American Journal of Plant Sciences, 2014, 5, 2448-2453
Published Online July 2014 in SciRes. http://www.scirp.org/journal/ajps
http://dx.doi.org/10.4236/ajps.2014.515259

Scientific Research

Chemical Constituents and Antimicrobial Activity of Essential Oil of *Senna podocarpa* (Guill. et Perr.) Lock

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Received 24 May 2014; revised 28 June 2014; accepted 19 July 2014

Abstract

The chemistry of essential obtained from the leaves of *Senna podocarpa* (Guill. Et Perr.) Lock from Nigeria is being reported. The hydrodistilled oil was analyzed by gas chromatography-flame ionization detector (GC-FID) and gas chromatography-mass spectrometry (GC-MS) techniques. The main constituents of the oil were 1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester phthalate (26.6%) and *β*-elemene (27.9%). There were significant amounts of caryophyllene oxide (7.3%) and urs-12-en-24-oic acid, 3-oxo-methyl ester (5.5%). The antimicrobial activity of the essential oil was evaluated against a panel of seven bacteria and two fungal strain using agar diffusion and broth microdilution methods. Results had shown that the oil exhibited moderate to strong antimicrobial activity against the tested microorganisms. The activity zones of inhibition (ZI) ranged between 10.0 ± 0.2 mm and 28.3 ± 2.9 mm while the minimum inhibitory concentrations (MIC) ranged between 0.3 mg/mL 5.0 mg/mL, respectively. The chemical constituents and antimicrobial activity of the essential oil of *Senna podocarpa* were being reported for the first time.

Keywords

***Senna podocarpa*, Essential Oil Composition, Mono (2-Ethylhexyl) Ester Phthalate, *β*-Elemene, Antimicrobial Activity**

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How to cite this paper: Adebayo, M.A., Lawal, O.A., Sikiru, A.A., Ogunwande, I.A. and Avoseh, O.N. (2014) Chemical Constituents and Antimicrobial Activity of Essential Oil of *Senna podocarpa* (Guill. et Perr.) Lock. *American Journal of Plant Sciences*, **5**, 2448-2453. http://dx.doi.org/10.4236/ajps.2014.515259

1. Introduction

Senna podocarpa (Guill. et Perr.) Lock., is a glabrous shrub usually 1 - 2 m high. The leaves are alternate, stipulate and pari-pinnate with 3 - 5 pairs of leaflets in a 15 - 30 cm long rachis. The flowers are yellowish up to 3 cm diameter in dense terminal spikes 20 - 30 cm long. The seeds (about 12 - 25) are black which cause transversal waving in pod valves [1]. The leaves and fruits are strongly purgative. The decoction of the leaves, roots and flowers is given for the treatment of veneral diseases in women. Fresh leaves are applied as poultices for the treatment of wounds, swellings, skin diseases and yawns [1].

A report indicated that the aqueous infusion of *S. podocarpa* pod was devoid of overt acute and sub-chronic toxic effects in mice and rats [2] [3]. The hydromethanolic leaf extracts of *S. podocarpa* have potent antioxidant properties and cytotoxic to the K562 leukaemia cell line [4] and the extracts may be useful in the management/improvement of anemic conditions [5]. Methanol extract of the plant was shown to displayed antimicrobial activity [6], antiviral activity [7], increased intestinal motility of rats [8]-[10] and possessed laxative property [11]. The phytochemical screening showed the presence of saponins, tannins, anthraquinone, phlobatanin, phenolics, flavonoids and alkaloids [4]-[6] [12]. In addition, compounds such as rhein, emodin, chrysophanol, rhein-anthroneglucoside, sennoside A and sennoside B have been characterized from the plant [13].

Till present, there is no report on the chemical constituents of its essential while only one report indicates that the leaf essential oil possesses moderate antimicrobial activity [14]. In this paper, we report the compounds identified in the leaf essential oil of *S. podocarpa* as well as its antimicrobial potentials. This research is part of continued interest on the chemical analysis of poorly studied species of Nigeria flora [15].

2. Materials and Methods

2.1. Plant Sample

Fresh leaves of *S. podocarpa* were collected in March 2013, from a location in Ore, Ondo State, Nigeria. The sample was taxonomically identified by Curators at the Herbarium of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria, where voucher specimen FHI 109,903 was deposited for future reference.

