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Antioxidant and antibacterial constituents of *Steganotaenia araliacea* stem bark

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Abstract

Steganotaenia araliacea Hochst (Apiaceae/ Umbelliferae) is a small tree used in traditional medicine especially in tropical Africa and savannah where it is mostly found. It is used in ethnomedicine to treat pneumonia, asthma, peptic ulcer, sore throat, fever, as a diuretic agent and other diseases of microbial origin. The antioxidant and anti-bacterial activities of the hexane, dichloromethane and aqueous extracts obtained from the stem bark of *Steganotaenia araliacea* were evaluated in terms of capacity to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical and to inhibit the growth of typed microorganisms used (namely *Staphylococcus aureus*, *Salmonella typhi* and *E. coli*). The hexane and dichloromethane extracts showed significant antioxidant activity, expressed as IC_{50}, 5.62µg/mL and 1.26µg/mL respectively. The antioxidant potential of the extracts, expressed as IC_{50}, ranged between 1.26 µg/mL and 5.62 µg/mL. (Ascorbic acid, gallic acid and rutin used as standard had 2.79 µM, 1.78 µM and 10.0 µM respectively). In the test for antibacterial activity, dichloromethane extract showed the highest inhibitory activity followed by hexane extract, especially against, *Salmonella typhi*. GC-MS analysis of the hexane and dichloromethane was carried out to determine their chemical constituents. Results revealed that both extracts contained similar compounds (including cumene, xylene, citronellol and long chain hydrocarbons). In addition the dichloromethane extract contains cadinanol, *ar*-curcumene and α-guaien, not found in hexane extract. These compounds may be contributing to the observed antioxidant activity of the extract.

Keywords: *Steganotaenia araliacea*; Antimicrobial; Antioxidant; GC-MS; Cadinanol; Citronellol

INTRODUCTION

Steganotaenia araliacea Hochst (Synonyms: *Peucedanum araliaceum* Benth. & Hook f.; *P. fraxinifolium* Hiern), commonly called carrot tree, belongs to the family Apiaceae / Umbelliferae. A decoction of the leaves is used in the management of diabetes mellitus in Kenya (Keter *et al*., 2012). The root is used to treat: snakebites in India (Selvanayahgam *et al.,* 1994); menstrual problems, abdominal pains, malaria and snakebites in Tanzania (Chhabra *et al.,* 1993); bilharzia, sore throat and swellings caused by allergies in East Africa. It is used in multicomponent prescriptions to treat heart palpitations, severe abdominal pains and gonorrhoea (Hedberg *et al*., 1983). The whole root as well as the root bark is used in the management of HIV/AIDS in Uganda (Lamorde *et al*., 2010). Its infusion is used in Uganda for the treatment of theileriosis (East Coast fever). The disease is endemic to east and southern Africa, caused by the protozoan *Theileria parva*, and transmitted by tick

