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Full Length Research Paper

Evaluation of cytotoxic and antimicrobial activities of *Struchium sparganophora* (Linn.) Ktze Asteraceae

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Struchium sparganophora **(Linn) Ktze, (Asteraceae) is a culinary herbs used as part of a traditional dish in Nigeria and a medicinal plants for the treatment of different ailments in Africa. Dried leaf, stem and root parts of this plant were extracted with n-hexane, chloroform and methanol respectively, concentrated under reduced pressure, freeze dried and evaluated for their antimicrobial and anti tumour activities. The antimicrobial test involved microdilution titre technique while cytotoxicity activities was evaluated using the 3,-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) assay. The extracts exhibited a broad spectrum of activity against Gram- positive and Gram-negative organism of minimum inhibitory concentration (MIC) of 50 to 6.25 mg/ml but this activity is less than that of anti-microbial standard drug amoxicillin which had MIC of 0.78 to 6.25 mg/ml except against *Klebsiella aerogenes* on which the extracts had a similar activity to that amoxicillin of MIC 6.25 mg/ml. The extracts showed a significant anti- fungal activity (P< 0.05) of MIC 6.25 mg/ml in comparison with the anti-fungal standard drug Fluconazole (MIC) 50 mg/ml. The extracts also showed to be cytotoxic to all cancer cell lines at the dose of 0.75 mg/ml and significant growth inhibitory activity to all the cancer cell lines used for the test at the dose of 1 mg/ml. The hexane extracts exhibited increase in most cancer cell lines growth at the doses of 0.1 and 0.25 mg/ml while the chloroform and methanol extracts did not show uniform activity. These activities may portend a beneficial effect to human consumption as it may hinder the development and growth of cancerous cells at a high dose.**

Key words: *Struchium sparganophora*, antimicrobial, cytotoxicity, ovarian, melanoma, HeLa.

INTRODUCTION

There is a general increase in research activities aim at discovering new anti-infective drugs due to global health problem pose by antibiotic and multi-drug resistance (Bax et al., 2000). Natural products from plant origin have been a source of many novel drugs in conventional use in Health care today e.g. Artemisinine from Annual Mugwort and taxol from *Taxus brevifola* (De Smet et al., 1993). At the same time, the use of traditional medicines has increased, as consumers seek complementary and or alternatives to prescribe 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 shrubs (chrysanthemum) Dahli, Zinnia and Marigold (Burkill, 1985). *Struchium sprganophora* (Linn) *K*tze, (Asteraceae) is culinary herbs used as part of a traditional dish in Nigeria, the leaves are boiled with water, drained completely of the water content and add to

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Table 1. Extraction yield in percentage w/w).

	Leaf	Stem	Root
Hexane	5	0.3	0.3
Chloroform	14	0.96	0.32
Methanol	14	9.6	2.4

soup and consumed as a vegetable. It is also widely used medicinally. Thus, decoction of the stem and root are employed in the treatment of headaches, gonorrhoea and as an antidote for poisons (Aka et al., 1995). In San Tome and Principe (STP) island (Gulf of Guinea), and in the treatment of measles tropically (Heywood et al., 1977).

The leaves have nutritive, antioxidant, antimicrobial and anti- malaria activities as reported by (Oboh 2006; Oboh et al., 2008; Madureira et al., 2002) also the aqueous extract of *S. sparganophora* (L) has an anti-inflammatory responses in Wistar Rats (Eko et al., 2008). Sesquiterpine lactone has been isolated from the plant as reported by Jakepovic et al. (1986).

This study provides evidence for its antimicrobial use of the different parts of the plant in the treatment of some infectious agents and its' cytotoxic activities on some cancer cell lines.

MATERIALS AND METHODS

Plant material

S. sparganophora leaf, stem and root were collected from Sagamu community in Sagamu local government area of Nigeria in the month of November.

The plant was identified and authenticated by Mr. I. K. Idewo of the Forest Research Institute of Nigeria, Ibadan, by comparing with Voucher specimens 105358. A specimen is kept in the Pharmacognosy Department, Faculty of Pharmacy, Olabisi Onabanjo University, Sagamu, Ogun State, Nigeria.

Extraction

The plant material was dried in an oven (40°C) and powdered. Then 200 mg leaf, 500 mg stem and 500 mg root were extracted sequentially with solvents of increasing polarity, *n*- hexane, chloroform and methanol with a soxhlet apparatus and the extracts obtained were concentrated under reduced pressure (Rotavapor-R, Buchi).

