An examination of the chemical structures and *in vitro* cytotoxic bioactivity of halomon related secondary metabolites from *Portieria hornemannii* found worldwide

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Received: 22nd August, 2015. Accepted: 23rd May, 2016. Published: 15th August, 2016.

Abstract

An examination of the chemical structures and in vitro cytotoxic bioactivity of halogenated monoterpenes isolated from Portieria hornemannii worldwide is presented here for the first time. It is anticipated that this analysis will be of valuable to the natural product chemist working in the field of drug discovery with reference to the rapid identification and possible characterisation of halogenated monoterpene secondary metabolites which demonstrate in vitro cytotoxic bioactivity.

Keywords: Halogenated monoterpenes; Halomon related compounds; *Portieria hornemannii.*

ISTJN 2016; 8:15-30.

1 Introduction

A large number of halogenated metabolites have been isolated from many genera belonging to red seaweeds (Rhodophyta) (Blunt *et al.*, 2011; Faulkner, 2002). Red seaweeds from

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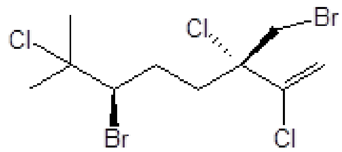
the families Plocamiaceae and Rhizophyllidaceae, in particular, produce a wide variety of halogenated and biologically active monoterpenes (Kladi *et al.*, 2004). It is believed that these compounds are produced by red alga as defensive mechanisms against predators that feed on the fronds of these marine alga (Paul *et al.*, 1987; Paul *et al.*, 2006; Paul and Pohnert, 2011). Of the family Plocamiaceae and Rhizophyllidaceae, species from the genera Plocamium and Portieria are an especially well established source of acyclic and cyclic polyhalogenated monoterpenes (Gunatilaka *et al.*, 1999). Metabolites from these genera are often of interest because of their diverse biological properties which range from ichthyotoxicity to anti-feedant, antimicrobial, cytotoxic and antifungal activity (Naylor *et al.*, 1983). From the family Rhizophyllidaceae, the genus Portieria hornemannii is renowned for producing 'exciting' chemistry. Ochtodene, which was isolated from P. hornemannii, exhibited selective solid tumour activity in cellular in vitro assays, but neither toxicity nor anti-tumour activity was observed in vivo (Gunatilaka *et al.*, 1999). Halomon was isolated from P. hornemannii (Fuller *et al.*, 1992). As a result of its successful selective in vitro activity against brain tumours, it was selected for pre-clinical drug development (Wise *et al.*, 2002). Compounds related to halomon, such as isohalomon, also demonstrated a unique differential cytotoxicity profile against several human tumour cell lines.

For an overview of secondary metabolites previously isolated from P. hornemannii see below. The structural elucidation of these compounds is not a trivial task because molecular ions are often not obtained, regardless of the ionisation method that is used. In addition to this, the location of chlorine and bromine groups on the ten carbon chain can be masked by the cumulative effects of numerous substituents on the monoterpene chain or ring (Naylor *et al.*, 1983) (Knott, 2012).

Since the isolation of 6*R*-bromo-3S-(bromomethyl)-7-methyl-2,3,7-trichloro-1-octene or otherwise known as halomon (**1**) (Fuller *et al.*, 1992), a lot of interest has been re-directed towards halogenated monoterpenes. Halomon was flagged by the National Cancer Institute (NCI) when it showed strong differential cytotoxicity towards the brain, colon and renal cell lines of the human *in vitro* tumour cell lines, while leukaemia and melanoma lines were relatively less sensitive (Fuller *et al.*, 1992). For example, compared to some of the less sensitive melanoma and leukaemia lines, several of the more sensitive brain, renal, and colon tumour cell lines were 1000-fold or more sensitive to halomon at the $\mathbf{GL}_{50}$ response level and 100-fold at the $\mathbf{LC}_{50}$ level (Fuller *et al.*, 1992). A major rate-limiting step in the further development of this compound, was the lack of reliable natural or synthetic sources from which halomon could be obtained. The reason for this is that *P. hornemannii*, from which halomon was first extracted, demonstrated extreme site-to-site and temporal variations with regards to the concentrations and types of halogenated monoterpenes that have been isolated during relevant extractions (Gunatilaka *et al.*, 1999) (Knott, 2012).



