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The in vitro pharmacological activities and a chemical investigation of three South African *Salvia* species

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Abstract

Salvia species (sage) are well known in folk medicine throughout the world. In South Africa sage is used against fever and digestive disorders. Three closely related South African species (*Salvia stenophylla*, *Salvia repens* and *Salvia runcinata*) were investigated for their anti-oxidant (DPPH assay); anti-inflammatory (5-lipoxygenase and cyclo-oxygenase assays); antimalarial (tritiated hypoxanthine incorporation assay); antimicrobial (disc diffusion and micro-dilution assays) properties and toxicity profile (tetrazolium-based assay). The solvent extracts exhibited anti-oxidant, antimalarial and antibacterial and poor anti-inflammatory properties. The essential oils exhibited anti-inflammatory and antimalarial properties, but displayed poor anti-oxidant and antimicrobial activity. The extract of *Salvia stenophylla* and the essential oil of *Salvia runcinata* displayed the highest toxicity profile. Overall, *Salvia runcinata* displayed the most favorable activity of all three taxa tested with an IC_{50} value of 6.09 (anti-oxidant); 29.05 (antimalarial) and 22.82 μg/ml (anti-inflammatory). Analytical procedures (GC–MS and HPLC-UV) were employed to generate chromatographic profiles for the essential oils and solvent extracts respectively. The HPLC analysis revealed the presence of rosmarinic acid in all three taxa while carnosic acid was only present in *Salvia repens* and *Salvia stenophylla*. The GC–MS analysis showed that oils were qualitatively and quantitatively variable. β-Caryophyllene was present in large amounts in all three taxa. Other components present include camphor, α-pinene and α-bisabolol. The results of the in vitro pharmacological activities provide a scientific basis to validate the use of these *Salvia* species in traditional medicine in South Africa.

Keywords: Anti-inflammatory; *Salvia*; Antimalarial; Antimicrobial; Essential oil; Anti-oxidant; GC–MS

1. Introduction

In many countries in the world, traditional medicine still plays a major role in primary health care, especially in the rural areas due to availability and cost. In South Africa, it is estimated that 80% of the black population consult with traditional healers (Jäger and van Staden, 2000). *Salvia* (Lamiaceae) is acknowledged worldwide as an important genus because of the beneficial uses of the essential oils produced by the foliage (Ahmed et al., 1994) and many *Salvia* species have been used in folk medicines making members of this genus a popular choice for researchers. There are about 900 *Salvia* species worldwide of which 26 are found in South Africa especially in the Cape region (Paton, 1991).

Until the discovery of antibiotics, *Salvia* was a frequent component of herbal tea mixtures, recommended to patients

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with tuberculosis to prevent sudation and was found to be an active ingredient in combined plant preparations for the treatment of chronic bronchitis. It has also been used as medication against perspiration, fever, rheumatism, sexual debility and in treating mental and nervous conditions as well as an insecticidal (Watt and Breyer-Brandwijk, 1962; Baricevic and Bartol, 2000).

In southern Africa, *Salvia stenopylla* Burch. ex Benth., *Salvia runcinata* L.f. and *Salvia repens* Burch. ex Benth. are used as disinfectants and as a purgative (Watt and Breyer-Brandwijk, 1962). A decoction of leaves is also used against fever, headache and digestive disorders (Amabeoku et al., 2001). Plant extracts may also be used to treat sores on the body, and decoctions for throat inflammation and female ailments (Watt and Breyer-Brandwijk, 1962).

Some *Salvia* species have been studied in many parts of the world and found to possess antibacterial (Ulubelen et al., 2001); anti-oxidant; anti-inflammatory; anticholinesterase (Perry et al., 2003) and anticancer (Li et al., 2002) activities. Due to the lack of studies on the medicinal properties of South African species, three related taxa (*Salvia stenophylla*, *Salvia repens* and *Salvia runcinata*) were chosen to validate the claims made by traditional medicine practitioners of the effectiveness of these plants.

