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Short Communication

Chemical composition and cytotoxicity of the essential oils of *Crinum ornatum* (Ait.) Bury

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The bulbs of *Crinum ornatum* were collected, dried and grounded. The powdered samples were subjected to distillation using a hydro-distiller (all-glass clevenger apparatus) to extract the essential oil present in the plant samples. GC and GC/MS analysis were carried out on the essential oil and was found to contain 18 compounds; hydrocarbons being the dominating group of compounds. They are 2,4-dimethylhexane (1.51%), methyl benzene(5.49%), cis-1,3- dimethyl cyclohexane (2.08%), cis-decahydronaphthalene (5.49%), trans-decahydronaphthalene (2.08%), undecanoic acid, ethylester (1.51%), caryophyllene (1.51%), dodecanoic acid (1.51%), 14-methylpentanedecanoic acid methylester (20.89%), 2,6,10,15-Tetramethylheptanedecane (3.14%), n-hexanedecanoic acid (13.06%), eicosane (2.61%), 9,12-octadecadienoic acid (13.06%), heneicosane (13.14%), eicosanoic acid, ethylester (5.22%), nonacosane(2.35%), tetratriancontane (2.61%) and tetratetracontane (10.45%) representing 97.71% of the total essential oil. The cytotoxicity result of LC_{50} (µg/ml) value of 1.701 obtained through the brine shrimp toxicity assay indicated that the oil is toxic.

Keywords: *Crinum ornatum*, essential oils, cytotoxicity, hydrodistillation, gas chromatography/ mass spectroscopy.

INTRODUCTION

Plants are used medicinally in different countries and are a source of many potent drugs (Srivastava et al., 1996). Essential oils are a group of secondary metabolites. The biological activity of the essential oils can be compared with the activity of synthetically produced pharmacological preparations and should be investigated in the same way. Oils are a class of volatile oils, extracted from plants, fruits, or flowers having each its characteristic odour. They are used in essences; perfumery and some have been shown to have medicinal properties. Essential oils are made up of many chemical constituents, alcohol, aldehyde, esters, ketones, phenols and terpenes (Ghani, 1990; Harborne, 1998).

Crinum ornatum (Amaryllidaceae) is a bulbous plant with fleshy, wide spreading rich green and glaucous leaves reaching 75 cm long by 6 cm wide. It grows well in damp site and is distinctly ornamental. The plant attracts considerable attention due to various medicinal properties as anti-tumor, immuno-stimulating, analgesic, anti-viral, anti-bacterial and anti-fungal (Burkill, 1985). Augustine and lycorine isolated from this plant showed moderate anti-malarial activity against *Lasmodim falciparum*, but the selectivity was very low compared to anti-malarial control compounds (Ghosal et al., 1985; Tram et al., 2002). Some members of the family also contain pharmacologically active chemical compounds; Crinamine from *Crinum jagus*, possessed strong anti-bacterial activity, while lycorine, hamayne and 6-hydroxycrinamine were inactive (Adesanya et al., 1992). Crinisine from bulb of *Crinum asiaticum* was also an effective insecticide (Tram et al., 2002).

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MATERIALS AND METHODS

Materials

Plant materials

Fresh bulbs of *C. ornatum* were collected in June, at Ife road in Ibadan North Local Government Area of Oyo State, Nigeria and specimens were identified and authenticated at Forestry Research Institute of Nigeria, Ibadan (No FHI 105367). The bulbs were air-dried under mild sunshine for 10 days until the weight was stable and ground into fine powder with a Hammer Mill (Ashai 7500) and

Table 1. Composition of the volatile oil from the bulbs of *Crinum ornatum* by GC-MS analysis*.

Peak no	Compound	RRI	% composition
1	2,4-dimethylhexane	688	1.51
2	Methyl benzene	794	5.49
3	Cis-1,3- dimethyl cyclohexane	842	2.08
4	Cis-decahydronaphthalene	1101	5.49
5	Trans-decahydronaphthalene	1101	2.08
6	Undecanoic acid, ethylester	1481	1.51
7	Caryophyllene	1494	1.51
8	Dodecanoic acid	1570	1.51
9	14-Methylpentanedecanoic acid methylester	1814	20.89
10	2,6,10,15-Tetramethylheptanedecane	1852	3.14
11	n-hexanedecanoic acid	1968	13.06
12	Eicosane	2009	2.61
13	9,12-Octadecadienoic acid	2093	13.06
14	Heneicosane	2109	3.14
15	Eicosanoic acid, ethylester	2375	5.22
16	Nonacosane	2904	2.35
17	Tetratriancontane	3401	2.61
18	Tetratetracontane	4395	10.45
	Total		**97.71%**

*Percentages calculated from flame ionization detection data. RRI, relative retention indices calculated against *n*-alkanes.

kept in non-absorptive nylon for subsequent use.