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(*Rhipicephalus appendiculatus*) in cattle (Tabuti *et al*., 2003). The bark is used to treat: asthma by the Zigula and Sukuma people of South Africa (Watt and Breyer-Brandwijk, 1962); leukaemia and malaria in Tanzania (Chhabra *et al.,* 1993; Gessler *et al.,* 1995); rheumatism by rubbing the ash into scarifications, dysentery and flatulence by taking a decoction mixed with milk in East Africa (Hedberg *et al*., 1983). The water extract of the leaf is used to treat: gonorrhoea, sore eyes and sore throat in Zimbabwe (Wild and Gelfand, 1959; Gelfand *et al.,* 1985); convulsions in Gambia (Irvine, 1961). The whole plant is used to treat lung and liver diseases of cows in East Africa. It is also reputed to cause abortion in goats (Watt and Breyer-Brandwijk, 1962). The combination of the whole plant and *Securidaca longepedunculata* is used for the treatment of Ascariasis in Uganda (Hamill *et al*., 2002). The central core of the root, when wrapped around the penis is claimed to increase the size of the latter (Buchanan, 1975). Pharmacognostic/ phytochemical screening of the plant revealed the presence of tannins, resins, flavonoids and saponins while alkaloids and anthraquinones were not found (Mohammed *et al*., 1999). The ethanol extract and chromatography fractions have been shown to possess antiviral (Beuscher *et al.,* 1994) and cytotoxic properties (Taafrout *et al.,* 1983a). The leaves and bark were also shown to have molluscicidal activity (Kupchan *et al.,* 1973; Kloos *et al.,* 1987). The leaf extracts demonstrated insect repellent/ antifeedant properties (Abubakar *et al.,* 2001). The powdered leaves showed a potent pesticidal activity against *Sitophilus zeamais* (Nukenine *et al*., 2007). The combination of the leaves and stem bark demonstrated an effective anthelmintic activity against Schistosomiasis (Molgaard *et al*., 2001) The 70% ethanol extract of the fresh root bark was however inactive in antibacterial tests against *B. subtilis* and *E. coli* at 100µg/mL. It was also inactive in antiviral (against rhinovirus Type 2), antifungal (*Penicillium crustosum*), and anti-yeast (*Saccharomyces cerevisiae*) test systems (Taniguchi *et al.,* 1978). Some essential oils were identified from the powdered leaves which include: α-pinene, β-caryophyllene, germacrene D, β-pinene, limonene, α-cubebene, α-copaene, cis-muuroladien, trans-α-bergamotene, α-guaien, α-caryophyllene, δ-cadinene, ar-curcumene, cis-calamene and caryophyllene (Nukenine *et al*., 2007). Several lignans have been isolated from the stem bark and entire plant. These include: araliangine (Taafrout *et al*., 1983a), neoisostegane (Hicks and Sneden, 1983; Taafrout *et al*., 1983b; 1984b), prestegane A (Taafrout *et al*., 1983c), prestegane B (Taafrout *et al*., 1984a), steganacin, steganangin (Kupchan *et al.,* 1973), steganol (Wickramaratne *et al.,* 1993), steganolide A (Taafrout *et al*., 1986), steganolides B, steganolide C (Robin *et al.,* 1986), steganone (Hughes and Raphael, 1976) and 10-demethoxystegane (Meragelman *et al.,* 2001). Also, triterpenoid glycosides (saponins) have been isolated from the leaves. These include: glycosides of barrigenol R1 (now known as barringtogenol C) and steganogenin with glucose, galactose and rhamnose in their sugar portions (Lavaud *et al.,* 1992). The specific saponins are: 21-*O*-angeolyl-3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-xylopyranos yl-(1→3)-β-D-glucuronopyranosyl] barringtoge nol; 21-*O*-tigloyl-3-*O*-[β-D-galactopyranos yl-(1→2)-β-D-galactopyranosyl-(1→3)-β-D-gluc uronopyranosyl] barringtogenol; 21-*O*-tiglo yl-3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-xylo pyranosyl-(1→3)-β-D-glucuronopyranosyl] barringtogenol; 3-*O*-[β-D-galactopyranos yl-(1→2)-β-D-glucuronopyranosyl]-28-*O*-β-D-glucopyranosyl-olean-12-en-28-oic acid and 3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucopy ranosyl-(1→3)-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl].

The present study aimed at investigating the antioxidant and antibacterial activities of the extracts from the stem bark of *S. araliacea* with a view to justifying some of its claimed uses.

EXPERIMENTAL

Plant material: The stem bark of *S. araliacea* was collected from mature trees in Jos, Nigeria between December and March. The plant was duly authenticated at the Federal College of Forestry Jos, as *Steganotaenia araliacea* and voucher specimens were deposited in the institution's herbarium. The stem bark was air-dried for a period of two weeks under a shade and the dried sample was pulverized.

Extraction: Air-dried and pulverized stem bark of *S. araliacea* was extracted by percolation with n-hexane, dichloromethane, and water in succession for 24 h to give the hexane, dichloromethane, and aqueous extracts, respectively. The extracts were evaporated to dryness and stored in a desiccator.

Antioxidant Screening: Qualitative assay: Reduction of DPPH (1,1-diphenyl-2-picrylhydrazyl) radical [molecular formula $C_{18}H_{12}N_5O_6$, mw=394]. TLC autographic assay: after developing and drying, TLC plates (with amounts of sample ranging from 0.1 to 100µg) were sprayed with 0.2% $^{w}/_{v}$ of DPPH solution in methanol. The plates were examined 30 min after spraying. Active extracts appeared as yellow spots against a purple background (Chacha *et al*., 2005). The samples were done in triplicate and the mean value of the three was recorded.

