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Full Length Research Paper

The essential oil composition and antimicrobial activity of leaves of *Schistostephium hippifolium*

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The essential oil from the leaves and branches of *Schistostephium hippifolium* were extracted by hydro-distillation during summer and winter months. Both essential oils were analyzed by gas chromatography- mass spectroscopy (GC-MS) respectively during their extracted period. Fourteen compounds were identified from essential oil extracted during the February, summer month in South Africa. The volatile oil constituted about 99.31% of the total essential oil composition. The GC-MS reveals the following major constituents in the oil: Chrysanthenyl acetate (32.17%), 1.8-cineole (24.85%), ethanone, 1- (1-cyclohexen-1-y1) (6.36%), camphor (6.02%), terpinen-4-ol (5.02%), α-pinene (5.13%), germacrene-D (4.89%) bicyclogermacrene (4.38%), linalool L (2.04%), trans - caryophyllene (1.80%), γ-terpinene (1.95 %). In June, a winter month in South Africa, 48 volatile compounds from the essential oil of *S. hippifolium* were identified which constituted about 99.99% of the total composition of the essential oil. The major compounds identified were the 1.8-cineole (18.31%), Germacrene-D (7.43%), bicyclogermacrene (5.24%), 3-cyclohexen-1-ol (6.78%), camphor (5.64%), bicyclogermacrene (5.24%), α.-pinene (2.52%), β-caryophylle (2.11%), linalool L (3.35%), caryophyllene oxide (1.88%), spathulenol (1.62%) and γ-terpinene (1.39%). In both essential oils (summer and winter), minor volatiles were identified. However, they were in low concentration. The crude extract from the aerial parts of the plant were screened for antibacterial activity against five Gram-positive species (*Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus, Micrococcus kristinae* and *Staphylococcus faecalis*) and five Gram-negative (*Escherichia coli, Pseudomonas aeruginosa, Staphlococcus flexneri, Klebsiella pneumonia* and *Serratiaf marcescens*) microorganisms. The minimum inhibitory concentration (MIC) values where highly active at values below two and less active at values above two. All the extracts tested displayed very good activity against both Gram-positive and Gram-negative bacteria with MIC ranging between 0.098 to 0.65 mg/l.

Key words: Essential oil, GC-MS, oil composition, antimicrobial activity, *Schistostephium hippifolium*.

INTRODUCTION

Literature has documented that indigenous people of South Africa were predominantly using traditional plants for centuries to treat medical related illness (Magwa et al., 2005; Burits et al., 2001, Rabe and van Staden, 1997). Such practices and usage was associated with witchcraft and heathen practices during the missionary era. As a result the reputation on the usage of traditional medicine by indigenous people of South Africa during colonial era was undermined. However, at the beginning of the last century an increase in the recognition of medical practices was observed as a result the World Health Organization recognized the importance of plants in the global health care (Burt, 2004; Choir et al., 2001; Also et al., 2000; Croteau, 1977). Recent studies have shown that the pharmaceutical, cosmetic and fragrance industries have changed their focus of research from synthetic products to natural or traditional use of medicinal plants. These companies and related industries make use of plant extracts to develop drugs, medicines for the treatment of various diseases or illnesses,

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improvement on nutrition, fragrance and aromatherapy (Lima et al., 2004; Nguefack et al., 2004; Tan et al., 2002; Mill et al., 2000; Theimer, 1989). Some of these species of aromatic plants have been investigated and demonstrated that they can assist in antihydrotic, splasmolytic and anti-flammatory treatments which include mental and nervous conditions (Lee et al., 2003; Burits et al., 2001; Gundidza, 1993). These species are common in large families of flowering and non-flowering plants such as Solanaceae, Lamiaceae, Liliaceae, Asteraceae and where many studies have been conducted (Mayekiso et al., 2008; Mhinana et al., 2010). These families consist of species which are of economic importance.