2.2. Extraction of Essential Oil

The air-dried plant sample (600 g) was pulverised and hydrodistilled for 4 h using a modified Clevenger-type apparatus according to the British Pharmacopoeia specification [16] to yield colourless essential oil. The distilled oil was collected over water and stored under refrigeration until analysis.

2.3. Analysis of the Oil

GC analysis of the oil was carried out on a Hewlett Packard HP 6820 Gas Chromatograph equipped with a FID detector and DB-5 column (60 m × 0.25 mm i.d., film thickness, 0.25 µm) at the split ratio of 1:25. The oven temperature was programmed from 50˚C (after 2 min) to 240˚C at 5˚C /min and the final temperature was held for 10 min. Injection and detector temperatures were maintained at 200˚C and 240˚C, respectively. Hydrogen was the carrier gas at the flow rate 1 mL/min. An aliquot of 0.2 µL of the diluted oil was injected into the GC. Peaks were measured by electronic integration. A homologous series of *n*-alkanes were run under the same conditions for determination of retention indices. The relative amounts of individual components were calculated based on the GC peak area (FID response) without using correction factors.

GC-MS was performed on a Hewlett Packard Gas Chromatography HP 6890 interfaced with a Hewlett Packard 5973 mass spectrometer system equipped with a DB-5 capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm). The oven temperature was programmed from 70˚C - 240˚C at the rate of 5˚C /min. The ion source was set at 240˚C and electron ionization at 70 eV. Helium was used as the carrier gas at a flow rate of 1 mL/min. The scanning range was 35 to 425 amu.

2.4. Identification of the Constituents

The identification of constituents was performed on the basis of retention indices (RI) determined with reference to the homologous series of *n*-alkanes, under identical experimental conditions, co-injection with standards (Sigma-Aldrich, St. Louis, MO, USA) or known essential oil constituents, authentic collection of Mass spectra data of known compounds and by comparing with MS literature data [17] [18].

2.5. Antimicrobial Assay

2.5.1. Microorganisms

Nine local isolates comprising of two gram-positive bacteria, five gram-negative bacteria strains and two fungi obtained from the Department of Microbiology, Lagos State University, Ojo, Lagos and Nigerian Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria, respectively were used for this study. These microorganisms were *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Kiebsiella* spp., *Proteus* spp., *Pseudomonas* spp., *Salmonella* spp., *Penicillium notatum* and *Rhizopus stolonifer*. The stock cultures were maintained at 4˚C in Müeller-Hinton agar (Oxoid, Germany).

2.5.2. Determination of Antimicrobial Activity

The antibacterial activity of essential oil of *S. podocarpa* was measured by disc-diffusion method [19]. The microorganisms were grown overnight at 37˚C in 10 mL of Mueller Hinton Broth (Oxoid Ltd, England) for 24 h. The cultures were adjusted with sterile saline solution to obtain turbidity comparable to that of McFarland no. 0.5 standard (1.0×10^8 CFU/mL). Petri dishes containing Müeller-Hinton agar (Oxoid Ltd, England) were inoculated with the microbial suspensions. Sterile Whatman No.1 (6 mm) discs paper was placed on the surface of the seeded agar plates and 10 µL of 40 mg/mL of each extract in dimethylsulfoxide was applied to the filter paper disk. The plates were incubated overnight at 37˚C for 24 h and the diameter of any resulting zones of inhibition (mm) was measured. Each experiment was carried out in triplicates. Standard antibiotic discs (gentamycin and nalidixic acid) and 1% DMSO solution ((positive and negative controls) were also run in parallel along with the extracts.

2.5.3. Determination of Minimum Inhibitory Concentration (MIC)

A broth microdilution method was used to determine the MIC of *S. podocarpa* oil [20]. Bacterial cultures were incubated in Müeller-Hinton (MH) broth overnight at 37˚C and a 1:1 dilution of each culture in fresh MH broth was prepared prior to use in the micro dilution assay. One hundred µL of bacterial culture of an approximate inoculum size of 1.0×10^8 CFU/mL was added to all well and incubated at 37˚C for 24 H. After incubation, 40 µL of 0.2 mg/mL *p*-iodonitotetrazolium violet (INT) solution was added to each well and incubated at 37˚C. Plates were examined after about 30 min of incubation. Microbial growth is indicated by the presence of a reddish colour, which is produced when INT, a dehydrogenase activity-detecting reagent is reduced by metabolically active microorganisms to the corresponding intensely coloured formazan. MIC is defined as the lowest concentration that produces an almost complete inhibition of visible microorganism growth in liquid medium. Solvent control (DMSO solution) and standard antibiotics (gentamycin and nalidixic acid) were included in the assay.