Preparation of extracts

The crude extracts and the standard drugs (Amoxycillin for anti-microbial and Fluconazole for anti-fungal) were dissolved in 20% v/v dimethylsulfoxide (DMSO) in peptone water (Oxoid) to attain the desired concentrations of 200 mg/ml.

Amoxycillin and Fluconazole were used as positive control while 20% DMSO in water and test extract was considered as a negative control.

Test microorganisms

The strains of bacteria used in this study were *Staphylococcus aureus* (NCTC 6571) *Klebsiella aerogenes* (Welcome Res. Lab.CN 345), *Escherichia coli* (NCTC 9001) and *Proteus vulgaris* (NCTC 8313). The fungal strain used was *Candida albicans* (ATCC10231).

Bacteria culture methods

All bacteria were subcultured from Nutrient agar (Oxoid) stock cultures stored at 25°C in a suspension of 0.1% v/v peptone water incubated at 37°C for 24 h.

Fungi culture methods

The same method (as per bacteria cultures) was used for the yeast culture (*C. albicans*) except that nutrient agar was replaced with sabouraus/dextrose agar (Oxoid).

Standardisation of all inocula

The turbidity of all inocula were standardised by adjusting to match 0.5 Mc Fardland standard by the addition of appropriate sterile broth. Mueller- Hinton broth (Oxoid) was used for the dilution of bacteria while malt extract broth was used for the fungus.

Internal standards and controls

Appropriate controls were set up which involved (1) a negative control, which contained the test material and 20% DMSO in peptone water but absence of test organism; and (2) a positive control involving the absence of test material but the presence of the organism and the standard drug Amoxycillin. The tests were carried out at different days to avoid cross-contamination and mix up of the test procedure.

Minimum inhibitory concentration (MIC) dilution assay

This involved microdilution titre technique as described by (Drummond and Waigh, 2000; Rahman and Gray, 2002). The activities were compared with the anti-bacteria standard drug Amoxycillin and the anti-fungal standard drug Fluconazole. The MIC in mg/ml are presented in the Table 1.

Primary cell cultures and cell lines

The primary cell lines include those obtained from Prof. William Stinson of University of Strathclyde, Department of Immunology, Ovarian cancer (CAOV-3), Melanoma (Sk-mel 28) derived from human hepatocarcinoma (ATCC No.HTB-72) and the normal fibroblast HeLa cells (ECACC) were used for the anti-tumour tests.

Table 3. Cytotoxicity testing of hexane extracts showing the growth percentage mean and standard deviation of three replicates of the cell lines.

Conc. (mg/ml)	HeLa			Ovarian			Melanoma		
	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
0.1	120 ± 10	140 ± 10	130 ± 10	120 ± 5	100 ± 10	135 ± 10	-	120 ± 10	80 ± 5
0.25	98 ± 10	*0	150 ± 2	*0	98 ± 20	130 ± 10	-	98 ± 2	100 ± 3
0.5	110 ± 2	-	*20 ± 5	-	75 ± 15	*15 ±5	-	*0	120 ± 5
0.75	-	-	-	-	-	-	-	-	-
1.0	*0	-	*0	-	-	*0	-	-	*0
Control	100	100	100	100	100	100	100	100	100

-- Cytopathic effect, * Significant.

Table 4. Cytotoxicity testing of chloroform extracts showing the growth percentage mean and standard deviation of three replicates of the cell lines.

Conc. (mg/ml)	HeLa			Ovarian			Melanoma		
	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
0.1	80 ± 5	100 ± 10	70 ± 10	120 ± 5	100 ± 10	130 ± 10	120 ± 5	80 ± 5	80 ± 5
0.25	110 ± 5	120 ± 10	80 ± 5	*0	98 ± 20	-	*20 ± 5	138 ± 10	80 ± 5
0.5	120 ± 10	140 ± 20	75 ± 23	-	75 ± 15	-	*0	140 ± 10	130 ± 2
0.75	-	-		-	-	-	-	-	-
1.0	*0	*0	*0	-	*0	*0	-	*0	*0
Control	100	100	100	100	100	100	100	100	100

-- Cytopathic effect, *Significant.

Culture methods

All cultures were maintained in a colour-free medium composed of 50% RPMI-1640 Medium (Roswell Park Memorial Institute Medium-tissue culture, Sigma-Aldrich Ltd, UK); supplemented with 10%v/v feotal calf serum (FCS) (Seralab, UK); 1% v/v L-glutamine (Gibco, UK) and containing 1% v/v of antibiotic mix:10,000 units penicillin and 10 mg streptomycin (Gibco, UK). Cultures were maintained at 37℃ in a humidified 5% CO_2 incubator. Cells were in the exponential growth phase at the time of testing with experiments conducted at the concentrations of 250,000-500,000 cells per ml. The viability of the cells exceeded 95% on all occasions as determined with 10 v/v Almar blue (Serotec UK) dye exclusion method.

