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Chemical Composition and Antibacterial Activity of Essential Oils from *Struchium sparganophora* Linn. Ktze Asteraceae

Lateef S. Kasim*, Kafayat O. Olaleye, A. B. Fagbohun, S. F. Ibitoye, O. E. Adejumo

Department of Pharmaceutical and Medicinal Chemistry, Olabisi Onabanjo University, Sagamu, Nigeria
Email: *kasimls@yahoo.co.uk

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Abstract

***Struchium sparganophora* Linn. Ktze Asteraceae is a culinary herb that is consumed in the Western part of Nigeria and has wide medicinal uses in traditional medicine. The present study was carried out to determine the chemical composition of the oils from its leaf and stem and their antibacterial activity. Essential oils were collected from the leaf and stem of *Struchium sparganophora* by hydrodistillation and analysed by GC and GC-MS. The antimicrobial activity was tested against Gram negative (G − ve) and Gram positive (G + ve) microorganisms obtained from the Medical Microbiology Unit, University College Hospital (UCH), Ibadan, Nigeria. Forty-six compounds were identified in the leaf representing 95.3% of the total oil while fifty-five compounds were identified in the stem representing 93.5% of the oil. *β*-caryophyllene, Germacrene A, a-humulene and Germacrene D represented the major components in both oils. Antibacterial activity of the oils against certain strains of bacteria showed that the different concentrations of the oils (100 - 10,000 ppm) from the leaf had activity ranging from 9.0 ± 1.0 to 14.3 ± 2.55 mm while that from the stem had activity ranging from 18.5 ± 2.2 to 20.0 ± 0.0 mm for both G − ve and G + ve microorganisms respectively.**

Keywords

Essential Oils, *β*-Caryophyllene, Germacrene A, Antibacterial Activity

1. Introduction

Plants have been an indispensable part of human lives either as source of food, shelter, ecological conservation and most importantly avenue for the discovery and synthesis of drugs. The use of ethno-botanical information

*Corresponding author.

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about different plants have greatly enhanced plant research [1] and thus now provide the model for more than 50% of Western drugs [2]. Essential oils from plants have been in the forefront of tremendous breakthrough in the classification and production of products with therapeutic properties.

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 isolated from the plant *Artemisia annua*, sweet wormwood, a herb employed in Chinese traditional medicine and taxol from *Taxus brevifola* [3]. 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 afflower), medicine (chamomile) and many as an ornamental plant [4].

Struchium sprganophora is a culinary herb 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 [5]. The plant is an antidote for poisons [6]. In Gulf of Guinea, it is used as anti-malaria [7] while it also has anti measles activities [4]. The leaves posses nutritive, antioxidant, antimicrobial and the antimalaria activities [8] and also the anti-oxidant properties of its polar and non polar leaf extracts have been reported [9]. Phytochemical composition of the leaf and the isolation of sesquiterpine lactone, luteolin, vernodalin, 3 methyl, 2, 6 hexacosedienol from it have been reported [10]-[12].

The objective of this study was to determine the chemical composition of the essential oils from the leaf and stem of *Struchium sparganophora* and their antibacterial activity.

2. Materials and Methods.

2.1. Plant Material

Fresh aerial parts of *Struchiun sparganophora* were collected during full flowering season at the riverbed in Asejire, Oyo state. It was identified and authenticated at the Federal Research Institute of Nigeria (FRIN) by Mr. Micheal by comparing with Voucher specimens 105358 and a specimen kept.

2.2. Isolation of Essential Oil

Fresh leaves and stems 108 g and 270 g were weighed respectively and hydrodistilled using a Clevenger-type apparatus. The oils gotten were dried over anhydrous Sodium sulphate and stored in vials at low temperature until analysis.

2.3. GC Analysis

The essential oils were subjected to GC-FID analysis carried out on an Agilent model 6890 gas chromatograph fitted with a flame ionization detector. Fused silica capillary Agilent tech HS-5ms column (30 × 0.25 mm, 0.25 um film thickness) was used for the separation. Helium was used as carrier gas at flow rate of 1ml/min. GC oven temperature was programmed at 60˚C (held for 2 minutes) and raised to 240˚C at a rate of 4˚C/min with final hold time of 20 minutes. Injection and detection temperature was set at 200˚C and 250˚C respectively.

2.4. GC-MS Analysis

The GC-MS spectroscopy was performed on an Agilent model 6890 instrument with split/spilt less injection interfaced to a 5973 mass selective detector operated at 70eV with a mass range of m/z 50 - 500. Oven temperature was programmed from 60˚C - 230˚C at a rate of 3˚C/min with injector temperature 200˚C and detector temperature of 250˚C. The volume injected was 1 µL, split ratio 1:60; nitrogen was used as carrier gas at 1 mL/min.

2.5. Identification of Constituents

Identification of individual constituent of the oils was achieved based on their linear retention indices (LRI) after co-injection of the sample with homologous series of normal alkane by comparison of the masses (MS) with

those reported in NIST 05 and Wiley libraries and those published in literature [13].

