Bioscience Research Communications
Vol. 14, No. 2, April 30, 2002
Printed in Nigeria

0795-8072/2002 $6.00 + 0.00

BRC 2001028/14215

Phytochemical and *in-vitro* antibacterial potential of *Struchium spargnophora* (Linn). (Compositae)

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(Received February 19, 2001)

ABSTRACT: The petroleum ether, chloroform, methanol and water extracts of the whole plant of *Struchium sparganophora* [Linn.] were screened phytochemically for the presence of secondary metabolites and for *in-vitro* antibacterial activity. The Phytochemical screenings showed the presence of alkaloids, cardiac glycosides, traces of saponins, flavonoids and hydrolysable tannins but absence of anthraquinone glycosides. Extracts of the plant material were screened for antibacterial properties using seven Gram positive and eight Gram – negative human pathogenic bacteria. All the crude extracts except aqueous extract at a concentration of 100mg/ml showed various degree but broad-spectrum activity against most Gram-positive and Gram-negative bacteria.

Of the four extracting solvents, the chloroform extracts gave the highest level of anti bacterial potency followed by the methanol extract and then petroleum ether extract in the agar cup diffusion technique. The aqueous extract showed no activity against all the tested organisms. Some ampicillin resistant strains of *Staphylococcus aureus* were sensitive to some of the extracts. Ampicillin, gentamicin and streptomycin were used as standard reference drug. Diameters of zones of inhibition were in the range of 10 – 25 mm for the extracts and drug. The antimicrobial activities observed are discussed in relation to the chemical constituents reportedly isolated from this plant and the traditional use.

Key words: Medicinal plants; *Struchium sparganophora*, Phytochemical; Antibacterial activities.

Introduction

The family *compositae* is a large family of the spermatophyte with over 20,000 species, which are mainly herbaceous. One of the genus in the family is *Struchium* and it is widely distributed pan-tropically (Burkill, 1935). *Struchium sparganophora* is a semi-succulent perennial herb, decumbent or erect to a height of 10 feet and usually found across damp sites. The leaves are evergreen, opposite, ovate to lanceolate leaflets with acute apex and entire margin. The common local names are "Ewuro odo" in Yorubaland and "Nti mgbada" in Igboland. It is very edible and is eaten by the Yoruba tribe in Nigeria as a potherb (Dalziel, 1937). Cattle in Java are also said to graze on it (Burkill, 1935; Dalziel, 1937). Decoctions of stems and leaves are used for headaches in Nigeria (Ainsle, 1937) and in Ghana (Dalziel, 1937; Irvine, 1930). The leaves, are mixed with the powdered seed of *Monodora myristica* (*Annonaceae*) as headache cure in Gabon (Walker, 1953). The leaf sap is instilled into the eye in cote d'voire for vertigo and fainting (Bouquet and Debray, 1974, Kerharo and Bouquet, 1950). A preparation is taken for

dysentery, diarrhoea and guinea-worm infection. The decoction is drunk at child birth to ease delivery, and is prescribed for sterility (Bouquet and Debray, 1974). A decoction is drunk in Nigeria for gonorrhea and as an antidote against serious poison (Ainslie, 1937).

The plant has been reported to contain sesquiterpene lactones, caffeic acid esters, methylated flavonoids and fatty acid amide. Plant material from Congo, when assayed for alkaloids has been reported to contain 0.1 – 0.3% of the whole plants (Dalziel, 1937). Pharmacologically, the leave of the plant has been reported to have diuretic properties attributed to the caffeic acid esters content.

Information on *in-vitro* anti-bacterial activity of *Struchium sparganophora* is not available in literature hence this study is aimed at evaluating the antibacterial potentials of this plant and to scientifically justify its relevance in traditional medicine.

Materials and Methods

The whole plants of *Struchium sparganophora* were collected from Onigambari forest reserve in Oyo State of Nigeria during rainy season. Mr. Gabriel Ibhanessebor of the Forestry Research Institute of Nigeria (FRIN), Ibadan, did the authentication. The herbarium voucher number of the plant is F.H.I. 105809. The whole plant was sun-dried and pulverized.

