

Full Length Research Paper

Antimicrobial agents from the leaf of *Struchium sparganophora* (Linn) Ktze, Asteraceae

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Three Antimicrobial agents from the leaf of *Struchium sparganophora* were isolated by chromatographic methods and characterized. The microbial inhibitory activity of these compounds was investigated against Gram-positive, Gram-negative bacteria and fungi. The antimicrobial activity of some of these compounds was similar in some of the micro-organisms used for the tests except *Klebsiella. aerogenes* and the fungus *Candida albicans* where all the compounds showed minimum inhibitory concentration (MIC) values between 50 and 6.25 µg/ml. Luteolin and 3 methyl 2, 6, hexacosadienol significantly inhibited the growth of the two fungi (P<0.05) while vernodalin did not show strong activity against *Aspergillus niger* with MIC of 100 ug/ml. These compounds contribute to the anti-infective use of this plant in folkloric medicine and also these compounds could be used as natural antimicrobial agents in the food preservation and human health.

Key words. *Struchium sparganophora*, antimicrobial, luteolin, vernodalin, 3 methyl, 2, 6 hexacosadienol.

INTRODUCTION

Increasing use of natural medicines as a result of consumers seeking complementary and/or alternatives to prescribed drugs has provoked a great interest in research into medicinal plants. Some of these herbal products used to improve overall health, prevent and cure diseases, are likely to act through a stimulation of receptor sites, the immune system, inhibit pathogenesis of disease condition or have a lethal effect on pathogens. The discovery of bioactive compounds from plant origin offers an attractive approach to the control of infectious or non infectious diseases.

Struchium sparganophora is a culinary herb in most African countries. There is lack of relevant scientific research carried out on this herb, despite its wide spread consumption in African countries and its ethnobotanical

use. This study provides evidence for its antimicrobial use in the treatment of some infectious agents.

There is an increase in research aimed at discovering new anti-infective drugs due to global health problem posed 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. Artemisinin from Annual Mugwort and taxol from *Taxus brevifolia* (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 artichoke) oil (Sun flowers and safflower), medicine (chamomile), and many as ornamental plants (Burkill, 1985). *S. sparganophora* (Linn) Ktze (Asteraceae) is a culinary herb used in Nigeria. The leaves are boiled in water drained completely and added

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Table 1. The yield of leaf extraction in grammes and percentage (w/w).

	Hexane	Chloroform	Methanol
Weight of extractive (g)	5	14	14
%W/W	2.5	7	7

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, gonorrhoea (Jakupovic et al., 1987). The plant is also used as an antidote for poisons (Akah and Ekekwe, 1995). In the Sao Tome and Principe (STP) island (Gulf of Guinea), it is used for the treatment of malaria (Heywood et al 1977; Madureira et al., 2002) and in the treatment of measles (Burkill, 1985).

The nutritive, antioxidant and antimicrobial and the anti-malaria activities of the leaves have been reported by (Oboh, 2006; Oboh et al., 2008; Madureira et al., 2002). Its phytochemical composition and the effect of its aqueous extract on cockroach crude extract- induced airway inflammatory responses in Wistar Rats has been reported by Eko et al. (2008). Sesquiterpene lactone has been isolated from the plant as reported by Jakupovic et al. (1987). This study provides evidence for its antimicrobial use of the leaf in the treatment of some infectious agents.

MATERIALS AND METHODS

General experimental procedures

All solvents were redistilled. Vacuum liquid chromatography (VLC) was first used to fractionate the extracts using solvents of different polarities with hexane –chloroform (100:0, 0:100) and then chloroform- methanol (100:0, 0:100). Fractions were combined according to their TLC patterns. Size exclusion chromatography was performed using Sephadex LH-20 (particle size 25 – 100 µm) from Pharmacia Fine Chemicals, Sweden. TLC was performed using silica gel GHLF-250 µm plates from Analtech, Inc. The TLC plates were examined under ultraviolet (UV) light and developed with visualizing agents, usually 1% anisaldehyde in concentrated sulphuric acid followed by heating in an oven at 11°C for few minutes. The combined fractions were concentrated using a rotating evaporator at 40°C. These were then subjected to gradient elution column chromatography (CL) and preparative thin layer chromatography (PTLC) which led to the isolation of the pure compounds.

Infrared spectra were obtained with the sample in KBr using a Matson Galaxy Spectrometer. Nuclear magnetic resonance (NMR) spectra (¹H, ¹³C, COSY, HMBC, HMQC, and NOESY) were acquired on a Bruker AMX-400 instrument.