Sample preparation

Freezed dried extracts were dissolved in RPMI-1640 Medium (Roswell Park Memorial Institute Medium-tissue culture, Sigma-Aldrich Ltd, UK) and the solution incubated at 37℃ for 30 mins to allow it to dissolve completely. The solution was filtered sterilised (0.22 µm) filter and stored at 4℃. This was carried out in a laminar flow cabinet. A high concentration 400 mg/ml was made up and this was diluted as required. 1 mg/ml of all test samples was first prepared from the stock solutions. After which a serial dilutions or varied concentrations of each test material were prepared in a cell culture medium from this stock solution to give test concentration range of 1.25 to 0.0625 mg/ml which were used for the growth curve. The cells were plated out into 96 well tissue culture plates at a concentration of 2.5-5 x 10^6 cells per ml and incubated with the extracts (1 mg/ml) or the varied concentrations (for the cytoxicity testing and for the growth curve) along with positive and negative controls (as stated *in vitro* cytotoxicity testing) for 24 h at 37℃, 5% CO_2 and 100% humidity.

***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 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 only and the results expressed as the mean of triplicate reading ± SD. The cell growth was calculated as follows:

Percentage cell growth = ((OD570 – OD600) of test agent / (OD570 – OD600) of control) x100

Cytotoxicity was expressed as CD_{50}, that is, the concentration to reduce the absorbance of treated cells by 50% with reference to the control (untreated cells) and $LD_{50,}$ is the lethal dose of the extracts. The Tables 3, 4 and 5 show the percentage growth at 48 h light phase contrast microscope at x 40 magnification. The total number of cells in 1 ml was calculated by multiplying by 2 x 10^4. The cell suspension was then diluted appropriately.

Table 5. Cytotoxicity testing of methanol extracts showing the growth percentage mean and standard deviation of three replicates of the cell lines.

Conc. (mg/ml)	HeLa			Ovarian			Melanoma		
	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
0.1	80 ± 5	100 ± 10	70 ± 10	120 ± 5	100 ± 10	130 ± 10	120 ± 5	80 ± 5	80 ± 5
0.25	110 ± 5	120 ± 10	80 ± 5	*0	98 ± 20	-	*20 ± 5	138 ± 10	80 ± 5
0.5	120 ± 10	140 ± 20	75 ± 23	-	75 ± 15	-	*0	140 ± 10	130 ± 2
0.75	-	-		-	-	-	-	-	-
1.0	*0	*0	*0	-	*0	*0	-	*0	*0
Control	100	100	100	100	100	100	100	100	100

-- Cytopathic effect, *Significant.

Table 2. The results of anti-microbial and the anti-fungal tests of the crude extracts showing the MIC in mg/ml.

Extracts	SA	EC	PV	PA	CA
Root hexane	25	12.5	12.5	12.5	50
Root chloroform	50	25	25	12.5	50
Root methanol	50	50	25	6.2	50
Stem hexane	25	25	-	-	6.25
Stem chloroform	50	50	-	6.25	12.5
Stem methanol	50	25	-	6.25	25
Leaf hexane	25	12.5	-	50	25
Leaf chloroform	50	25	-	12.25	25
Leaf methanol	50	25	-	25	50
Solvent	>100				
Fluconazole					50
Amoxycillin	0.78	3.12	6.25	0.25	

SA = *Staphylococcus aureus*; EC = *Escherichia coli*; PV = *Proteus vulgaris*; PA =*Pseudomonas earuginosa; CA = Candida albican*.

RESULTS AND DISCUSSION

Extractions

The extracts were dried on a Rotary evaporator to give a percentage yield as shown in Table 1.

The results of anti microbial test

The results of antimicrobial and antifungal tests in terms of MIC are presented in Table 2. All the extracts inhibited the growth of most of the test organisms used in this experiment but the hexane extracts showed exceptional activity having the least activity at the MIC 25 mg/ml for Gram- positive organism *S. aureus* and the highest activity at MIC 6.25 mg/ml for Gram- negative organism *P. aerogenes* similar to Amoxycillin (MIC) 6.25 mg/ml for Gram- negative organism *P. vulgaris*. The activity of root hexane and leaf hexane extracts were the same for the *S. aureus* and *E. coli* having MIC 25 and 12.5 mg/ml respectively. Among all the hexane extracts only the root hexane extract showed antibacteria activity against all the microorganism used in the test while the leaf and stem hexane extracts activity can not be defined for *P. vulgaris*. Comparison of the susceptibility of all the bacteria to the various extracts indicated that *S. aureus* was the most resistant organism to the extracts having six of the extracts with MIC 50 mg/ml and having its highest sensitivity at the MIC 25 mg/ml. This observation was at variance with the previous reports of some workers (Buwa and Staden, 2006; Parekh et al., 2005).