2.6. Microorganisms and Media

2.6.1. Preparation of Test Organisms

The microorganisms used in this study were *Salmonella typhi*, *Bacillus cereus*, *Proteus mirabilis*, *Pseudomonas aeruginosa and Bacillus subtilis* (**Table 1**). They were obtained from the Medical Microbiology Unit, University College Hospital (UCH), Ibadan, Nigeria and were subsequently maintained as stock stains.

2.6.2. Preparation of Culture Media

Mueller Hinton agar (Becton Dickinson® M.D USA) was used for direct sensitivity testing for the bacteria while sabouraud extract broth was used for the fungus. 16g of powder was dissolved in 400 ml distilled water and shaken until it was evenly dispersed. It was then sterilized in the autoclave at 121˚C for 15 minutes. 60 g sabouraud dextrose agar powder was mixed with 1 litre distilled water until evenly dispersed. It was then heated up with repeated stirring and boiling for complete dissolution 0.20 ml solution was then transferred into universal bottles while hot and then sterilized in the autoclave at 121˚C for 15 minutes. Nutrient broth was prepared thus: 13 g of powder was dissolved in 1 liter of distilled water and dispersed. Then 5 ml of the solution was transferred into bottles and sterilized at 121˚C for 15 minutes.

2.6.3. Agar-Well Diffusion Assay

Agar-well diffusion method by Durodola, 1977 [14] and Cheruiyot *et al.*, 2009 [15] was used for this microbiological assay.

A concentrations of (100 - 10.000) ppm of the essential oils was prepared from the stock solution. Cultures of *Salmonella typhi*, *Bacillus cereus*, *Proteus mirabilis*, *Pseudomonas aeruginosa and Bacillus subtilis* were inoculated separately on the surface of Mueller Hinton agar plates by surface spreading using a sterile cotton swab and each bacterium evenly spread over the entire surface of agar plate to obtain a uniform inoculum. The sensitivity testing of the oils was done using the agar well diffusion method [16] whereby, wells of 6 mm diameter and 5 mm depth were made on the solid agar using a sterile glass borer and Gentamicin (Sigma, UK) (10 µg/ml) in distilled water was used as a positive control. Distilled water was used as negative control. All the tests were ran in triplicates for quality results. The set up was incubated for 24 hr at 37˚C for anti-microbial test and the zones of inhibition were measured using a ruler (AIM®) and a pair of divider, and then results reported in millimeters (mm). The inhibition was measured as a basis for activity [17].

3. Results and Discussions

Hydrodistillation of essential oil from leaf and stem of *Struchium sparganophora* gave a colourless liquid with characteristic smell of a yield of 0.15 ± 0.0% w/w and 0.08 ± 0.01% w/w respectively

The GC and GC/MS analysis of these oils resulted in the identification of 46 compounds in the leaf representing 95.3% of the oils while 55 compounds were identified in the stem representing 93.5% of the total oil as shown in **Table 2**.

Major constituents of the essential oil from the leaf were *β*-caryophyllene (44.5%), humulene (5.6%), Caryophyllene oxide (4.2%), Germacrene A (3.4%) and Germacrene D (2.9%) indicating that sesquiteterpene hydrocarbon represented 61.2% of total oil.

In the stem, the major compounds were *β*-caryophyllene (26.1%), Caryophyllene oxide (4.9%), Humulene (4.2%), and Selinene (2.4%). This also indicated that sesquiterpene hydrocarbon accounted for 37.1% of its total oil. There was appreciable presence of methylsalicylate in both oils.

Oils from the leaf of same family Astereacea showed similarity in some type of compounds present in them but at different concentrations e.g. *Vernonia scorpiodes*, *Vernonia migeodi and Vernonia perrotetti* had *β*-caryophyllene and Germacrene D as part of their major constituents [18]. Comparing the oils from *Struchium sparganophora* with those from some members in the Asteraceae family showed they have at least two of these compounds in common. Germacrene D, *β*-caryophyllene and 1,8-cineole [19]-[21].

Table 3 and **Table 4** showed the result of the antibacteria assay. The essential oil from the leaf had activity against the strains as indicated by their inhibition zones. The essential oil from the stem had the highest activity

Table 1. List of the test organisms used.

Microorganism	Description
Salmonella typhi	UCH NO12 Gram negative bacilli
Bacillus cereus	UCH PO13 Gram positive cocci
Proteus mirabilis	UCH NO13 Gram negative bacilli
Pseudomonas aeruginosa	UCH NO14 Gram negative bacilli
Bacillus subtilis	UCH FO11

Table 2. Chemical composition of the leaf and stem essential oils of *S. sparganophora*.