Plant collection

S. sparganophora leaf, stem and root were collected from Sagamu community in Sagamu local government area of Nigeria. The plant was authenticated by Mr. I. K. Idewo of the Forest Research Institute of Nigeria, Ibadan, with Voucher specimen (FHI 105358).

Extraction

The leaf of *S. sparganophora* was dried in an oven (40°C), powdered and 200 g leaf was extracted sequentially with solvents of increasing polarity, *n*- hexane, chloroform and methanol in a soxhlet apparatus and the extracts obtained were concentrated under reduced pressure (Rotavapor-R, Buchi) and weighed as shown in Table 1.

Isolation of luteolin

The methanol extract (28 g) obtained from sequential extraction with solvents of increasing polarity, *n*- hexane, chloroform and methanol was first subjected to vacuum liquid chromatography (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 fraction chloroform-methanol (9;1) was successively chromatographed on Sephadex LH-20 using the gel permeation and column chromatography with solvent system chloroform- methanol(8;2) as shown in Figure 1, The separation afforded 35 mg pure yellow crystal which gave a single yellow spot at R_f 0.4 on TLC analysis (45:8:9 toluene-methanol-acetic acid). The ¹³C-NMR spectrum (dimethylsulfoxide (DMSO-d₆) of this compound corresponded to the published spectrum of luteolin (Figure 2) and the ¹H-NMR spectrum (DMSO-d₆) assignments for it were also consistent with literature data (Markham and Geiger, 1993) with all shift values as enunciated in the compilation of flavone ¹³C NMR data prepared by (Harborne et al., 1975), which is often used for making assignments and confirming flavone structures. The I.R data showed a strong absorption at 3413 cm⁻¹ characteristic of the presence of the hydroxyl and at 1690 cm⁻¹ characteristic of the presence of carbonyl group.

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 vacuum liquid chromatography (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 which were formally chromatographed on Sephadex LH-20 using the gel permeation and then chromatographed on a column of silica gel (Merck,) packed in chloroform and eluted with solvent system chloroform-methanol(9;1) yielded the colourless oil (42.2 mg) as shown in Figure 1, The ¹³C-NMR spectrum and ¹H-NMR spectrum (pyridine-d₆), 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 (Figure 3) structure were consistent with the published data by (Kupchan et al., 1969; Ganijian et al., 1983) of vernodalin although this compound was first isolated from *Vernonia*

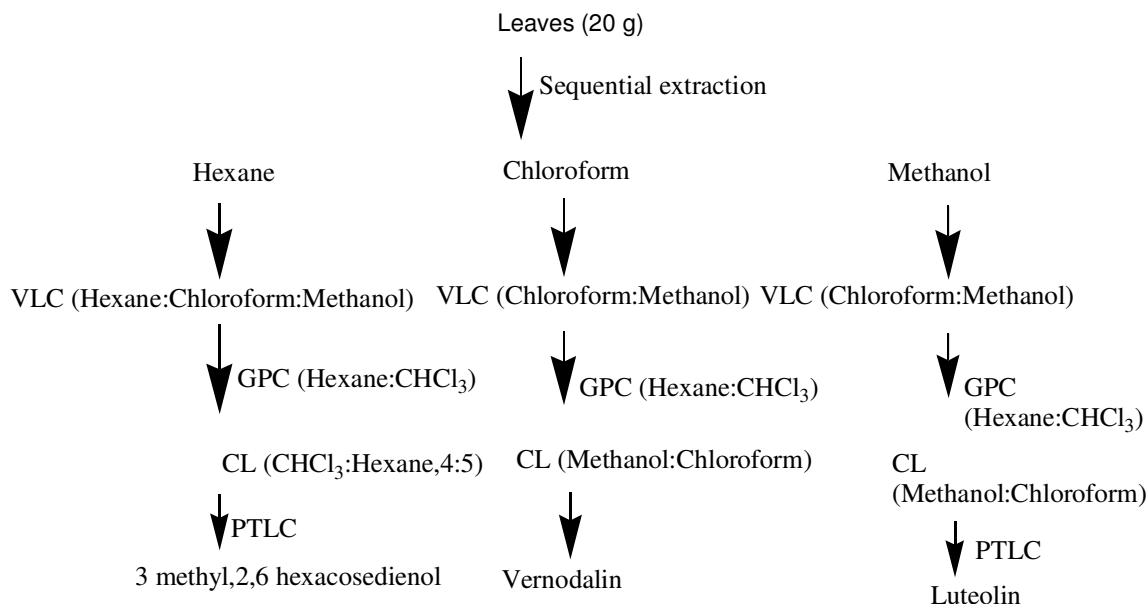


Figure 1. Isolation of luteolin, vernodalin and 3 methyl 2, 6, hexacosdienol. Gel permeation chromatography (GPC), vacuum liquid chromatography (VLC), column chromatography (CL), preparative thin layer chromatography (PTLC).