2 Findings

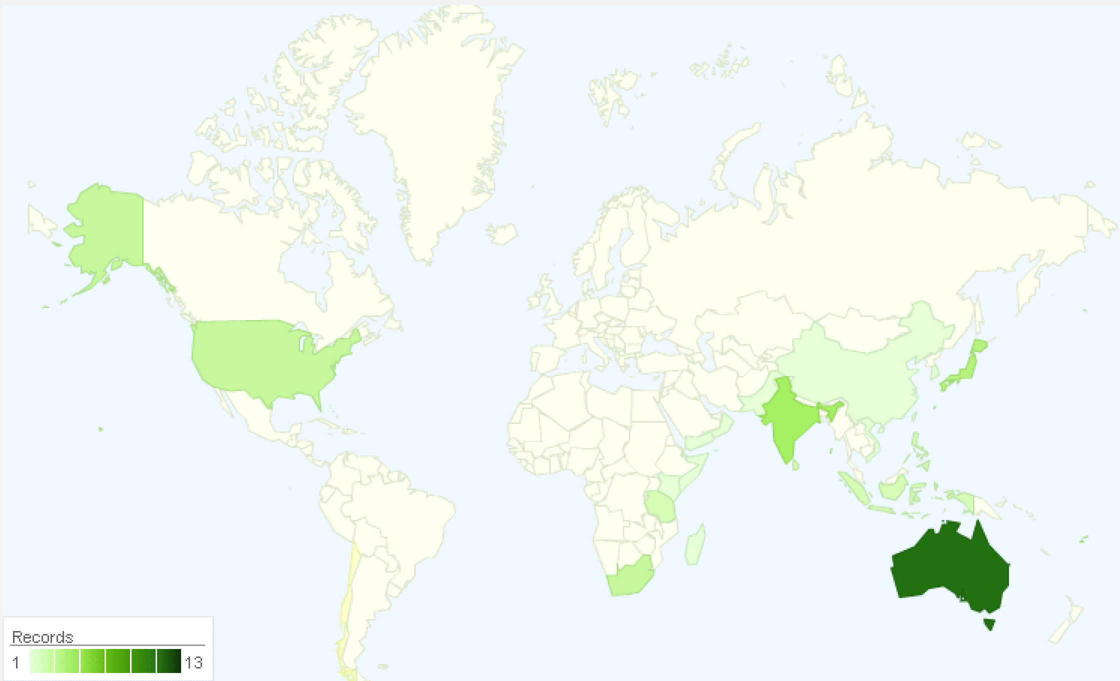
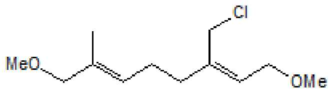


Figure 1: Worldwide distribution of the number of different kinds of *P. hornemannii* found in each country (Guiry and Guiry, 2011).

Various kinds or subspecies of *P. hornemannii* are found around the world (Figure 1). Both the genus *Portieria* as well as the genus *Chondrococcus* belong to the family Rhizophyllidaceae. *Chondrococcus* is currently regarded as a taxonomic synonym of Portieria. From the structures below (Knott, 2012), it can be seen that *P. hornemannii* exhibits notable site-to-site variation in secondary metabolite production and that *P. hornemannii* contains both acyclic and cyclic polyhalogenated monoterpenes. Additional comments on delineating the nature of these structural trends cannot be extrapolated at this stage, as there seem to be many variations of *P. hornemannii*.



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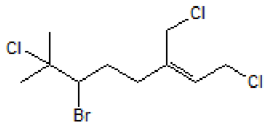

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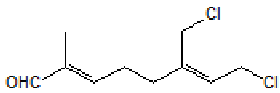

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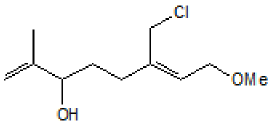

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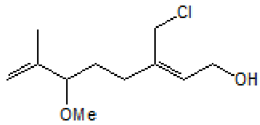

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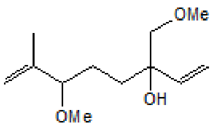

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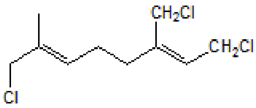

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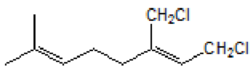