2.6. Statistical Analysis

The mean and standard deviation of three experiments were determined for zones of inhibition. Statistical analysis of the differences between mean values obtained for experimental groups were calculated using Microsoft excel program, 2003 [21]. Data were subjected to one way analysis of variance (ANOVA). *P* values ≤0.05 were regarded as significant and *P* values ≤0.01 as very significant.

3. Results and Discussion

3.1. Chemical Constituents

The yield of the oil obtained from the hydrodistillation procedure was 1.13% (v/w), calculated on a dry weight basis. **Table 1** indicates the list of 19 compounds identified in the leaf oil of *S. podocarpa*. These amounted to 93.7% of the total oil content. Monoterpene compounds were conspicuously absent in the oil. Sesquiterpene hydrocarbons (42.0%), oxygenated sesquiterpenes (8.7%), phthalate (26.6%), triterpenes (10.5), diterpenes (2.8%) and alkanes (2.1%) were the main classes of compounds identified in the oil. The major compounds were identified as 1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester phthalate (26.6%) and *β*-elemene (27.9%). There were significant amounts of caryophyllene oxide (7.3%), urs-12-en-24-oic acid, 3-oxo-methyl ester (5.5%), *β*-caryophyllene (3.1%), *γ*-muurolene (3.0%) and (3*β*)-lup-20(29)-en-3-ol, acetate (3.0%).

Although literature citation on the composition of the essential oil of *S. podocarpa* is scarce, the oil contents of some *Senna* species have been documented. The major components of the oil of *Senna alata* were ar-tur-

Table 1. Essential oil constituents of *Senna podocarpa*.

Compounds[a]	RI[b]	RI[c]	Percentage (%)
α-copaene	1371	1372	1.0
(*E*)-4-tetradecene	1380	1379	2.1
β-elemene	1389	1393	17.9
β-caryophyllene	1426	1417	3.1
α-humulene	1454	1452	0.9
γ-muurolene	1479	1477	3.0
γ-elemene	1489	1482	0.7
β-selinene	1491	1489	2.0
α-selinene	1495	1494	1.3
δ-cadinene	1527	1522	1.4
α-calacorene	1541	1543	0.7
(*E*)-nerolidol	1564	1561	0.5
Caryophyllene oxide	1588	1583	7.3
Humulene epoxide II	1603	1606	0.9
Phytol	2118	1942	2.8
1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester	2549	2553	26.6
Urs-12-en-24-oic acid, 3-oxo-, methyl ester	2720	2710	5.5
Lanosta-8,24-dien-3-ol, acetate, (3*β*)	3331	3339	2.0
Lup-20(29)-en-3-ol, acetate, (3*β*)	3381	3372	3.0
Total			**93.7**

[a]Elution order on DB-5 column. [b]Retention indices on DB-5 column. [c]Literature retention indices.

merone (13.5%), *β*-caryophyllene (7.3%), (*E*)-phytol (7.0%) and 6,10,14-trimethyl-2-pentadecanone (6.8%). while the quantitatively significant constituents in *Senna occidentalis* leaf oil were (*E*)-phytol (26.0%), hexadecanoic acid (17.3%) and 6,10,14-trimethyl-2-pentadecanone (9.9%). (*E*)-phytol (30.8%), pentadecanal (21.7%) and 6,10,14-trimethyl-2-pentadecanone (3.8%) were identified as principal components of *Senna hirsuta* from Nigeria [22]. The quantitatively significant constituents of the leaf oil of *Cassia alata* from Nigeria [23] were 1, 8-cineole (39.8%), *β*-caryophyllene (19.1%) and caryophyllene oxide (12.7%). The essential oil obtained by hydrodistillation of leaves of *C. alata* collected in Gabon [24] was found to contained linalool (23.0%), borneol (8.6%) and pentadecanal (9.3%) as the major constituents. The analysis of chemical compositions of essential oils of *Cassia fistula* growing in Egypt [25] showed the main components of the flower were (*E*)-nerolidol (38.0%), 2-hexadecanone (17.0%) and heptacosane (12.8%), while leaf was characterized by the abundance of phytol (16.1%) together with the hydrocarbons, tetradecane (10.5%) and hexadecane (8.7%).