Moreover the stem hexane, chloroform, and methanol extracts as well as leaf hexane and chloroform extracts showed significant anti-fungal activity ($P<0.05$) having MIC of 6.25, 12.5, 25, 25 and 25mg/ml, respectively, than all other extracts which showed similar activity to the standard antifungal drug Fluconazole. The antifungal activity showed by *S. sparganophora* was similar to other species of the family and this highlights its chemical relationship with other species of the Asteraceae family which have been shown to possess antifungal activities (Moody et al., 1998).

The result of the cytotoxicity tests showed that all the

leaf, stem and root hexane, chloroform and methanol extracts in Tables 3, 4 and 5, had similar cytotoxic activities on ovarian cell lines which was significant ($P<0.05$) at a dose higher than 0.25 mg/ml in comparison to other cell lines but all the extracts had a lethal effect or inhibited the growth of all the cell lines at the dose of 0.75 mg/ml though the leaf hexane extract was cytopathic to melanoma cell lines in all the doses used for the experiment.

However, all extracts increase the cell growth above the hundred percent growth for the control or slight reduction in this percentage growth in all the cell lines between the dose of 0.1 and 0.5 mg/ml except stem and leaf hexane extracts as well as leaf and root chloroform extracts that significantly inhibited the growth of HeLa and ovarian cell lines at the dose of 0.25 mg/ml. The leaf, stem and root chloroform extracts had LD_{50} of 0.75 mg/ml for HeLa cells and the leaf and root chloroform extracts had LD_{50} less than 0.25 mg/ml for ovarian cancer cells and the leaf chloroform extract had LD_{50} of about 0.5 mg/ml for melanoma cell lines. Though the leaf chloroform extract of *Vernonia amygdalina* of the same family, (Asteraceae) showed similar activity which was attributed to the presence of sesquiterpines lactones (Kupchan et al., 1969).

Moreover, the leaf and root methanol extract showed significant activity ($P < 0.05$) on ovarian cancer cell lines at a dose of 0.25 mg/ml just like leaf methanol extract on melanoma but none of the leaf, stem and root methanol extracts showed this activity on HeLa cell lines at the same dose.

Therefore, at this preliminary stage it may be suggested that the *S. sparganophora* extracts posses anti-microbial activities and can also be used as an anti-tumour promoting agent at a higher dose which renders them suitable as potential therapeutic agents, moreover, they may be good for human consumption because of its inhibitory effect on the growth of cancer cell lines at higher doses. Differences in the profiles of these biological activities of the various extracts suggest the presence of different constituents or similar compounds having different mode of actions. The biological activities of any medicinal plant are direct reflections of the effect and nature of the phytochemicals it contains. *S. sparganophora* has been observed to contain protocatechuic acid, p-coumaric acid and caffeic acid (Salawu et al., 2006). Because of their relative polarities due to phenolic compositions, these compounds are likely to be found in the aqueous fraction of the plant. Protocatechuic acid has been reported to be one of the allelopathic constituents of *Delonix regia* (Chou and Leu, 1992). Also, in experimental animals, Protocatechuic acid has been reported to prevent chemically induced carcinogenesis (Tanaka et al., 1993). The probable presence of this compound may have contributed to the biological activities of *S. sparganophora*.

Certain medicinal plants in the Asteraceae family have been known to possess related cytotoxic activities. The allelopathic effects of *Ambrosia cumanensis* H.B.K. (Compositae) and also Piqueria trinervia (Compositae) leaves and roots have been revealed to inhibit proliferative activities of malignant cells (Anaya and Amo, 1978: De la Parra et al., 1981). Also, the antiproliferative effects of some Asteraceae species against human cancer cell lines have been observed (Evans, 2002). Moreover *Ageratum conyzoides* L. (Asteraceae) leaves have been implicated for anticancer effects (Adebayo et al., 2010). Although, this investigation requires further tests using non cancer human cell lines, the results obtained so far have indicated the claimed ethnomedicinal use of these plants in treating tumor-related ailments as well as antiinfective agent.

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