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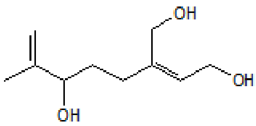

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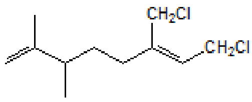

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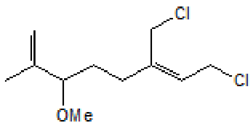

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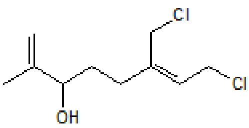

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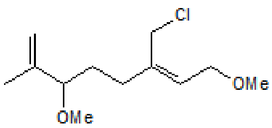

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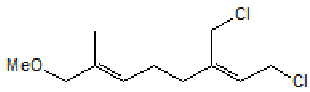

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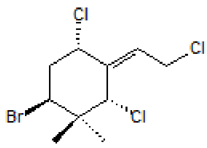
Australia (Wright *et al*., 1991)



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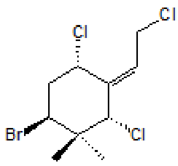

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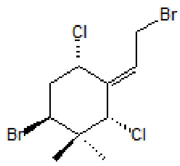

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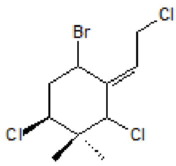

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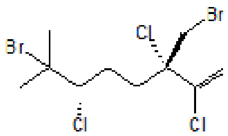
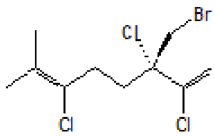
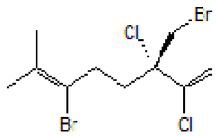
Guam (Gunatilaka *et al*., 1999)

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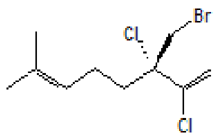
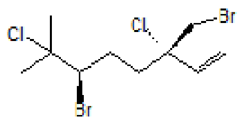


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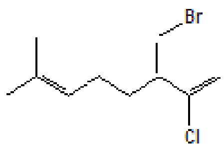
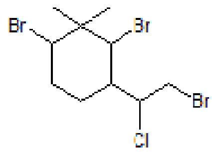
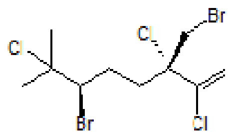
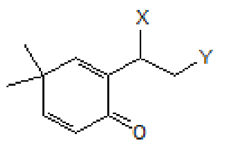
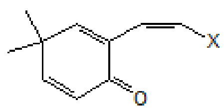




26 **27** **1**

Hawaii (Fuller *et al*., 1994)

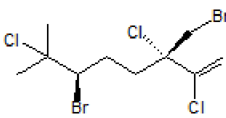



a) X = Br Y = Br
b) X = Cl Y = Br
c) X = Cl Y = Cl

d) X = Br
e) X = Cl

28 a, b, c **29 d, e**

Japan (Kuniyoshi *et al*., 2003)



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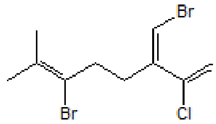

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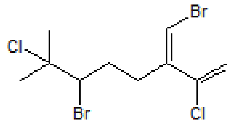

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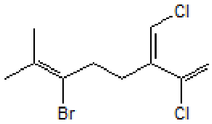

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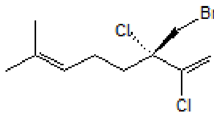

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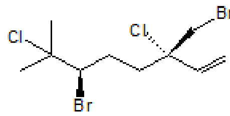

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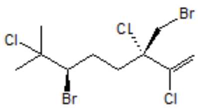
Madagascar (Andrianasolo *et al.*, 2006)



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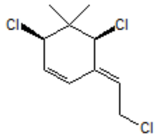

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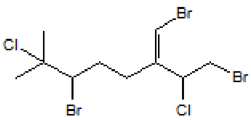
Philippines (Fuller *et al.*, 1992)



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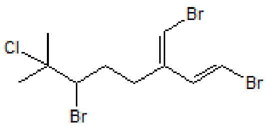

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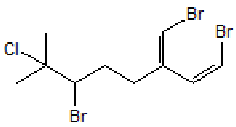

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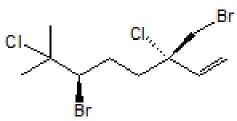