2. Materials and methods

2.1. Plant collection

Plants were collected from the wild at three localities in South Africa during 2002; *Salvia stenophylla* from Sannieshof (26° 37′ 16″ S, 25° 34′ 57″ E), *Salvia repens* from Lady Grey (30° 41′ 57″ S, 27° 06′ 03″ E) and *Salvia runcinata* from Klerkskraal Dam (26° 15′ 17″ S, 27° 09′ 20″ E). The taxonomic identification was confirmed by the National Botanical Institute (Pretoria) and voucher specimens were deposited in the Department of Pharmacy and Pharmacology, University of the Witwatersrand. Gono-Bwalya (2003) investigated 30 samples of the above mentioned three species and confirmed the chemical uniformity in each of the taxa, hence a single collection of each species was selected for this comprehensive study.

2.2. Preparation of solvent extracts

Aerial parts were air-dried at room temperature and 5 g of the ground material was submerged in methanol and extracted for 3 h in a water bath at 30–40 °C and then filtered. This procedure was repeated twice.

2.3. Preparation of essential oils (EO)

Aerial parts of the plants were hydro-distilled for 4 h using a Clevenger apparatus. The oils obtained were kept at 4 °C until further analyzed for biological activity.

2.4. Determination of different pharmacological properties

2.4.1. Anti-oxidant activity

The anti-oxidant activity was assessed using a modified quantitative 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Shimada et al., 1992). The solution of DPPH was prepared with HPLC grade methanol and DPPH (Sigma–Aldrich). For each test sample, different concentrations were plated out in a 96-well plate with control wells containing DMSO. The absorbances were read at 550 nm after 30 min of incubation and the percentage of decolourisation determined. Vitamin C was used as the positive control. The IC_{50} values (concentration at which 50% of decolourisation was obtained) were determined using Enzfitter® software.

2.4.2. Anti-inflammatory activity

The inflammatory reaction can be prompted by a variety of mediators including eicosanoids derived from the cyclooxygenase (COX) and 5-lipoxygenase (5-LOX) pathways. Anti-inflammatory activity was determined using both 5-LOX (Baylac and Racine, 2003) and COX-2 (Zschocke and van Staden, 2000) assays. For the 5-LOX assay, the IC_{50} value (concentration at which 50% of the enzyme was inhibited) of each sample test was determined from the plot of percentage activity against concentration.

2.4.3. Antimalarial activity

The antimalarial activity of plant extracts and essential oils were assessed using the tritiated ^{3}H-hypoxanthine incorporation assay (Desjardins et al., 1979; van Zyl and Viljoen, 2002). The concentration required to inhibit 50% ^{3}H-hypoxanthine incorporation (IC_{50} value) was determined from the sigmoid dose response curve generated by the Enzfitter® software. Quinine was used as the positive control.

2.4.4. MTT toxicity assay

To investigate the toxicity profiles of the plant extracts and essential oils, the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay was performed on Graham cells (transformed human kidney cells) (Mosmann, 1983; van Zyl and Viljoen, 2002). The absorbance was read at the test wavelength of 540 nm and the reference wavelength of 690 nm using Ascent® software and the IC_{50} values (concentration at which 50% of cells were viable) were determined.

2.4.5. Antimicrobial activity

Preliminary antimicrobial screening was done with both the volatile and non-volatile extracts using six Gram-positive bacteria; Enterococcus faecalis (ATCC 29212) *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 2223), *Bacillus cereus* (ATCC 11778), *Bacillus subtilis* (ATCC 6051), methicillin resistant *Staphylococcus aureus* (MRSA) and eight Gram-negative bacteria; *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 8739), *Klebsiella pneumoniae* (NCTC 9633), *Salmonella*

typhimurium (ATCC 14028), *Salmonella enteriditis* (clinical strain), *Yersinia enterocolitica* (ATCC 23715), *Serratia odorifera* (ATCC 33132), *Proteus vulgaris* (clinical strain), two yeasts; *Cryptococcus neoformans* (ATCC 90112) and *Candida albicans* (ATCC 10231), two moulds; *Aspergillus niger* (clinical strain) and *Alternaria alternata* (clinical strain).