Preparation of Plant Extracts:

Coarsely powdered plant sample weighing 100g were successively extracted using soxhlet with petroleum ether (60-80°C), chloroform, methanol and water in succession for 12 hours each. The different extracts were filtered and concentrated *in-vacuo* and each stored at 40°C until needed for analysis. Each extract was separately resuspended in 50% methanol to a concentration of 100mg/ml for the antibacterial assay.

Organisms:

The organisms used in this study consisted of 7 Gram-positive bacteria (*Staphylococcus aureus* NCTC 6571, *S. aureus* (Oxford strain), *Corynebacterium pseudot, Bacillus subtilis, B. megatarium, Micrococcus luteus* ATCC 9341 and *Myco faecalis* ATCC 114506) and 8 Gram-negative bacteria (*Escherichia coli, Actinomadura madurae* ATCC 19425, *Neisseria gonorrhoae* ATCC 19424, *N. meningitides* ATCC 13077, *Klebsiella pneumoniae* ATCC 10031, *Klebsiella sp., Salmonella typhi* ATCC 6539 and *S. galinarium*).

Media

Media employed in the study include eugon broth pH 7.0±0.2 and eugon agar pH 7.0±0.2 for the America Type Collection Culture bacterial strain, and nutrient broth and nutrient agar pH 7.3±0.2 for other bacteria. All media are products of Difco Laboratories, USA.

Antimicrobial Agents

The following chemotherapeutic agents were included in the test as positive controls, Gentamicin sulphate 10µg/ml (Formulations Plc, England); Ampicillin 2.5µg/ml (SmithKline Beecham Pharmaceutical Brentford, England), and Streptomycin sulphate 10µg/ml (Antibiotics S. A-IASI Romania), 50% methanol was used as negative control.

Phytochemical Screening:

The qualitative chemical analysis of the powdered sample was carried out for the presence of various secondary metabolites such as alkaloids, anthraquinone, saponins, cardiac glycosides, cyanogenetic glycosides, flavonoids, steroidal nucleus and tannins using the method adopted in similar surveys (Harborne, 1991) as stated below:

(i) *Alkaloids*: Powdered plants material with 10% acetic acid in ethanol, leaving to stand for at least 4 hour. The extracts were concentrated to one-quarter of the original volume and the alkaloids were precipitated by dropwise addition of conc. NH_4OH. This was collected by centrifugation and washed with 1% NH_4OH. Residues were then dissolved in a few drops of chloroform or chloroform-ethanol. The concentrates were then tested for alkaloids with Dragendorff reagent.

(ii) *Anthraquinones*: Powdered plants material was boiled with 10% HCl for a few minutes, filtered and allowed to cool. This was then partitioned against equal volumes of chloroform. Formation of a rose-pink color in the aqueous layer on addition of 10% ammonia solution indicated the presence of combined anthraquinones.

(iii) *Saponins*: This was determined by observing the formation of persistent foams during plant extraction and concentration of plant extracts. The presence of saponins was confirm by the ability of the extracts to haemolyse red blood cells.

(iv) *Cardiac glycosides*: Samples was extracted with 10ml. Of 80% methanol for 5 minutes on a steam bath, filtered and diluted with an equal volume of distilled water. A few drops of lead acetate solution were added, shaken and filtered after a while. Filtrate was then extracted with methylene chloride (two times) and was evaporated to dryness on a steam bath. Then about 1ml. Of 2% 3,5-dinitrobenzoic acid in ethanol was added to the residue and the solution was made alkaline with 5% NaOH. The formation of a brownishpurple color is indicative of the presence of unsaturated lactones.

(v) *Cyanogenetic glycosides*: About 1g of powdered sample was boiled with distilled water and moist sodium picrate paper held inside the tube with a cork. A color change from yellow to brick-red of the picrate paper is positive for cyanogenetic glycosides.

(vi) *Steroidal nucleus*: A few grams of the powdered material were heated over a Bunsen flame with 10% HCl and $FeCl_3$ for 15 minutes, after which it was filtered and cooled. Then extracted with 2 volumes of $CHCl_3$. The organic phase was concentrated to a small volume and acidified with 5ml, acetic anhydride. The formation of a reddish-brown ring at the interface on carefully pouring conc. H_2SO_4, is indicative of the presence of steroidal nucleus.