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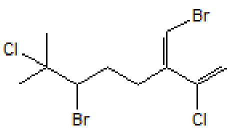

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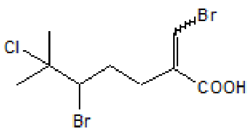

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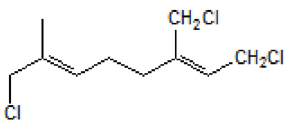
South Africa (Knott, 2012)

Although *Chondrococcus hornemannii* and *Portieria hornemannii* are the same thing, molecular sequencing has shown that *P. hornemannii* is a complex species, and seems to contain as many as more than 90 species. For example, there are about 20 in the Philippines and apparently 4 in South Africa (Bolton, 2016). For this reason, the compounds presented above and below, have not all been grouped together, but rather presented on a per paper title (for example *Portieria* above and *Chondrococcus* below) and a per location basis.

The genera *Chondrococcus* (below) has also shown considerable structural and geographical variation with regards to its monoterpene content (Coll and Wright, 1987). *Chondrococcus hornemannii* from different locations around the world have also yielded a considerable number of different halogenated monoterpenes.



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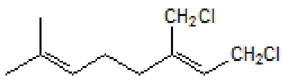

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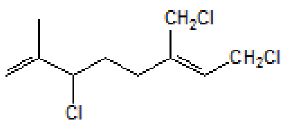

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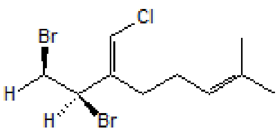

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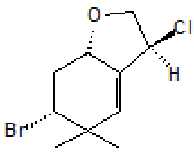

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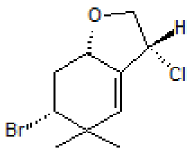

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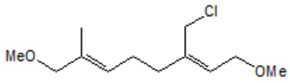
Australia (Coll and Wright, 1987)



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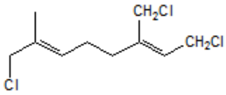

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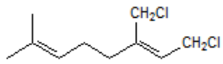

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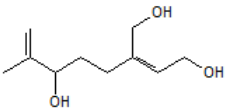

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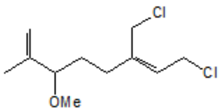

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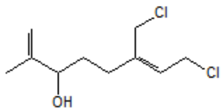

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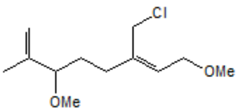

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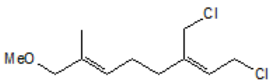

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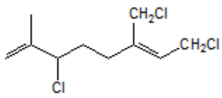

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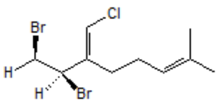

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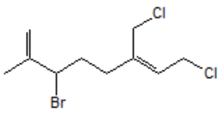

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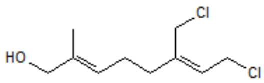

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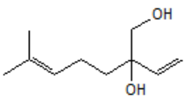

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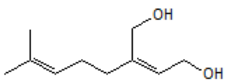

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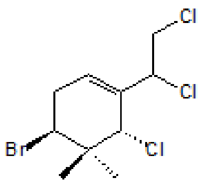
Australia (Coll and Wright, 1989)



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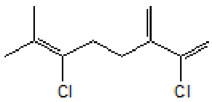
Bora Bora (Crews *at al.*, 1984)



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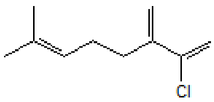

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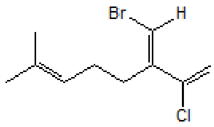

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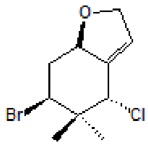

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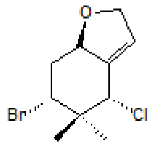

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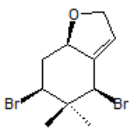
Hawaii (Burreson *et al*., 1975)



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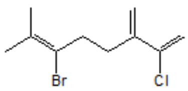

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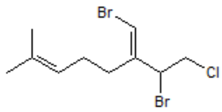

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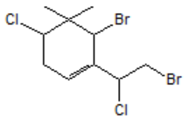

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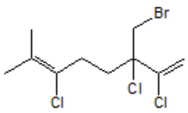