It could be seen that each oil sample has its own compositional pattern different from others. A noteworthy observation is the fact that mono (2-ethylhexyl) ester phthalate and *β*-elemene were not previously described as major constituents of any *Senna* oil. Mono (2-ethylhexyl) ester phthalate was the main constituents of essential oil of *Polygonum chinense* [26] and *Iris germanica* [27] where it was shown to exhibit good antimicrobial activities. The triterpene compounds of the oil of *S. podocarpa* have been found in other oil samples. 3*β*-lup-20(29)-en-3-ol-acetate is an important constituent of the volatile oil of *Ficus auriculata* [28] while lanosta-8,24-dien-3*β*-ol-acetate could be seen prominent in the oil of propolis [29].

3.2. Antimicrobial Activity

The results of antimicrobial study (**Table 2**) showed that *S. podocarpa* exhibited broad spectrum of antimicrobial activity against some of the tested microorganisms with IZ and MIC highest in *R. stolonifer* (28.3 ± 2.9 mm and 0.3 mg/mL), *E. coli* (15.3 ± 0.5 mm and 0.6 mg/mL) and *P. notatum* (21.0 ± 1.5 mm and 0.6 mg/mL). However, moderate activity could be observed against *B. subtilis* (12.0 ± 1.0 mm and 1.3 mg/mL), *S. aureus* (11.7 ± 1.2 mm and 1.3 mg/mL), *Pseudomonas* spp. (17.0 ± 0.5 mm and 1.3 mg/mL), *Kiebsiella* spp. (13.0 ± 0.7 mm and 2.5 mg/mL) and *Proteus* spp. (12.3 ± 1.3 mm and 2.5 mg/mL). The leaf oil of *S. podocarpa* displayed poor activity against *Salmonella* spp. with IZ of 10.0 ± 0.2 mm and MIC of 5 mg/mL.

Table 2. Antimicrobial activity of the essential oil of *Senna podocarpa.*

Microorganisms	*S. podocarpa*		Gentamycin[d]		Nalidixic acid[d]	
	IZ[b]	MIC[c]	IZ	MIC	IZ	MIC
B. subtilis	12.0 ± 1.0	1.3	20.7 ± 1.2	0.3	23.7 ± 1.6	0.3
S. aureus	11.7 ± 1.2	1.3	28.3 ± 2.1	0.6	24.3 ± 1.5	1.3
E. coli	15.3 ± 0.5	0.6	24.7 ± 1.2	0.3	15.7 ± 1.2	1.3
Kiebsiella spp.	13.0 ± 0.7	2.5	10.7 ± 1.5	2.5	23.7 ± 1.6	0.3
Proteus spp.	12.3 ± 1.3	2.5	12.3 ± 2.5	1.3	20.3 ± 0.6	0.6
Pseudomonas spp.	17.0 ± 0.5	1.3	15.7 ± 2.1	2.5	15.7 ± 1.2	1.3
Salmonella spp.	10.0 ± 0.2	5	22.0 ± 2.0	0.6	13.7 ± 2.1	2.5
P. notatum	21.0 ± 1.5	0.6	14.3 ± 1.3	1.3	15.7 ± 1.2	1.3
R. stolonifer	28.3 ± 2.9	0.3	20.7 ± 1.2	0.3	13.7 ± 2.1	2.5

[a](n = 3, X ± SEM). [b]IZ: Inhibition zones diameter (mm) including diameter of sterile disc (6 mm), with values given as mean ± SD (3 replicates). [c]MIC values are given as (mg/ml). [d]Methanolic solutions of Gentamycin and Nalidixic acid, 5 mg/mL.

The oil acted more strongly against *R. stolonifer*, *E. coli* and *P. notatum* with MIC of 0.3, 0.6 and 0.6 mg/mL respectively. The antimicrobial activity of essential oil is related in most cases to its major components and the synergistic effects of the minor constituents should also be taken into consideration [23]. Referring to literature, mono (2-ethylhexyl) ester phthalate has shown considerable antimicrobial activities [26] [27]. In addition, essential oils with considerable amount of *β*-elmene have displayed potentials antimicrobial properties [30]. The present antimicrobial effects justify the use of *S. podocarpa* plant for treatment of various ailments. Therefore, it is recommended as a plant of phytopharmaceutical importance.

Acknowledgements

Authors are grateful to Mrs. Ogunwande Muslimat for the type setting of the manuscript. The effort of Mr. Daniel Mojiji of the University of Lagos, Nigeria, in the GC and GC/MS analysis was highly appreciated.

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