(vii) *Tannins*: Plant material was boiled with water for a few minutes. This was filtered and diluted with more water. Bluish-black color formation on addition of a few drops of ferric chloride, is indicative of the presence of tannins.

Determination of Antimicrobial Activity:

The eugon agar plates and nutrient agars plates were each seeded with 0.6ml of a 1:100 dilution of an overnight culture of each ATCC strains and other bacterial isolate respectively. The seeded plates were allowed to dry in the incubator at 37°C for 20 minutes. A standard cork borer of 8mm diameter was used to make equidistant and uniform wells on the surface of the agar and into different wells were placed 60µl of the different extracts re-suspended in 50% methanol at a final concentration of 100mg/ml. The plates were incubated at 37°C for 24 hours after which diameter of zones of inhibition were measured. Since each of the extracts were reconstituted in 50% methanol before being tested, 50%methal was included in each plate as a solvent control besides the chemotherapeutic agents included as positive controls. This method is similar to previous procedures (Kavanagh, 1977). The antimicrobial studies were done in triplicates and diameters of zones of inhibition (mm) are expressed as means and standard errors on means. Student's "T" test was used to test probability at $P<0.05$.

Results

The color and percentage yield of the petroleum ether, chloroform, methanol and aqueous extract of the whole plant of *Struchium sparganophora* are presented in Table 1. The phytochemical screening (Table 2) revealed that the various secondary metabolites present are alkaloids, cardiac glycosides, flavonoids and traces of saponin glycosides and hydrolysable tannins while anthraquinone glycosides and essential oils are absent. All the extracts except the aqueous extract at concentration of 100mg/ml showed various range of antibacterial activity (Table 3) but no activity was observed when lower concentrations of 10mg/ml and 20mg/ml were first tested. The chloroform and methanol extracts had some activity on some of the micro-organmisms resistant to ampicillin and gentamicin. The chloroform extract showed the greatest antibacterial activity. Generally, the order of susceptibility of the bacterial to the extracts is *Actinomadura madurae* > *S. aureus*(oxford strain) > *B. subtilis* > *S. aureus* NCTC 6571 > *Klebsiella sp.* > *Salmonella galinarium* > *E. coli* = *B. megaterium* > *Neisseria meningitis* > *S. typhi* > *N. gonorrhoea*. There is no activity at all on *Myco feacalis* and *K. pneumoneae*. The methanol extract also showed an appreciable level of antibacterial activity. The least antibacterial activity was witnessed in the aqueous extract.

Table 1: Characteristic of Crude Extracts of *Struchium sparganophora*

Extracting solvet	Morphological part	Colour	Percentage yield(w/w)
Petroleum ether	Whole plant	Dark shinning mass	1.14
Chloroform	Whole plant	Dark green	0.56
Methanol	Whole plant	Blackish brown	2.05
Water	Whole plant	Dark brown	1.19

Table 2: Phytochemical screening of *Struchium sparganophora*

Phytochemical compound	Observation
Alkaloids	+++
Anthraquionone glycoside	-
Tannins	+
Cardiac glycosides	++
Saponin glycosides	++
Flavonoids	+++
Essential oil	-

Key:
+++ = High concentration; ++ = Medium concentration; + = Low concentration;
- = Absent.

Table 3: Antimicrobial Activity Crude Extract of *Struchium sparganophora* Diameter of Zone of Inhibition (mm)