Quantitative assay: The antioxidant activity (free radical scavenging activity) of hexane, dichloromethane and aqueous extracts on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined according to the method described in (Brand-Williams, *et al*., 1995) with slight modifications. 12.5mg of each extract was dissolved in methanol using 25mL volumetric flask. The following concentrations of hexane, dichloromethane and aqueous extracts were prepared 500, 250, 125, 62.50, 31.25, 15.62, 7.8125, 3.91, 1.95, 0.98, 0.49, 0.245, 0.1225 and 0.06125µg/mL. All the solutions were prepared with methanol as solvent. 2mL of each prepared concentration was mixed with 4mL of 50µM DPPH solution in methanol. Experiment was done in triplicate. The mixture was vortexed for 10s to homogenise the mixture and test tubes were incubated for 30min at room temperature in the dark, after 30min of incubation the absorbance was measured at 515nm on a UV-Vis spectrophotometer (Shimadzu, UV-1620PC, Japan). Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Gallic acid and rutin were used as standards with the following concentrations 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.7812, 0.391 and 0.195µM. Blank solution was prepared by mixing 2mL of methanol with 4mL of 50µM DPPH solution. The difference in absorbance between the test and the control (DPPH in methanol) was calculated and expressed as % scavenging of DPPH radical. The capability to scavenge the DPPH radical was calculated by using the following equation

$$\% \text{ inhibition} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

Finally, the IC_{50} value, defined as the concentration of the sample leading to 50% reduction of the initial DPPH concentration, was calculated from the separate linear regression of plots of the mean percentage of the antioxidant activity against concentration of the test extract (µg/mL).

Antibacterial screening: Agar cup diffusion method (B.P. 1988) was used to determine the activity of the extracts on the following typed organisms: *Escherichia coli*; *Salmonella typhi* and *Staphylococus aureus*. Briefly, a 0.1mL of a 10^{-2} dilution of an overnight broth culture of each organism (containing an inoculum

size 10^6 – 10^7 cells/mL) seeded into molten but cooled 20mL nutrient agar (pH 7.4 Oxoid) was used. The extracts prepared (using methanol-water) as 10 and 20mg/mL of hexane, dichloromethane and aqueous were dropped into the 3mm diameter wells bored in the agar. Gentamicin (Beecham, England) 25mg/mL was used as a positive control, and 50% methanol as negative control. The plates were incubated at 37°C for 24 hr and the diameter of the zone of inhibition was measured to determine the antibacterial activity. The tests were performed in duplicates and averaged values were recorded.

GC-MS Analysis: The component of both hexane and dichloromethane extracts were identified by GC-MS analysis using GCMS-Q2010 PLUS Shimadzu (Japan). The column was programmed at 60°C for 5min to 140°C at a rate of 2.3°C/min, then kept at 280°C for 30min. Helium was used as a carrier gas. Mass spectra were taken at 70eV. The GC/MS data system contains the NIST library which was used to verify the identity of individual components by mass spectral comparison.

RESULTS AND DISCUSSION

Table 1 shows that the percentage yields of dichloromethane extract from *Steganotaenia araliacea* was higher than both the hexane and aqueous extracts. This means that the majority of the extractable constituents are present in the dichloromethane extract.

Table 1: Yield from extraction of *S. araliacea* stem bark

Extract	Weight	% Yield*
Hexane	10.23	1.71
DCM	20.13	3.36
Aqueous	15.5	2.58

* From 0.6 kg of bark; DCM = dichloromethane

Table 2: Antibacterial activities of *S. araliacea* bark extracts (*zone of inhibition diameter in mm)

Extract	Concn.	Test organism		
		S. aureus	*E. coli*	*S. typhi*
Hexane	10 mg/mL	10.0	11.0	15.0
	20 mg/mL	17.0	19.0	22.0
DCM	10 mg/mL	10.0	7.0	20.0
	20 mg/mL	20.0	10.0	25.0
Aqueous	10 mg/mL	9.0	7.0	NA
	20 mg/mL	10.0	6.5	NA
Gentamicin	100µg/mL	28.0	30.0	30.0

* cup size = 3mm; NA = not active; DCM = dichloromethane

Table 3: Test for antioxidant activity (DPPH asay)

Extract	TLC (µg)	IC_{50} (µg/mL)	Potency
Hexane	0.5	5.62	5.25
DCM	0.1*	1.26	5.899
Aqueous	0.5	2.24	5.65
Rutin	< 0.10	10.0µM	5.0
Ascorbic acid	< 0.15	2.79µM	5.55
Gallic acid	< 0.10	1.78µM	5.75

