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Full Length Research Paper

The chemical composition and antibacterial activity of the leaf extract of *Salvia repens* Burch. Ex Benth

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The chemical composition of the essential oil of *S. repens* contain the following major constituents: 1 – camphor (0.4%) *para*–cymene (34.1%), sabinene (23.9%), *1–β*–pinene (16.9%),, myrcene (3.9%), - terpinene (1.8%), *trans- β*–Ocimene (1.3%), terpinene-4-ol (0.7%), nopol (0.4%), α-terpinolene (15.89%), β caryophllene (0.6%). The antibacterial activity of the aerial parts of *Salvia repens* has shown that the acetone extract inhibited the growth of *Bacillus cereus*, *Streptococcus pyrogens* and *Escherichia coli* bacteria tested at minimum inhibitory concentration (MIC) of 0, 5 mg/ml. The methanol extracts effectively inhibited the growth of both *Staphylococcus epidermidus* and *Micrococcus kristinae* at minimum concentration of 0, 5 mg/ml. At 0. 1 mg/ml the methanol extract inhibited the following *B. cereus*, *S. pyrogens* and *E. coli* bacteria whose inhibition concentration was below 0. 5 mg/l. The activity of the water extracts of the plants against Gram-negative and Gram-positive bacteria has shown inhibition at 1.0 and 2.5 mg/l respectively. The extract from the methanol also suggest that, the plant extract has intensive activity at low concentration compare to acetone and water. The antibacterial activity suggests a possibility of *S. repens* plant for use on medicinal applications.

Key words: *Salvia repens*, essential oil composition, antibacterial medicinal plant, *para*–cymene, sabinene, *1– β*–pinene, α-terpinolene.

INTRODUCTION

Plant species have been utilized as a source of food, fragrance and medicine for millennia throughout the world (Nguefack et al., 2004). The family Lamiaceae has been extensively known to have immense medicinal, pharmacological and industrial properties. Many of these species within the Lamiaceae family has a potential of possessing essential oils which can be supplied to industry as raw material for different application in preparation of insecticides, antiseptics, perfumes, spices and many other commodities. *Salvia* (sage family) is one of the large genus of Lamiaceae family and feature prominently in the pharmacopoeias of many countries throughout the world. It contains about 500 species of which are *Salvia dominica* L., *Salvia fruticosa* Mill, *Salvia sclarea* L. and *Salvia officinalis* has been extensively studied (Bisio et al., 1999; Grierson and Afolayan, 1999;

Werker et al., 1985 a, b). *Salvia repens* is also a member of of sage family with promising medicinal and pharmacological properties. It is used by indigenous people of South Africa to treat sore and decoction of the roots is used for the treatment of stomach ache and diarrhoea (Auge et al., 2003). The purpose of the study was to investigate whether this plant can be a suitable candidate for pharmacological and industrial applications. In this study the chemical composition of the essential oil and antimicrobial activity of the plant will be reported.

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MATERIAL AND METHODS

Plant material: *S. repens* was collected from a site at a Fort Beaufort farm, about 35 km from the University of Fort Hare Alice, in the Eastern Cape. The plant was identified at the Schonland Herbarium at Rhodes University, Grahamstown, and a voucher specimen was deposited in the Giffen Herbarium at the University of Fort Hare in Alice.

Essential oil extraction (water distillation): Plant samples were collected from the site in Fort Beaufort.

Table 1. Essential oil composition of *S. repens* Burch ex Benth

Constituents	Retention time (min)	% of total
1 – beta – pinene	4. 843	16. 8
myrcene	4. 947	3. 9
gamma-terpinene	5. 240	1. 8
para – cymene	5. 698	34. 1
sabinene	5. 802	23. 9
trans- beta – Ocimene	6. 010	1. 3
alpha-terpinolene	6. 884	15. 9
l. –camphor	8. 218	0. 4
terpinene	8. 947	0. 3
nopol	9. 527	0. 6
beta- caryophllene	14. 984	0. 4

The leaves were removed from stems, and were weighed. The weighed mass of fresh sample of leaves, about 550 g, was placed in a cleavenge flask to which 1000 ml of distilled water was added and subjected to high temperatures for a period of 2 h.

This process was repeated three times using consistent mass in order to obtain statistical accepted results. Essential oil collected from cleavenger flask from the distilled sample was stored at -10^{O}C and was ready for oil analysis.

Gas chromatography analysis: The essential oil was cooled and analyzed on a Hewlet Packard, Series II Gas Gromatograph, with a flame ionization mass selective detector and a Hewlett Packard 2971 Series. 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 - 40:1. The temperature programmes, initial temperature was 50^{O}C and accelerated to a temperature of 240^{O}C in the temperature range of 50 - 240^{O}C at an acceleration of 3^{O}C/min. Idetification of chemical compounds was achieved by mass spectroscopy.

Preparation of extract: *S. repens* leaves were oven-dried at 60^{O}C for overnight. Dried leaves (100 g) were shaken separately in acetone and methanol, and then the water extract was freeze-dried and shaken on an orbital shaker for 24 h. The extracts were filtered using a Buchner funnel and Whatman no. 1 filter paper, and each filtrate was concentrated to dryness under reduced pressure at 40^{O}C using the rotary evaporator. Each extract was resuspended in the respective solvent to yield a 50 mg/ml stock solution.

Antibacterial assays: The acetone, methanol and water extracts were individually tested against 10 laboratory isolates of bacterial species provided by the Department of Microbiology and Biochemistry, Rhodes University.