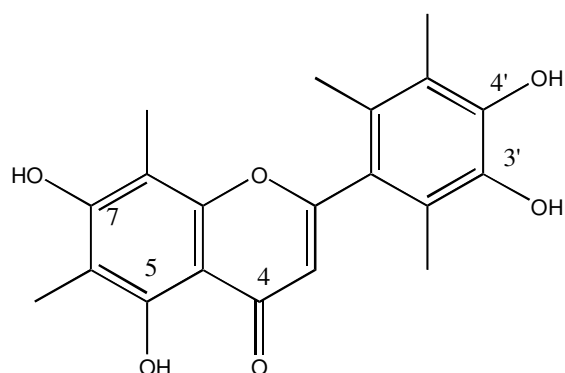


Figure 2. Luteolin

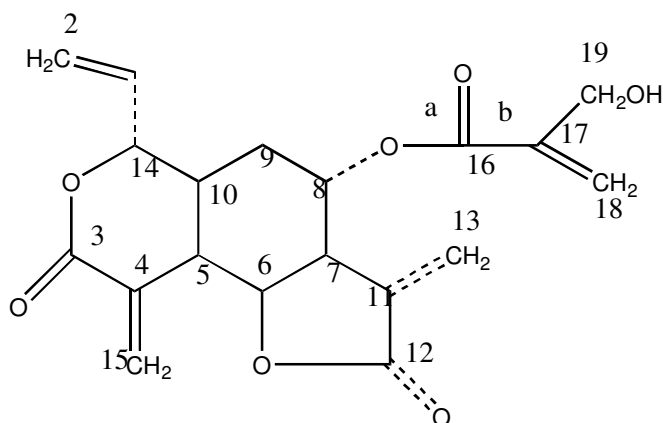


Figure 3. Vernodalin

amygdalina (Kupchan et al., 1969, Ganijian et al., 1983) but this is the first time to be isolated from *S. sparganophora*

Isolation of alcohol

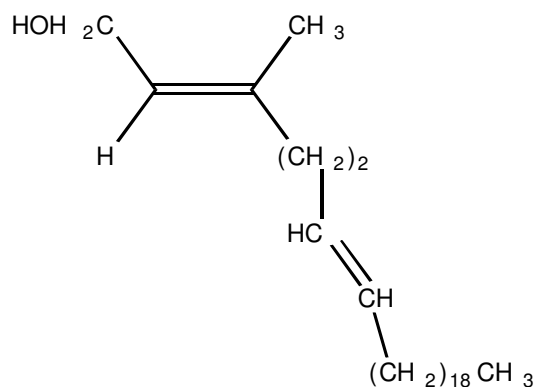
The 3 methyl, 2, 6 hexacosdienol (Figure 4) was isolated as shown in Figure 1. The hexane extract (5 g) was first subjected to vacuum liquid chromatography (VLC) with solvent of increasing polarity *n*-hexane-chloroform (100:0, 0:100) and TLC characteristics of the fractions were examined and identical fractions pooled together. The fractions which were formally chromatographed on Sephadex LH-20 using the gel permeation was rechromatographed 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 ¹³C 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 ¹H NMR spectrum (270MHz, Pyridine-d₆) showed signals at δ 5.78 (2H brs, J=5, 33 Hz, H-1); δ 4.51 (3H d, J=6, 33 Hz, H-3'); δ 3.9 (1H, s, H-2); δ 2-5 (4H, t, J=7.3, 7.2 Hz, 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 C₂₇H₅₂O and therefore partially identified as 3 methyl, 2, 6 hexacosdienol.

Antimicrobial susceptibility testing

The strains of bacteria used in this study were *Staphylococcus aureus* (NCTC 6571) and *Klebsiella aerogenes* (Welcome Res. Lab.CN 345), *Escherichia coli* (NCTC 9001) and *Proteus vulgaris* (NCTC 8313). The fungal strain used was *Candida albicans* (ATCC10231) and *Aspergillus niger* (NCPF3149)

Table 2. MIC values for isolated compounds from the leaf of *Struchium sparganophora* against some bacteria and two fungi in µg/ml.