Potency = $-\log IC_{50}$; * = reacted instantaneously, DCM = dichloromethane

Table 4: GC-MS analysis: compounds identified from the hexane and dichloromethane extracts of *S. araliacea.*

s/n	Dichloromethane extract	Hexaneextract
1	O-Xylene	1-Hepten-3-ol
2	2-Ethylhexanal	o-xylene
3	Phenylacetaldehyde	3,7-dimethylundecane
4	Cumene	Cyclohexanemethanol
5	Hex-3-enyl acetate	psi Cumene
6	Citronellol	Undecane
7	Curcumyl 2-methylbutyrate	4-Heptanal
8	N-acetylmethyl anthranilate	Cyclogeraniolane
9	Cyclogeraniolane	Trans-1,4-menthol
10	Cadinanol	4-Ethylguiacol
11	α-guaien	Citronellol
12	ar-Curcumene	Methylcitronellate
13	Trichocoleine	β-Citronellene
14	Muuroladien	2-hydroxy-3,5-dimethoxy-9,10-dihydrophenanthrene

With respect to the antibacterial activity recorded in Table 2, dichloromethane extract showed a significant antibacterial activity on *S. aureus, S. typhi* and *E. coli* at a concentration of 20mg/mL, this was followed by the hexane extract. The aqueous extract showed no significant antibacterial activity on the microorganisms. This agrees with the results reported by Taniguchi *et al*., 1978 that 70% ethanol extract of the fresh root bark was inactive in antibacterial tests against *B. subtilis* and *E. coli* at 100μg/mL. The results reported by Alemika *et al*. (2004) had also indicated that both the dichloromethane and ethyl acetate extracts showed significant antibacterial activity against *S. aureus, B. subtilis* and *E. coli* while the polar extracts showed little or no activity against the typed organisms. Since many plant phenolics have been found to be responsible for several biological properties, including antibacterial properties (Yaltirak *et al*., 2009), it was expected that the antibacterial activity of *S.* araliacea would be related to its phenolic compounds majorly resided in the dichloromethane extract.

The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples (Ebrahimzadeh *et al.,* 2008). DPPH is a stable nitrogen-centered free radical the color of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron- donation. Substances which are able to perform this reaction can be considered to be antioxidants and therefore radical scavengers (Dehpour *et al.,* 2009). The antioxidant activity of hexane, dichloromethane and aqueous extracts of *S. araliacea* was measured by DPPH radical-scavenging activity. The results obtained by these studies are given in Table 3. According to DPPH assay, dichloromethane extract showed maximum activity with IC_{50} = 1.26 μg/ml followed by the aqueous extract with IC_{50} = 2.24 μg/ml and the activity was comparable to those of standard ascorbic acid, rutin and gallic acid (IC_{50} of 2.79μM, 10.0 μM and 1.78μM respectively). Results were also transformed into potency (-log IC_{50}) values (Anesini *et al*., 2008). The potency values in Table 3 show that the dichloromethane extract has the maximum potency value which makes it a good candidate for further investigation. It is noteworthy to mention that dichloromethane extract bleached the DPPH immediately suggesting that it could be classified as a fast kinetic antioxidant (Brand-Williams *et al*., 1995, Erasto *et al*., 2004). From the present result it may be postulated that some hydrogen donors in antioxidant principles of dichloromethane extract of S. *araliacea* reduce the radical when it reacts with

hydrogen donors in antioxidant principles. DPPH radicals react with suitable reducing agents, the electrons become paired off and solution looses colour stochiometrically, depending on number of electrons taken up by the extract. It has been shown that free radicals and other related species are the major cause of oxidation of biomolecules, leading to cell injury and death. The emergence of phenolics and related compounds have been of great interest as they are present in large quantities in natural products having significant antioxidant activity. The extracts of *S. araliacea* exhibit a high antioxidant activity especially the dichloromethane extract, compared to ascorbic acid, gallic acid and rutin. Therefore, the results show that the dichloromethane extract of *S. araliacea* possesses compounds with antioxidant properties which could be isolated and then used as antioxidants for the prevention and treatment of free radical related disorders.

GS/MS analysis resulted in the identification of 14 compounds each from the hexane and dichloromethane extracts. The results are shown in Table 4. These results are also supported by the work of Nukenine *et al*. (2007) who reported the presence of essential oils in the powdered leaves of *S. araliacea*.

In conclusion, the antibacterial activities recorded have justified the traditional use of S. araliacea in the treatment of some of the infectious diseases related to *S. aureus*, *E. coli* and *S. typhi*. The antioxidant activity of the extracts, especially the dichloromethane extract, shows the presence of phenolics, resins, flavonoids and saponins. This points to the need for further studies with the view to isolating the specific active components of the plant using bioassay-guided fractionation approach.

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