CYTOTOXICITY OF ISOLATED COMPOUNDS FROM THE EXTRACTS OF *STRUCHIUM SPARGANOPHORA* (LINN) KTZE ASTERACEAE

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ABSTRACT

Chemical investigation of the leaves of *Struchium sparganophora* by the application of VLC, CL and PTLC resulted in isolation of three compounds. The cytotoxicity activity of these compounds on malignant human cultured cells was examined. Vernodalin showed a significant cytotoxic activity on the melanoma and ovarian cancer cell lines ($P<0.05$) while the conjugated 3 methyl, 2, 6 hexacosedienol and luteolin caused cell death after 48h reculture without them.

These compounds portend an effective remedy if subjected to structural modification to enhance its' efficacy and the dietary importance of this plant as a culinary herb in west Africa countries is evidence by the presence of these antitumour compounds in this plant.

Keywords: *Struchium sparganophora*, cytotoxicity, luteolin, vernodalin, 3 methyl, 2, 6 hexacosedienol.

INTRODUCTION

Struchium sparganophora is a culinary herb in most African countries. There is a severe lack of relevant scientific research carried out on this herb, despite its wide spread consumption in African countries and its ethnobotanical use. Natural products from plant origin have been a source of many novel drugs in conventional use in Health care today e.g. Artemisinin from Annual Mugwort (A. annua) and taxol from *Taxus brevifola* (De Smet PAGM *et al*., 1993). At the same time, the use of traditional medicines has increased, as consumers seek complementary and or alternatives to prescribed drugs.

Plants belonging to the family Asteraceae constitute one of the largest Plant families. It contains over 40 economically important species; they are used as food (lettuce and Jerusalem artichopa), oil (Sun flowers and safflower), medicine (chamomile) and many as an ornamental plants (Burkill, 1985). *Struchium sprganophora* is a culinary herb used in Nigeria. The leaves are boiled in water drained completely and added to soup or consumed as a vegetable. It is also widely used medicinally in a number of countries: decoction of the stem and root are employed in the treatment of headaches, gonorrhea (Jakupovic *et al.*, 1987). The plant is an antidote for poisons (Akah and Ekekwe, 1995). In Gulf of Guinea, it is used as anti malaria (Madureira *et al.*, 2002) while it also has anti measles activities (Burkill, 1985).

The nutritive, antioxidant and antimicrobial and the anti-malaria activities of the leaves have been reported by (Oboh, 2006) and also the anti-oxidant properties of its polar and non polar extracts was reported by the same author and his colleagues (Oboh *et al.*, 2008). Its phytochemical composition and the effect of its aqueous extract on cockroach crude extract-induced airway. Inflammatory response in Wistar Rats has been reported by (Eko *et al.*, 2008). Sesquiterpine lactone has been isolated from the plant as reported by Jakupovic *et al.*, 1987. This study provides evidence of the cytotoxicity activity of three isolated compounds on human malignant cells.

MATERIALS AND METHODS

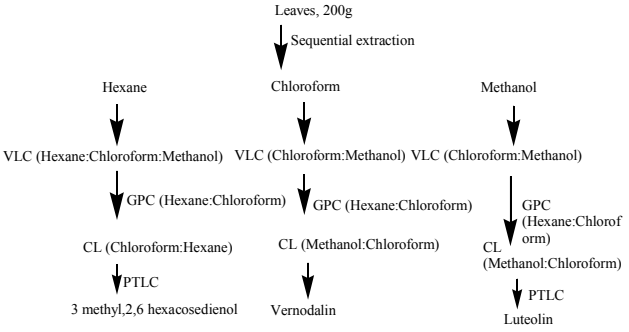

Fig: Procedure of isolation of 3 methyl 2, 6, hexacosedienol, vernodalin and luteolin

Plant collection

Struchium sparganophora leaf was collected from Sagamu community in Nigeria and identified by Mr. IK Idewo at the Forest research Institute of Nigeria (FRIN) with a voucher number (FHI 105358).