		%Composition	
Retentive index (RI)	Compound	Leaf	Stem
865	(E)-2-Hexanal	2.3	3
868	1-Hexanol	nil	tr
900	n-Nonane	nil	1.4
902	Heptanal	nil	0.4
926	Tricyclene	0.3	nil
939	α-Pinene	nil	0.4
953	Camphene	nil	tr
976	sabinene	0.3	1.1
980	β-pinene	2.5	0.6
985	6-Methyl-5-hepten-2-one	nil	tr
991	Mycrene	0.5	tr
999	Decane	0.7	nil
1003	Octanal	tr	tr
1007	E-3-hexanal acetate	0.7	0.4
1008	1-Hexyl acetete	0.2	nil
1011	p-3-Carene	nil	tr
1026	Pseudocumene	0.3	nil
1028	p-Cymene	tr	tr
1031	Limonene	1	0.9
1035	1,8-Cineole	1.9	0.7
1060	trans-decahydronaphthalene	nil	tr
1100	n-Undecane	0.3	1.2
1103	Nonanol	1.1	1
1106	E-2-nonenal	tr	1
1181	Naphtalene	tr	tr
1190	Methylsalicylate	12.3	31.7
1199	n-Dodecane	0.4	0.6
1206	Decanal	o.4	0.6
1299	n-Tridecane	tr	tr
1306	Undecane	tr	tr
1316	(E,E)-2-4-decadienal	nil	tr
1376	α-Coperene	0.4	tr
1385	Geranyl acetate	nil	tr
1391	β-Elemene	0.7	0.6
1399	Cyperene	nil	tr
1400	n-Tetradecane	0.7	1.3
1405	Z-Caryophyllene	nil	tr

Continued

1408	Dodecanal	nil	tr
1419	*β*-Caryophyllene	44.5	26.1
1428	E-*α*-lonone	0.3	tr
1439	Trans-*α*-Bergamotene	nil	tr
1455	*α*-humulene	5.6	4.2
1461	Alloaromadendrene	0.7	tr
1475	*β*-Chamigrene	tr	0.4
1480	Germacrene D	2.9	1
1485	*β*-Selinene	1	2.4
1487	(E)-*β*-ionone	1.2	nil
1492	Valencene	nil	tr
1496	*α*-Selinene	0.3	tr
1500	n-Pentadecane	0.4	0.8
1504	Germacrene-A	3.4	1.7
1508	(E,E)-*α*-farnesene	nil	tr
1509	*β*-Bisabolene	1.7	nil
1513	Tridecanal	nil	0.8
1524	d-Cadinene	tr	tr
1565	Trans-nerolidol	tr	0.7
1582	Caryophyllene oxide	4.2	4.9
1600	n-Hexadecane	0.4	1.6
1608	Humulene epoxide 11	tr	nil
1652	Bisabolol 11	nil	0.4
1700	n-Heptadecane	0.3	0.4
1719	Pentadedecanal	2.1	nil
1800	n-Octadecane	tr	0.8
1848	Hexahydro-farnesylactone	tr	nil
1900	n-Nonadecane	nil	0.9

tr = traces

Table 3. Antibacterial activity of essential oil from the leaf after 24 hours (mm ± SD).

	Essential oil (ppm)			Gentamicine
Microorganism	10000	1000	100	10 µg/ml
Salmonella typhi	13.3 ± 2.52	11.7 ± 2.52	10.3 ± 0.82	11.2 ± 3.0
P. aeruginosa	11.7 ± 3.06	8.0 ± 2.0	nil	10.8 ± 2.0
Proteus mirabilis	14.3 ± 2.55	10.5 ± 0.71	10.0 ± 0.0	10.0 ± 0.0
Bacillus cereus	11.5 ± 2.12	10.5 ± 0.71	nil	10.8 ± 2.0
Bacillus subtilis	13.0 ± 0.00	10.0 ± 0.0	nil	10.8 ± 2.0

Table 4. Antibacterial activity of essential oil from the stem after 24 hours (mm ± SD).

	Essential oil (ppm)			Gentamicin
Microorganism	10000	1000	100	10 µg/ml
Salmonella typhi	20.00 ± 0.0	18.5 ± 2.12	nil	16.8 ± 2.0
P. aeruginosa	18.5 ± 2.12	18.0 ± 0.6	nil	15.8 ± 2.0
Proteus mirabilis	nil	nil	nil	10.0 ± 0.0
Bacillus cereus	nil	nil	nil	10.8 ± 2.0
Bacillus subtilis	nil	nil	nil	10.8 ± 2.0

against *Salmonella typhi* and *Peseudomonas aeruginosa* but had no activity against *Bacillus subtilis.* The activities of both oils were higher than that of the standard Gentamycin.

Asteraceae generally have antibacterial activity [22] [23]. The activity of essential oil is related to the composition of the oil [24], functional group and period of collection of the leaves [22].

The antibacterial activity exhibited by the essential oil from *Struchium sparganophora* could be attributed to the presence of *β*-caryophyllene, Germacrene D, a-humulene, Caryophyllene oxide and 1,8-cineole which had been confirmed to have antibacterial activity [25] [26].

Components are listed in order of their elution from RTX-5 column. Linear retention indices (LRI) were calculated using a homologous series C_6-C_{28} n-alkanes. Percentage was obtained by FID peak area normalization without the use of response factor values represent the average of three measurements, n = 3. The masses (MS) were gotten by comparison of the MS with those of the computer mass libraries NIST 05 library, Wiley and Adams [13].

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