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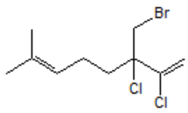

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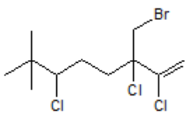

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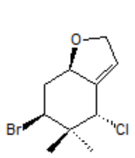
Hawaii (Burreson *et al*., 1975)



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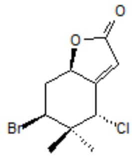

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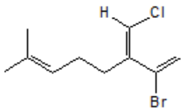
Hawaii (Woolard and Moore, 1978)



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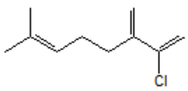

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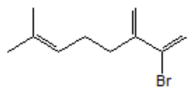

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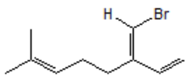

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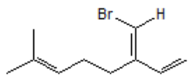

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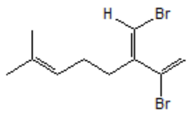

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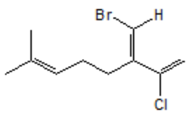

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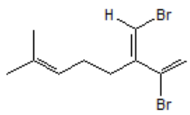

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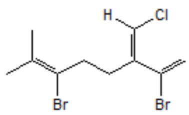

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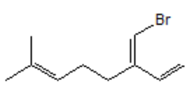
Japan (Ichikawa *et al.*, 1974)



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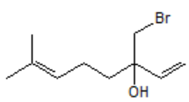

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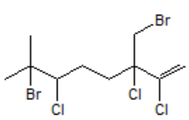

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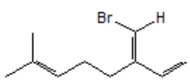

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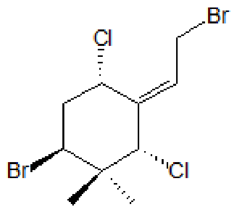
Sri Lanka (Woolard *et al.*, 1976)

2.1 Examination of cytotoxic compounds isolated from Portieria species

Secondary metabolites isolated from *Portieria* species comprise of a number of diverse and volatile halogenated compounds. These low molecular weight halogenated metabolites have shown an exciting range of biological properties. An examination of cytotoxic secondary metabolites previously isolated from these red algae was compiled. Publications in which pure compounds were isolated and whose chemistry tested for positive for cytotoxic behaviour are recorded below.

Ochtodene (**18**) from *Portieria hornemannii* demonstrated selective anti-tumour activity in cellular *in vitro* assays. However, this activity was not seen *in vivo* (Gunatilaka et al., 1999). No IC_{50} data was published.



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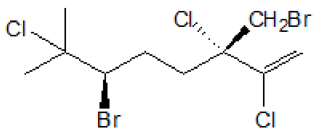
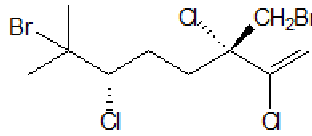
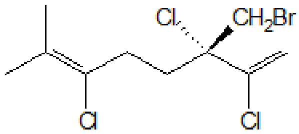
The following four compounds (**1**, **20**, **21** and **22**) were isolated from *Portieria hornemannii*. These compounds uniformly exhibited the same unique differential cytotoxicity profile as that of halomon against the NCI panel of 60 human tumour cell lines. All four compounds demonstrated comparable potency to each other (Fuller *et al.*, 1994) (Table 1).



Halomon (**1**)



Isohalomon (**20**)



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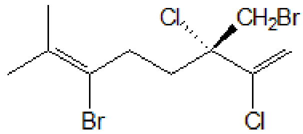

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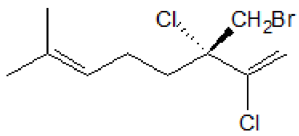
The cytotoxic profiles of the following four compounds (**23**, **24**, **25**, **26**) are less potent than that of halomon, and they also lack differential cytotoxicity (Fuller *et al.*, 1994.)



