CHAPTER 5

CYTOTOXICITY OF CAESPITATE, A PHLOROGLUCINOL ISOLATED FROM HELICHRYSUM CAESPITITIUM
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 °C with a rotary evaporator. After determining the yield (8.4 g (w/w)), the extract was stored at 4 °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°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 ID₅₀ 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 10⁵ 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 % CO₂ humidified incubator at 37°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°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 ID₅₀ 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.

<table>
<thead>
<tr>
<th>TREATMENT (g/ml)</th>
<th>TOXICITY</th>
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<tbody>
<tr>
<td></td>
<td>DAY 3</td>
</tr>
<tr>
<td>Control (MEM medium)</td>
<td>100% growth</td>
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<tr>
<td>100.0 g/ml DMSO</td>
<td>100% growth</td>
</tr>
<tr>
<td>100.0 g/ml caespitate</td>
<td>100% toxic</td>
</tr>
<tr>
<td>50.0 g/ml caespitate</td>
<td>No toxicity</td>
</tr>
<tr>
<td>25.0 g/ml caespitate</td>
<td>No toxicity</td>
</tr>
<tr>
<td>12.5 g/ml caespitate</td>
<td>No toxicity</td>
</tr>
<tr>
<td>6.0 g/ml caespitate</td>
<td>No toxicity</td>
</tr>
<tr>
<td>3.0 g/ml caespitate</td>
<td>No toxicity</td>
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<tr>
<td>1.5 g/ml caespitate</td>
<td>No toxicity</td>
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<tr>
<td>0.7 g/ml caespitate</td>
<td>No toxicity</td>
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<tr>
<td>0.3 g/ml caespitate</td>
<td>No toxicity</td>
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<tr>
<td>0.1 g/ml caespitate</td>
<td>No toxicity</td>
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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.


