity of South African Helichrysum species *South African Journal of Botany* 64(5): 293-295.

MATHEKGA, A.D.M., MEYER, J.J.M., HORN, M.M., and DREWES, S. E. 2000. An acylated phloroglucinol with antimicrobial properties from Helichrysum caespititium. *Phytochemistry* 53: 93-96..

MOSMANN, T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods* 65: 55-63.

SCUDIERO, D. A., SHOEMAKER, R. H., PAUL, K. D., MONKS, A., TIERNEY, S., NOFZIGER, H., CURRENS, M. J. SENIFF, D. and BOYD, M. R. 1988. Evaluation of a soluble tetrazolium formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines. *Cancer research* 48: 4827-4833.

WATT, J.M. and BREYER-BRANDWIJK, M.G. 1962. The medicinal and poisonous plants of Southern and Eastern Africa. 2 nd edn. E and S. Livingstone, London.