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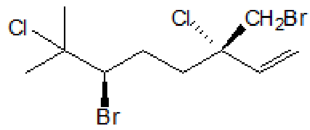

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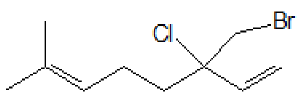

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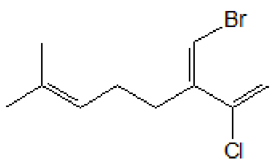

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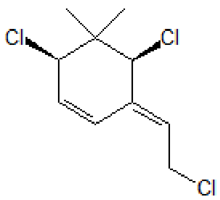
Looking at compounds **1**, **20**, **21**, **22**; the pharmacological results suggest that the halogen at C-7 is not essential for activity and that hybridization of C-6 and C-7 to sp^2 or sp^3 is also not a critical factor for cytotoxic activity. Fuller (1994) goes on to suggest that a halogen at C-6 is essential for the cytotoxic activity demonstrated by these compounds. In addition to this, a halogen at C-2 is also required for 'halomon-like' activity. During these pharmacological bioassays, carbocyclic compounds demonstrated considerably less cytotoxic activity than compounds **1**, **20**, **21**, **22**. Unlike compounds **1**, **20**, **21**, **22**; carbocyclic compounds also exhibited no differential response on the tumour cell line (Table 1).

The cyclic compound below (**35**) was found to be considerably less potent than halomon, by at least 1 order of magnitude for three different bioassays (Fuller *et al.*, 1992). However, the second cyclic compound (**19**) had a potency comparable to that of halomon. Unfortunately, this compound showed little differential response to cell lines when compared to halomon and was consequently not investigated any further (Fuller *et al.*, 1994).



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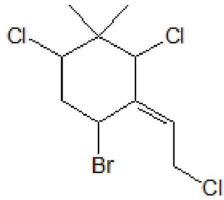

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Carbocyclic halogenated monoterpenes from Rhodophyta have been reported to show general toxicity to brine shrimp assays and in vitro inhibition of murine leukaemia cells (Fuller *et al.*, 1992). Nonetheless, these results show that carbocyclic halogenated monoterpenes are not particularly effective against solid tumour cell lines. The difference in activity between linear and cyclic halogenated compounds, suggests that linear compounds are not merely acting as electrophiles or alkylating agents (Fuller *et al.*, 1992).

Table 1: Results of comparative testing of compounds **1**, **20**, **21**, **22**, **23**, **24**, **25**, **26**, **35**, **19** in the NCI **in vitro** primary anti-tumour screen (Fuller *et al.*, 1994).

	Mean panel response values ($\times 10^{-6}$M)	
Compound	**GL$_{50}$**	**LC$_{50}$**
1	0.678	11.5
20	1.32	16.2
21	0.741	17.0
22	0.691	13.5
23	47.0	>100
24	26.1	>100
25	33.1	>100
26	19.5	>100
35	20.0	>100
19	1.15	20.0

Of the several cyclic monoterpenes that were isolated from Portieria hornemannii in Japan, a mixture of the following two cyclohexadienones (A-B) (**28a** and **28b**) showed significant cytotoxicity against P-388 murine leukaemia cells (IC_{50} μg/tml), A-549 human lung carcinoma (IC_{50} 0.5μg/ml) and HCT-8 human colon adenocarcinoma (IC_{50} 0.5μg/ml) (Kuniyoshi *et al.*, 2003).

Portieria hornemannii extracted from Madagascar, yielded the following four compounds which were later tested on a DNMT-1 enzyme inhibition assay. This assay is used to identify compounds which may be useful in the reversal of tumour growth. Compounds **1** and **30** had comparable activities (1.25 and 1.65 μM, respectively) and are low micromolar inhibitors of DNA methyl transferase-1; while compounds **31** and **34** were only weakly active (55.0 and 21.9 μM, respectively) (Andrianasolo *et al.*, 2006).

3 Conclusion

In the search for new or novel halogenated monoterpenes from different *Portieria hornemannii* collections, it is important to know what compounds have already been characterised or discovered. Furthermore, with the large number of metabolites that have already been isolated from *Portieria hornemannii*; an effective, reliable and rapid literature analysis of all these compounds is essential. Being able to provide this information both rapidly and accurately as seen above, is extremely valuable to the natural product chemist who is researching halogenated monoterpenes. It is anticipated that this examination which illustrates the secondary metabolites found in *Portieria hornemannii* species around the world will be a useful reference for those involved with marine drug discovery.

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***** Note this analysis forms part of my PhD thesis which was completed at Rhodes University, Grahamstown, South Africa, (Knott, 2012).***