	<i>S.aureus</i>	<i>E.coli</i>	<i>P. vulgaris</i>	<i>K. aerogenes</i>	<i>C.albicans</i>	<i>A. niger</i>
Dienol	100	50	100	50	50	50
Vernodalin	100	50	50	25	50	100
Luteolin	50	100	100	6.25	50	25
Solvent	>100	>100	>100	>100	>100	>100
Amoxycillin	0.195	1.625	50	1.6	N/A	N/A
Flucannazola	N/A	N/A	N/A	N/A	50	50

**Figure 4.** 3 methyl 2, 6, hexacosdienol.

Bacteria culture methods

All the bacteria were cultured on Nutrient agar (Oxoid) at 25°C in a suspension of 0.1% v/v peptone water (Oxoid). The bacteria were revived following incubation at 37°C for 24 h. The same method (as per bacteria cultures) was used for the yeast culture except that nutrient agar was replaced with Sabouraus/dextrose agar (Oxoid) and incubated for 72 h.

Standardisation of all inocula

The turbidity of all inocula were standardised by adjusting them with the addition of the appropriate sterile broth to match the 0.5 McFarland standard (10^8 CFU/mL) and diluted to 1:100 for the broth microdilution procedure. Mueller-Hinton broth (Oxoid) was used for the dilution of bacteria while Malt extract broth was used for the fungus. Appropriate controls were set up including, a negative control which, contained the compounds only and a positive control consisting of the organism and the anti-biotic amoxycillin (Sigma, UK) or the anti-fungal fluconazole (Pfizer, U.K); both dissolved in 20% (v/v) DMSO in peptone water. The tests were carried out on different days to avoid cross-contamination.

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 amoxicillin or fluconazole. The tests were carried out at different days to avoid cross-contamination and mix up of the test procedure.

MIC dilution assay

This involved microdilution titre technique as described by (Drummond and Waigh, 2000; Rahman et al., 2002).

RESULTS

Data and statistical analysis

Analyses of the data were performed using Student's t test. Values were considered to differ significantly if the *P* value was less than 0.05 or 0.001.

Discussion

The antimicrobial activities of the crude extract of this plant were carried out by Oboh (2006). The chemical investigation of the leaves of *S. sparganophora* by the application of VLC, CL and PTLC led to the isolation and characterization of three compounds. The isolated compounds were tested for antimicrobial activity using microdilution titre technique as described by (Drummond and Waigh, 2000; Rahman et al., 2002). The method is good due to its reproducibility of results. The MIC is the lowest concentration of the compound at which the microorganism tested does not demonstrate visible growth.

The MIC values result (Table 2) showed that all the compounds had activity against some of the test organisms with MIC values ranging from 100 µg/ml for Gram-positive organism *S. aureus* and 6.25 µg/ml for Gram-negative organism *K. aerogenes*, although these values were higher than the control amoxycillin with MIC values of 0.195 and 1.65 µg/ml for the two organisms respectively but luteolin and 3 methylhexacosdienol possessed a significant activity ($P < 0.05$) against *C. albicans* and *A. niger* in comparison to the control fluconazole with MIC value of 50 µg/ml for both organisms. Luteolin inhibitory activity against Gram-positive organism *S. aureus* is in conformity with the works of Yoichi et al. (2000), and Lv et al. (2009).

Also the minimal inhibitory concentration of vernodalin against *S. aureus* was 100 µg ml⁻¹ and this was in

conformity with the works of Reid et al. (2001), Vernodalinal also showed the same degree of antifungal activity with MIC value 100µg/ml on *A. niger*. The conjugated 3 methyl, 2, 6 hexacosadienol was effective against both the Gram- positive and Gram-negative organisms with MIC values of 100 and 50 µg/ml respectively. All these compounds seem to portend a good antimicrobial and antifungal remedy if subjected to structural modification to enhance their efficacy and these compounds might be responsible for the folkloric use of the leaves of this plant as anti infective agent.

Conclusions

These isolated compounds could be a possible source to obtain new and effective medicines to combat infections caused by strains of microorganisms from community as well as hospital settings when subjected to structural modification to enhance their efficacy. They could be used also as natural antimicrobial agents in food preservation.

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