The family Solanaceae for example is one of the large families of flowering plants with 85 – 90 genera and consists of 2800 to 3000 species with a cosmopolitan distribution (Zomlefer, 1983; Thorne, 1983; Kubitzski, 1977). Many of these species are found in Australia and Central to South America (Roddick, 1991; Cunningham et al., 1981; Robertson, 1981; Salmon, 1980). Many species are available as food products, like *Solanum tuberosum* - potato, *Lycopersicon esculentum* - tomato, chilli peppers of which the potato is the most important food crop compared to rice, wheat and maizes (Hawkes 1990, 1991). This family is also well-known for containing the toxic alkaloid nicotine, tobacco (*Nicotiana tabacum*) is grown for chewing, smoking, or snuff. Some of these species are regarded as a source of medicinal drugs, stimulants and poisons (Roddick, 1991), for example madrake (*Mandragora officinarum*). A large number of them also contribute to and are still extensively used in biotechnological and molecular research.

The Liliaceae family on the other hand consists of about 280 genera and 4000 species distributed worldwide (Antunes and Sevnate – Pinto, 1991; Ascensão et al., 1985; Fahn and Shimony, 1998). The family is an important component of the early spring flora in the deciduous forests of eastern North America. The Liliaceae is a very diverse family that Botanists separate into numerous smaller families such as Alliaceae, Asparagacease, Ruscaceae, and Trilliaceae (Pooley, 2003; Leistner, 2000; Germishuizen and Meyer, 2003).

It is also of economic importance among many well known garden and house ornamentals, and as a the result they are regarded as the chief economic value in other countries where over 160 genera are represented in American Trade (Valant- Vetschera et al., 2003; Antunes and Sevnate – Pinto, 1991; Ascensão et al., 1985; Fahn and Shimony, 1998).

In addition, most of the species which are either C3 or C4 subtypes have been developed biotechnologically into cultivars to constitute the stable food and animal fodder in many countries. The Asteraceae family is also recognized as probably the largest family of flowering plants (Pooley, 2003; Leistner, 2000; Germishuizen and Meyer, 2003). More than 2 5000 species world-wide, grow from sea-level to the highest mountain peaks (Zomlefer , 1986; Willis, 1973). In Southern Africa it is regarded as one of the biggest family of flowering plants with 246 genera and 2 300 species (Salmon, 1986; Mark et al., 1979; Johnson, 1975). The economic value of the representatives of this family can be divided into various categories: Food for man, poisonous and medicinal; weeds, wood and garden plants.

Schistostephium a genus belonging to the Asteraceae family and is characterized by three species which are regarded as related to *Artemisia afra* by the indigenous people of the Eastern Cape. These species are *Schistostephium heptalobium*, *Schistostephium hippifolium* and *Schistostephium fabeliforme*. There has been a conflicting and confusing idea about *Artemisia afra* and these species of *Schistostephium*. They cannot be distinguished from the Artemisia species in terms of traditional medicine. *S. hippifolium* can be described as a shrub that can grow up to 1.8 m tall characterized by small bright yellowish flowers and can be found in slopy rocky areas of the Eastern Cape Province of South Africa. It has an aromatic smell and bitter taste when utilized for flu and coughs.

In addition, there is no documentation in literature to our knowledge, which deals with the essential oil composition and antibacterial activity of *S. hippifolium*. This study will therefore focus on the essential oil composition of *S. hippifolium* and its behavioral pattern with the change of its composition during summer and winter seasons and the screening for antibacterial activity.

MATERIALS AND METHODS

During the summer and winter months, vegetative shoots of *S. hippifolium* were collected from a farm in Fort Beaufort. The locality was between 32° 46' 58'' to 32° 47' 2''S and 26° 37' 30'' to 26° 38' 00''E in the Eastern Cape Province, South Africa. The plant material was collected from the site and pressed at the Fort Hare Herbarium. It was identified by the curator of the Schonland Herbarium at Rhodes University, Grahamstown. A voucher specimen was deposited at the Griffen Herbarium, University of Fort Hare, Eastern Cape.

Gas chromatography and mass spectroscopy analysis

Fresh leaves were removed from stem and branches and subsequently weighed. The weighed mass (550 g) of sample of leaves was subjected to hydro-distillation for 3 h using a Cleavenger Unit according to British Pharmacopoeia. This process was repeated three times using consistent mass in order to obtain statistical accepted results.