Test Microorganism	SSP	SSC	SSM	SSA	GN	Amp	Str	M
Staphylocossus aureus NCTC 6571	R	21±0.1	NT	R	10±0.2	R	NT	R
Staphylococcus aureus (Oxford strain)	R	23±0.3	16±0.3	R	12±0.3	R	17±0.4	R
Corynebacterium pseudot	12±0.2	R	15±0.2	R	R	R	21±0.3	R
Bacillus subtilis	10±0.2	22±0.3	11±0.3	R	15±0.2	15±0.2	19±0.3	R
Bacillus megaterium	12±0.3R	15±0.2	14±0.2	R	R	12±0.3	19±0.4	R
Myco faecalis ATCC 114506	R	12±0.4	R	R	R	R	R	R
Micrococcus luteus ATCC 9341	R	R	R	R	R	R	22±0.2	R
Actinomadura madurae ATCC 19425	R	25±0.4	15±0.2	R	R	R	16±0.3	R
Klebsiella sp.	R	18±0.3	12±0.3	R	R	R	21±0.2	R
Salmonella galinarium	R	18±0.4	R	R	R	R	18±0.2	R
Escherichia coli	15±0.2	15±0.4	11±0.2	R	R	R	17±0.1	R
Neiseria gonorrhoea ATCC 19424	R	11±0.7	R	R	R	R	18±0.4	R
Neiseria meningitides ATCC 13077	R	13±0.3	22±0.6	R	R	R	14±0.4	R
Salmonella typhi ATCC 6539	R	12±0.2	R	R	R	R	18±0.3	R
Klebsiella pneumoniae ATCC 10031	R	R	R	R	R	R	R	R

SSP *Struchium Sparganophora* petroleum ether extract;
SSC *Struchium Sparganophora* chloroform extract;
SSM *Struchim Sparganophora* methanol extract;
SSA *Struchium Sparganophora* aqueous extract;
Gn Gentamicin (10µg/ml); Amp – Ampicillin (25µg/ml); Str – Streptomycin (10µg/ml)
M Methanol (50%); NT – Not tested; R – Resistant.

Discussion

The presence of alkaloids, cardiac glycoside, flavonoids and saponin glycosides was noted to various extends in the whole plant material screened for secondary metabolites. This is consistent with previous reports on plants of the compositae family (Burkill, 1985; Adeniyi and Odufowoke, 2000). Anthraquinone glycosides and tannins are not detected in the present studies (Table 2). The result of antibacterial studies as shown in Table 3 revealed that all the extracts except the aqueous extract ehbiited appreciable antibacterial properties, inhibiting the growth of most of the bacterial at 100mg/ml. The chloroform extract was most active, followed by the methanol and then petroleum ether extract. The susceptibility of the two species of *Neisseria* tested to these extracts is of particular significant. *Neisseria gonorrhoea* is the causative agent for the sexually transmitted disease gonorhoea while *N. meningitis* is noted for causing meningitis (Sleigh and Timbury, 1981). The antibacterial activity is broad-spectrum as both the Gram-positive and Gram-negative bacteria were sensitive to the extracts. Some of these bacterial have been implicated in disease such as dysentery, wound sepsis, diarrhoea, upper and lower respiratory tract infections, urinary tract infections, meningitis, typhoid fever etc. The susceptibility of ampicillin resistant strains of *Staphylococcus* and *Bacillus* to the extract shows that it has a potent antibacterial activity. The antibacterial activity may be due to the presence of some active compounds like flavonoids in the plant. Mori *et al* in 1987 reported the antibacterial activity and mode of action of plant flavonoids against some strains of *Proteus vulgaris* and *Staphylococcus aureus*.

In conclusion, all extracts have displayed antibacterial activities. The antibacterial activities observed from the extracts thus justify some of the ethnopharmacological claims about the plant in the treatment of dysentery, diarrhoea and guinea worm infection (Bouquet and Debray, 1970; Ainsle, 1937; Burkill, 1985 and Irvine, 1961). In view of the recent increase in bacterial developing resistant to the most commonly antibiotics most notable by R-plasmid DNA acquisition, there is an urgent need for development of potent, non-toxic, new antibiotics, more importantly from natural sources. The plant used in this study provides good opportunities for drug development in this area. In this regard, the chloroform extracts been the most active was analyzed by thin layer chromatography (silica gel; F254) whereby four main compounds were detected, one of them an alkaloid (Drangendoff positive). Study is however in progress for further purification by bioactivity, characterize and identify the specific active compounds in this plant responsible for its antibacterial activity.

ACKNOWLEDGEMENT: This work was supported by the International Foundation for Science Grant: F2884-1 from Sweden given to B.A. Adeniyi.

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