Five species were Gram positive (*Bacillus cereus*, *Staphylococcus epidermidis, Staphylococcus aureus*, Micrococcus *kristinae* and *Streptococcus pyogenes*) and five Gram negative (*Escherichia coli, Salmonella pooni, Serratia marcescens, Pseudomonas aeruginos*a and *Klebsiella pneumoniae*). (Table 2) Each organism was maintained on nutrient agar plates and was recovered for testing by growth in nutrient broth for 24 h. Before use, each bacterial culture was diluted 1:100 with fresh sterile nutrient broth. Test organisms were streaked in a radial pattern sterile nutrient agar plates containing filtered extracts at final concentrations of 0.1, 0.5, 1, 0 and 5.0 mg/ml and plates containing nutrient agar and extracts served as controls. After inoculation, the plates were incubated at 37^{O}C for 24 h.

Each treatment was performed in triplicate and complete inhibition of bacterial growth was required for an extract to be declared bioactive. Chloramphenicol and streptomycin were used as standard controls in the experiment.

RESULTS AND DISCUSSION

The chemical composition of the essential oil of *S. repens* appeared to contain the following major constituents: l. –camphor (0.4%), *para*–cymene (34.1%), sabinene (23.9%), *1*–*β*–pinene (16.9%), myrcene (3.9%), –terpinene (1.8%), *trans- β*–ocimene (1.3%), terpinene-4-ol (0.7%), nopol (0.4%), *α*-terpinolene (15.9%), *β* – caryophllene (0.6%) (Table 1). The 1-camphor, para-cymene and sabinene are regarded as having strong insecticidal properties. These compounds are also associated with toxic activities. The same characteristic features are also observed in other species of *Salvia* such as *S. dominica* which has a high percentage of linayl-acetate in its chemical constituents (Bayrak and Akgül, 1987; Werker et al., 1985b) . In addition, *Salvia aurea*, the predominant component is camphor (Serrato-Valenti et al., 1997), while the oil of *Salvia candidissima*, contain these main components; β - pinene α - pinene and cineole. Borneol was the major component of *Salvia cryptantha* essential oil of which the same oil was containing a considerable amount of camphor and 1, 8 cineole. The *S. fruticosa* essential oil was found to contain 1, 8 cineole as a major constituent. However in other publication campor and thujone are reported as major components of *S. fruticosa* (Lima et al., 2004).

S. officinalis essential oil contained camphor as the main component. α - Thujone and β - thujone were also important components (Croteau et al., 1981; Lima et al., 2004). All these volatile constituents are associated with insecticidal properties.

This appears to have an important role in inter-plant allelopathy owing to their phytotoxicity (Muller and Muller, 1964; Muller et al., 1968) . *Salvia runcinata* and *Salvia stenophylla* also display a high toxicity profile (Kleining, 1989). The presence of these compounds in *S. Repens*

Table 2. Antibacterial activity of extracts from *Salvia repens*.

	MIC (mg/ml)[a]			
Bacterial species	**Gram +/-**	**Acetone**	**Methanol**	**Water**
Bacillus cereus	+	0.5	0.1	5.0
Staphyloccocus epidermidus	+	5.0	0.5	na
Staphylococcus aurens	+	5.0	5.0	na
Micrococcus kristinae	+	2.5	0.5	na
Streptococcus pyrogens	+	0.5	0.1	5.0
Escherichia coli	_	0.5	0.1	0.1
Salmonella pooni	_	5.0	5.0	na
Serratia marcescens	_	5.0	5.0	2.5
Pseudomonas aeruginosa	_	5.0	5.0	na
Klebsiella pneumoniae	_	na[b]	na[b]	na

[a] Minimum Inhibitory Concentration (mg/ml of three replicates)

[b]na- not active

suggests a mode of defence strategy against herbivores and insects. It is believed that these essential oils contain complex mixture of different volatile oils which are associated with anti-microbial properties as well as flavanoids (Grierson and Afolayan, 1999) . It is on this basis that pharmaceutical companies and related industries exploit these chemical extract and essential oil which are obtained specifically from *Salvia* species. Some of these plant-derived phyto-chemicals extracts continue to benefit nutritional, perfumery and aromatheraptetic industries (Auge et al., 2003; Kleining 1989; Nguefack et al., 2004; Tan et al., 2002).

Antibacterial testing

The defensive strategy within the *S. repens* was further confirmed by the positive response towards bacterial activity. Although the traditional healers and indigenous people did not know a scientific validation, it can be argued that they have experience from the past generations to sense the appropriate herbal properties which could be used in their healing system. Indeed, they confirmed the use of *S. repens* as an anti-bacterial agent. Many products, which contain the ingredients of *Salvia* extracts, have industrial application. The typical examples are: *Salvia namaensis* of which it has been identified as a plant that is used by traditional healers to treat various infections (Grierson and Afolayan 1999) . In addition, *Salvia triloba* oil can be considered as an alternative to food preservatives which eliminates the growth of the important food borne pathogens and spoilage bacteria. *Salvia lavandulaefolia* which is the Spanish sage may be relevant in the treatment of dementia of the Alzheimer's type (Savelev et al., 2003).

S. officinalis is cultivated in several countries mainly to obtain dried leaves to be used as raw material in medicine, perfumery and food industry (Santos-Gomes et al., 2003). These aspects which are highlighted in many *Salvia* species as well as in *S. repens* are further confirmed by the similarities of many volatile compounds which occurred in these species. Furthermore, these evidence opens an avenue for research on the commercial application of *S. repens*.

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