The fresh essential oil was collected, allowed to cool and was analyzed immediately on a Hewlet Packard HP 5973 mass spectrometer interfaced with an HP-6890 Gas Chromatograph. The column consisted of a cross-linked 5% pH ME Siloxane on 30 m x 0.25 mm x 0.25 μm film thick and the column head pressure was 55 Kpa.

The carrier gas used was Helium and the flow was 35 cm/s-split flow 30 to 40:1. The temperatures were programmed at initial

Table 1. Chemical Composition of the essential oil of the leaf of *S. hippifolium* in Summer (February).

Compound	Composition (%)
Chrysanthenyl acetate	32.17
1.8-Cineole	24.85
Ethanone, 1- (1-cyclohexen-1-y1)	6.36
Camphor	6.02
Terpinen-4-ol	5.02
α.-Pinene	5.13
Germacrene-D	4.89
Bicyclogermacrene	4.38
1, 2-Benzenedicarboxylic acid,	2.41
Linalool L	2.04
γ-Terpinene	1.95
Bicyclo [3.1.1] hep-3-en-2-one	1.82
Trans – caryophyllene	1.80
(+) Spathulenol	1.19

temperature of 50°C and accelerated to a temperature of 240°C at an acceleration of 3°C per min. Identification of chemical compounds was achieved by mass spectroscopy.

Determination of antimicrobial activity

Preparation of the leaf extracts

Three separate samples of 60 g each were extracted with 600 ml of 100% acetone, 100% ethanol and pure distilled water respectively. All three samples were placed on the orbital shaker for 24 h and filtered through Whatman no.1 filter paper using a Buchner funnel. The acetone and ethanol filtrates were evaporated to small fractions in a rotary vapor to total dryness and subsequently taken in front of a fan. Changing of the mass of the sample was recorded; this was done by weighing the plant extracts until a constant dry weight was obtained. The water extract was taken to total dryness in a freeze-drier.

Bacterial strains and preparation

Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus, Micrococcus kristinae, Staphylococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Staphlococcus flexneri, Klebsiella pneumonia, Serratia marcescens were laboratory isolates obtained from the department of Biochemistry and Microbiology, University of Fort Hare. All bacteria were maintained on nutrient agar plates and revitalized for bioassay by culturing in fresh nutrient broth for 24 h. The bacterial culture was then diluted 200 µl in a 19.8 ml nutrient broth to make sure that the bacteria were at the start of the log phase when the assay commenced.

Micro-dilution assay

The micro-dilution method (Eloff, 1998) was used to determine the minimal inhibitory concentration (MIC) of the plant extracts with antibacterial activity. All extracts were re-suspended at 50 mg/ml with extracting solvents like water and ethanol. The concentration of ethanol was reduced to 25% to prevent the destruction of extracts.

All extracts were initially tested at 12.5 mg/ml in 96-wells microplates and serially diluted two-fold to 0.0098 mg/ml. The diluted bacteria culture 100 µl was added to each well. The antibiotic neomycin (100 mg/ml) was added as a standard in each assay and bacteria-free wells included as blanks. The micro-plates were covered and incubated at 37°C overnight. The solution of *p*-iodonitrotetrazolium violet (02 mg/ml) (INT) at 40 µl was added in every well and incubated at 37°C for 30 min. Minimal inhibition concentration values were recorded as the lowest concentration of extracts that completely inhibited bacterial growth, that is, the clear wells. The red colour wells were an indication of bacterial growth while, the colourless INT is reduced to red-coloured product by biological organism's (Eloff, 1998).

RESULTS AND DISCUSSION

Hydro-distillation of leaves and branches of *S. hippifolium* yielded an essential oil which was light blue in colour for both the summer and the winter oils. The volume of the oil was 0.6 g/ml during the summer month and 0.9 g/ml during the winter month. Fourteen compounds were identified by the GC-MS spectra from the summer essential oil and amounted to 99.31% of the oil (Table 1), while 48 compounds were identified by GC-MS in the winter oil which amounted to 99.99% of the total composition of the oil (Table 2). Both essential oil were characterized by the presence of hydrocarbons, aldehyde and terpenoids. The change in the essential oil composition might be associated with the change of the season. However, other environmental factors cannot be excluded.