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Extraction

The leaf of *Struchium sparganophora* was dried in an oven (40°C), powdered and 200grams leaf was extracted with, *n*- hexane, chloroform and methanol sequentially in a soxhlet apparatus and the extract of each solvent was concentrated under reduced pressure (Rotavapor-R, Buchi), weighed and expressed in grammes and percentage (w/w) as shown table 1.

Isolation of luteolin

The methanol extract (28 g) was subjected to vacuum liquid chromatography (VLC) with solvent of increasing polarity chloroform- methanol (100:0, 0:100) and the thin layer chromatography (TLC) characteristics of the fractions were examined and identical fractions pooled together. The fraction chloroform-methanol (90:10) was chromatographed on Sephadex LH-20 and column chromatography isocratically with solvent system chloroform- methanol (8:2) as in figure. The separation yielded 35mg pure yellow crystal of R_f 0.4 on TLC plate of a mobile phase toluene-methanol-acetic acid (7.2 :1.3:1.5). The ^{13}C-NMR spectrum (DMSO-d_6) as well as ^{1}H-NMR spectrum (DMSO-d_6) of this compound were in conformity with that of luteolin (Harbone *et al*., 1975) and were also consistent with literature data (Markham and Geiger, 1993) The IR data showed a strong absorption at 3413cm^{-1} characteristic of the presence of the hydroxyl and at 1690cm^{-1} characteristic of the presence of carbonyl group (Owen *et al*., 2003) and the GC-MS established the molecular formula as $C_{15}H_{12}O_6$.

Isolation of vernodalin

The chloroform extract (28 g) obtained from sequential extraction with solvents of increasing polarity, *n*-hexane and chloroform was first subjected to VLC with solvent of increasing polarity chloroform-methanol (100:0, 0:100) and TLC characteristics of the fractions were examined and identical fractions pooled together. The fractions was chromatographed on Sephadex LH-20 was rechromatographed on a column of silical gel (Merck,) packed in chloroform and eluted with solvent system chloroform- methanol (9:1) yielded the colourless oil (42.2 mg) as in figure. The ^{13}C-NMR and ^{1}H-NMR spectra (pyridine-d_6), the IR spectra, Mass spectrometry EIMS and the functionality supported by the presence in the mass spectrum prominent peaks at m/e 57 and 85 attributable to the fragmentations at a and b positions of vernodalin structure were consistent with the published data of vernodalin (Kupchan *et al.,* 1969, Ganijian *et al.,* 1983).

Although this compound was first isolated from *Vernonia amygdalina* but this is the first time to be isolated from *S. sparganophora.*

Isolation of dienol

The 3 methyl, 2, 6 hexacosedienol was isolated as shown in fig.

The (5 g) hexane extract was subjected to VLC with solvent of increasing polarity *n*-hexane-chloroform (100:0, 0:100) and TLC characteristics of the fractions were examined and identical fractions pooled together. The fractions was chromatographed on Sephadex LH-20 and later chromatographed on a column of silical gel (Merck) packed in n-hexane and eluted with solvent system chloroform-n-hexane (4:5) yielded the constituent which was further purified by PTLC to give the alcohol (13.5mg) which reacted with vanillin/sulphuric acid reagent to give a brownish colour. The ^{13}C J-modulated spectrum showed the presence of four of methylene carbon at δ 33.55, 33.48, 28.69 and 23.44. The methyl carbon at δ 20.43 and at 16.73. The ^{1}H NMR spectrum (270MHz, Pyridine-d_6) data were δ 5.78 (2H, br, J= 5Hz, H-1); δ 4.51 (3H d, J=6Hz, H-3'); δ 3.9 (1H, s, H-2); δ 2.-5 (4H, t, J= 7.2Hz, H-4) and δ 1.74 (1H, m, H-5). Based on the COSY- spectrum the unambiguous linkages between the protons were established. The high resolution ES-MS suggested an empirical formula $C_{27}H_{52}O$ and therefore partially identified as 3 methyl, 2, 6 hexacosedienol.

Primary cell cultures and cell lines

The Primary cell lines used for the anti-tumour tests were, Ovarian cancer (CAOV-3), Melanoma (Sk-mel 28) and the HeLa cells (ECACC 84113001).