Method

Isolation of essential oils

The oil was obtained by hydrodistillation on a Clevenger type apparatus for 3 h in accordance with the British Pharmacopeia specifications (1980). The essential oil was collected, dried over anhydrous sodium sulphate and stored at 4°C until analysis. The oil yield was calculated relative to the dry matter.

Analysis of the essential oils

Gas chromatography: The oils were analyzed by GC using a Shimadzu model QP2010 chromatograph. An HP-Innowax FSC column (30 m x 0.25 mm, with 0.25 µm film thickness) was used with Helium as carrier gas at a flow rate of 1 ml/min. The GC oven temperature was kept at 60°C (hold for 0 min), and programmed to reach 140°C at a rate of 5°C/min, then kept constant at 280°C for 10 min being the final hold time. The split ratio was adjusted to 50:1. The injector temperature was set at 200°C. The percentage compositions were obtained from electronic integration measurements using flame ionization detector (FID), set at 250°C. *n*-alkanes were used as reference points in the calculation of relative retention indices (RRI). Relative percentages of the characterized components are given in Table 1.

Gas chromatography-mass spectrometry: The essential oils were analysed by GC-MS using a Shimadzu model QP2010 gas chromatograph system with split/ splitless injector interfaced to a 5973 mass selective detector. Innowax FSC column (30 m x 0.25 mm, 0.25 µm film thickness) was used with helium as carrier gas (1 ml/min). GC oven temperature and conditions were as described above. The injector temperature was at 250°C. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 30 to 500.

Identification of components: Identification of constituent of the oil was achieved on the basis of their retention indices determined with a reference to a homologous series of *n*-alkanes and by comparison of their mass spectral fragmentation patterns (NIST database/ chemstation data system) with data previously reported in literature (Adams, 2001; Joulain and Konig, 1998; Mclafferty and Staufer, 1989).

Brine shrimp lethality test

The brine shrimp lethality test (BST) was used to predict the presence, in the oils, of cytotoxic activity (Meyer et al., 1982). The shrimp's eggs were hatched in sea water for 48 h at room temperature. The nauplii (harvested shrimps) were attracted to one side of the vials with a light source. Solutions of the extracts were made in DMSO, at varying concentrations (1000, 100 and 10 µg/ml) and incubated in triplicate vials with the brine shrimp larvae. Ten brine shrimp larvae were placed in each of the triplicate vials. Control brine shrimp larvae were placed in a mixture of sea water and DMSO only. After 24 h, the vials were examined against a lighted background and the average number of larvae that survived in each vial was determined. The concentration killing fifty percent of the larvae (LC_{50}) was determined using the Finney computer programme.

RESULTS AND DISCUSSION

The air dried powdered bulbs of *C. ornatum* were utilized to obtain volatiles by means of hydrodistillation. The essential oil, light yellow with characteristic smell was further analyzed both by GC and GC/MS systems using a polar column, resulting in the identification of only 18

constituents in the hydrodistilled sample, representing 97.71% of the total essential oil. The essential oil yield was 0.30% (w/w) which is low. The oil yield of *C. ornatum* seems to have depended on the age of plant used and the mode of extraction. Overall, hydrocarbons were found in the sample as the dominating group of compounds; 2,4-dimethylhexane (1.51%), methyl benzene (5.49%), cis-1,3-dimethylcyclohexane (2.08%), cis-decahydronaphthalene (5.49%), trans-decahydronaphthalene (2.08%), undecanoic acid, ethylester (1.51%), caryophyllene (1.51%), dodecanoic acid (1.51%), 14-methylpentanedecanoic acid methyl-ester (20.89%), 2,6,10,15-Tetramethylheptanedecane (3.14%), n-hexanedecanoic acid (13.06%), eicosane (2.61%), 9,12-octadecadienoic acid (13.06%), heneico-sane (13.14%), eicosanoic acid, ethylester (5.22%), nonacosane (2.35%), tetratriancontane (2.61%) and tetratetracontane (10.45%) (Table 1) were identified as the main constituents of the hydrodistilled samples. The LC_{50} (µg/ml) result of 1.701 from the Brine shrimp toxicity assay with upper confidence limit and lower confidence limit of 2.137 and 0.4678 respectively further corro-borated the presence in the oil hydrocarbon molecules thereby accounting for the high toxicity of the oil.

CONCLUSION AND RECOMMENDATION

A total of eighteen chemical components were detected by GC and GC/MS in *C. ornatum* oil and were identified by spectral comparison to be mainly hydrocarbons. Brine shrimp lethality test was carried out to know the toxicity of the oils to living organisms (shrimps). The oils of *C. ornatum* was discovered to be toxic. The toxicity was assayed using brine shrimps at 10, 100 and 1000 ppm resulting in LC_{50} ((µg/ml)) value of 1.701. *C. ornatum* used in this study was chosen on the basis that it is used traditionally for treatment of a wide array of diseases. The study is premised on justifying its use in traditional medicine.

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