The chemical composition of the essential oil of summer showed the following dominant compounds chrysanthenyl acetate (32.17%), 1.8-cineole (24.85%), ethanone, 1- (1-cyclohexen-1-y1) (6.36%), camphor (6.02%), terpinen-4-ol (5.02%), α.-pinene (5.13%), germacrene-D (4.89%), bicyclogermacrene (4.38%), 1,2-

Table 2. Chemical Composition of the essential oil of the leaf of *S. hippifolium* in Winter (June).

Compound	Composition (%)
1.8-Cineole	18.31
Germacrene-D	7.43
Terpinen-4-ol	6.78
Camphor	5.64
Bicyclogermacrene	5.24
Linalool L	3.35
α.-Pinene	2.52
β-Caryophyllene	2.11
caryophyllene oxide	1.88
(+) spathulenol	1.62
γ.-Terpinene	1.39
(-)-Bornyl acetate	0.92
Nopol	0.82
Sabinene	0.81

benzenedicarboxylic acid (2.41%), linalool L (2.04%). while in winter, cineole 1.8 (18.31), germacrene-D (7.43%), terpinen-4-ol (6.78%), camphor (5.64%), bicyclogermacrene (5.24 %), linalool L (3.35 %), α-pinene (2.52 %) and β-caryophyllene (2.11%) were identified as major constituents of the essential oil.

In winter, the major compounds which were identified were: 1.8-cineole (18.31 %), germacrene-D (7.43%), bicyclogermacrene (5.24%), 3-cyclohexen-1-ol (6.78%), camphor (5.64%), bicyclogermacrene (5.24%), α.-pinene (2.52%), β-caryophylle (2.11%), linalool L (3.35%), caryophyllene oxide (1.88%), spathulenol (1.62%), γ-terpinene (1.39%) with some minor volatiles which were in low concentration.

It appears that 1.8 Cineole can be regarded as the major constituent of the essential oil of *S. hippifolium*. This can be attributed by its high percentage composition in both GC-MS analysis of the summer and winter oils. Such a compound has been demonstrated to have an antibacterial, antifungal and antioxidant properties (Mayekiso, 2009; Magwa et al., 2005; Filipowicz et al., 2003; Lee et al., 2003; Gundidza, 1993; Grierson and Afolayan, 1999). However, its presence in this particular species is associated with a first line of defense (Harborne, 1990). It has been reported that 1.8 Cineole is also used as an ingredient in many mouth wash and cough suppressants (Magwa et al., 2006; Wikipedia, 2009; McGaw et al., 2005). Other compounds that seem to be a the characteristic feature of the *S. hippifolium* essential oil are Germacrene-D (20.28) which is associated with the neural activity in some insects and influences where they deposit their eggs, bicyclogermacrene a type of serquiterpenes or a volatile organic hydrocarbon followed by Camphor, Linalool and α-pinene.

Some of these compounds were identified in the essential oil of the leaves of *Schistostephium heptalobium* however, α- thujone was identified as a major constituent in the essential oil of S*. heptalobium* with the presence of 1.8 Cineole (Mayekiso et al., 2008). Most of these identified complex mixture of different volatiles contribute well in cosmetics companies (α – terpinolene), food flavorings (α - pine, terpineol and caryophyllene), fragrance industry (linalool and L – camphor) and as insect pheromones (germacrene – D) (Sader et al., 2002; Rabe and van Staden, 1997; Dudai et al., 1988).

The general screening results for antibacterial activity are shown in Tables 3 and 4. The three extracts were tested against five Gram–negative and five Gram–positive bacteria where they showed great activity on both. However, extracts displayed a very good activity against Gram–positive bacteria compared to Gram-negative bacteria. The antibacterial activity is regarded high if the MIC values are between 0.1 and 1.00 mmg/l. The MIC values for acetone extract were very high especially against *S. aureus*, Gram-positive bacteria followed by *S. epidermodis*, *S. faecalis* and *B. cereus* with MIC values ranging between 0.65 to 0.098 mg/l. The water extract showed good activity against *S. epidermidis* and *S. aureus* with MIC values of 0.62 and 1.17 mg/l respectively. The ethanol extract showed the best activities in *S. aureus* (0.195 mg/l) and *S. epidermidis* (0.098 mg/l).