Sample preparation

The cell preparation was carried out according to Haves, (2002) method. A high concentration (1mg/ml) of the compounds was made and this was diluted as required. 1µg/ml of all test compounds was first prepared as the stock solutions. After which a serial dilutions of concentration of 1.25-0.0625 × 10ng/ml of each compounds were prepared and used for the growth curve.

In vitro cytotoxicity testing

The MTT cytotoxicity/proliferation assay (Mosman, 1983) was used to measure the toxicity of the test materials by determining the absorbance of the cells in culture. Two exposure periods of 24 h and 48 h were chosen for determining the *in vitro* cytotoxicity of the test materials along with a positive control containing the cell lines and the medium, and the negative control containing the medium and the sample.

The percentage cell growth was calculated against the medium as mean of triplicate reading ± SD. The results were stated as concentration to reduce the absorbance of treated cells by 50% with reference to the control (untreated cells) or Cancer cell growth inhibition (CD_{50}) and the lethal dose LD values (µg/ml) of the compound derived from the growth curve are expressed as in table.

RESULTS

Solvent extracts of the leaf

The solvents, hexane, chloroform and methanol yielded 5g, 28g and 28g extracts respectively which are 2.5%w/w, 14%w/w and 14%w/w of the powdered leaf respectively.

Cytotoxicity results of the isolated compounds

Table: Cancer cell lines inhibition (CD_{50}) and the lethal dose (LD) values of pure compounds in (µg/ml) after 48h reculture without the compounds

Cell Line	3methy2,6 hexacosedienol		vernodalin		Luteolin	
	CD_{50}	LD	CD_{50}	LD	CD_{50}	LD
HeLa	-	=	-	=	-	-
Melanoma	-	-	-	0.005	0.0005	-
Ovarian	-	-	-	0.008	0.0004	-

- Cytopathic

DISCUSSION

The isolated compounds were tested for their cytotoxic activity or anticarcinogenic actvity as described by (Mosman, 1993). The conjugated 3 methyl, 2, 6 hexacosedienol and luteolin caused cell death after 48h reculture without them as in table 1 this might be attributed to the dose used for the test but it has been reported that luteolin inhibited a series of solid tumour (renal A-549, ovary SK-OV-3, melanoma SK- MEL-2, XF-498, HC15, gastric HGC-27 (Ryu *et al*., 1994; Matsukawa *et al*., 1993) and leukemia (P388, CEM-C1 and CEM-C7) (Post and Varma 1992) cell lines. Furthermore, Luteolin has been shown to be an antitumour promoter (Middleton *et al.,* 1987) and exhibit antimutagenic activity (Pettit *et al.*, 1996). The conjugated 3 methyl, 2, 6 hexacosedienol may assist in the cancer chemoprotective properties of this vegetable. For example, luteolin is especially abundant (3-13 mg/kg) in certain vegetables (Ramanathan, *et al*., 1994).

The flavonoids have been reported to be an important source of antioxidants in the human diet and may exceed that of β-carotene and vitamin E (Pettit *et al.,* 1996). However luteolin has been reported to be a mutagen (Nagao *et al*., 1981) by using *Salmonella typhimurium* procedure but this is at variance with many reported work on luteolin. All the compounds caused HeLa cells death (cytopathic) while only luteolin and 2, 6 hexacosedienol caused the death of all cancer cell lines the use of lower doses may produce a more beneficial result.

Vernodalin showed a significant ($P<0.05$) cytotoxic activity on. the melanoma (Sk-mel 28) and ovarian cancer cell lines (CAOV-3) table 3 and this in conformity with the work of (Kupchan *et al*., 1969). Moreover other sesquiterpine lactones apart from vernodalin has also been reported to poses tumour inhibitory effect e.g. vernolepin and vernomygdin (Kupchan *et al*., 1969). This compound portend an effective remedy if subjected to structural modification to enhance their efficacy and the dietary importance of this plant as a culinary herb in west Africa countries is evidence by the presence of these anti-tumour compounds in this plant

CONCLUSIONS

These isolated compounds could be a possible source to obtaining new and effective antitumour agents to combat malignant growth when subjected to structural modification to enhance their efficacy and their side effects.

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