Regarding the Gram-negative bacteria (Table 4), the ethanolic extracts exhibited very good activity against all the tested bacteria with the exception of *P. aeruginosa*.

Good antibacterial activity was also detected with acetone extracts with the MIC values ranging from 0.52 to 0.098 mg/l. Poor inhibitory activity was detected against all organisms with aqueous extracts.

The extracts from *S. hippifolium* exhibited very good

Table 3. Determination of antibacterial activity of *S. hippifolium* using a micro-dilution bioassay.

Solvent	Yield (mg/ml)	MIC value of Gram-positive bacteria in mg/ml				
		S. aureus	*S. epidermidis*	*B. cereus*	*M. kristinae*	*S. faecalis*
Water	3000	1.17	0.62	9.4	6.3	5.2
Ethanol	4600	0.195	0.098	3.38	3.16	2.60
Acetone	3400	0.098	0.195	1.56	2.08	0.65
Neomycin	-	0.0030625	0.195	0.006125	0.39	N.A

N.A = Not active.

Table 4. Determination of antibacterial activity of *S. hippifolium* using a micro-dilution bioassay.

Solvent	Yield (mg/ml)	MIC valuesof Gram-negative bacteria in mg/ml				
		E. coli	*P. aeruginosa*	*S. flexneri*	*K. pneumonia*	*S. marcescens*
Water	3000	4.69	4.69	4.23	4.69	0.52
Ethanol	4600	1.11	2.34	0.13	0.65	0.46
Acetone	3400	0.52	0.29	0.098	0.16	0.16
Neomycin	-	0.39	N.A	0.098	0.39	N.A

N.A = Not active.

antibacterial activities. The extracts inhibited most of the tested bacteria including the Gram-negative bacteria. A study undertaken by Vlietinck et al. (1995) has shown Gram-positive bacteria to be more susceptible to plant extracts as compared to Gram-negative bacteria. The effectiveness of a drug is to some extent challenging due to the out-membrane structure of Gram-negative bacteria (Delamare et al., 2005; Page et al., 1997). The high MIC value observed may be due to the great cell wall structure because Gram-negative bacteria consist of two layers (inner and outer layer). The inner layer consists of peptidoglyean and the outer layer polysaccharides with proteins. The cell wall of Gram-positive bacteria ranges from 10 – 80 nanometers, but for Gram-negative bacteria it ranges from 7 to 8 nanometers thick (Raven et al., 2005).

Page et al. (1997) suggest that the outer layer of Gram-positive bacteria is very tough and causes a challenging effect for any drug type; however, this is not the case with the plant extract tested in this study. Good activity was observed against all the tested Gram-negative bacteria.

Poor activity was detected with water extract against both bacteria suggesting that for research purposes water is not the most effective solvent for extracting the active compounds from plants. Drugs used by traditional healers are mostly prepared with water, as the healers generally do not have access to more lipophilic solvents. This is of concern, as it is possible that traditional healers do not extract all the active compounds that might be present in a plant. Nevertheless, negative results do not mean absence of bioactive constituents nor that the plant is inactive, plant extracts may act in other ways by stimulating the immune system, or by creating internal condition that are unfavorable for the multiplication of the microorganisms.

The ethanolic and acetone extracts showed the best activities. This activity may have contributed to the presence of α – pinene, 1.8–cineole, γ-terpinene and linalool. These compounds are monoterpene which may be a significant factor affecting bacteria in nature.

The results obtained from this study are in line with the work done by Madlokazi (2006) on *S. heptalobium*, a sister plant to *S. hippifolium*, which showed a strong antibacterial activity of the plant. *S. heptalobium* was found to contain compounds such as α – pinene and 1.8 cineole. The screening of crude extracts from *S. hippifolium* has shown that this plant is a potentially rich source of antibacterial agents.

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