For the disc diffusion assay, 1 ml of the microbial suspension yielding an approximate inoculum size of 1×10^6 CFU/ml was inoculated into 100 ml of Tryptone Soya agar (Oxiod) and poured onto another 100 ml layer of solidified Tryptone Soya agar. Sterile 6 mm paper discs impregnated with essential oils or methanol extracts were placed on the seeded agar. The plates were allowed to pre-diffuse for 1 h at 4 °C before incubation overnight at 37 °C. Inhibition zones were measured after 24 h (bacteria), 48 h (yeasts) and 7 days (moulds) using vernier callipers from the edge of the disc. Neomycin (30 μg/disc), nystatin (30 μg/disc) (Oxoid) were used as the bacterial and fungal positive controls, respectively.

The methanol extracts with good activity were selected for the well-dilution minimum inhibitory concentration (MIC) assay (Eloff, 1998) against *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus* and *Bacillus subtilis*. Test sample free control wells were used to observe the normal bacterial growth. Ciprofloxacin (0.01 mg/ml) (Oxoid) was used as the positive control. To indicate the organism's growth, *p*-iodonitrotetrazolium violet (INT, Sigma) solution was added to the wells after 18 h of incubation. The plate was then observed after 6 h. MIC's were determined as the lowest concentrations preventing visible microbial growth.

2.5. High performance liquid chromatography (HPLC)

The detection and identification of non-volatile compounds were performed using a Waters 2690 HPLC System (Phenomenex Aqua C18 column, 250 mm × 2.1 mm) equipped with both a 996 photodiode array (PDA) detector and a Thermabeam mass selective detector (TMD). The TMD detector was operated in electron impact mode with the ionizer at 70 eV with a gain of 10 and scanning a mass range of 50–550 amu. The flow rate of the HPLC was 0.2 ml/min and the gas flow through the nebulizer 30 l/h. The nebulizer temperature was 80 °C, the expansion region 90 °C and the source temperature 225 °C. The TMD was operated in positive mode with no flow splitting and HPLC flow rate of 0.2 ml/min. Standards of rosmarinic acid and carnosic acid were injected separately and co-injected with samples in order to identify and confirm the presence of those compounds in the extracts by comparing different retention times, UV and MS data.

2.6. Gas chromatography coupled with mass spectroscopy (GC–MS)

GC–MS was used to analyze the essential oil samples. A Hewlett-Parkard GCD system was used with electron impact ionisation of 70 eV. The system was equipped with Innowax FSC column (60 mm × 0.25 mm) and helium used as the carrier gas. Analysis was then carried out using GC, oven temperature was held isothermal at 60 °C for 10 min and programmed to 220 °C at a rate of 4 °C/min. Split flow was adjusted to 50 ml/min. The injector temperature was at 250 °C, the mass range was from 35 to 425 *m/z*. Relative percentage amounts of the separated compounds were calculated automatically from the peak areas of the total ion chromatogram. The identity of the components was assigned by comparison of their retention indices and spectral data with corresponding data in the Başer library of essential oil constituents.

2.7. Statistical analysis

Unless otherwise specified, data were expressed as mean ± standard deviation of triplicate experiments. The differences between the IC_{50} values among taxa for each assay were compared using one-way ANOVA. All statistical tests were performed using Statistica software at 95% confidence limits.

3. Results

3.1. Anti-oxidant activity

The anti-oxidant activity of the methanol extracts and essential oils tested as compared with vitamin C are displayed in Table 1. The methanol extract of *Salvia runcinata* exhibited the best activity while *Salvia stenophylla* displayed the lowest activity. A statistical significant difference was observed between the activity of *Salvia stenophylla* and other taxa ($P < 0.002$) while no difference was noted between *Salvia runcinata* and *Salvia repens* ($P < 0.43$). In contrast with the methanol extracts, the essential oils showed very poor activity with all IC_{50} values being greater than 100 μg/ml (Table 1).

3.2. Anti-inflammatory activity

In the 5-LOX assay, the essential oil of *Salvia runcinata* exhibited the highest activity compared with the other two taxa (Table 1). Effective anti-oxidant treatment has also been found to be anti-inflammatory. In our study, the methanol extracts, which exhibited potent anti-oxidant activity, displayed poor anti-inflammatory activity in the 5-LOX assay (Table 1).

All the essential oils exhibited good activity against COX-2 (Table 1). In contrast with the 5-LOX assay where the essential oil of *Salvia runcinata* was a strong inhibitor, it was less active against the COX-2 enzyme while *Salvia repens* with moderate inhibition of 5-LOX was the most active against COX-2 assay.

Table 1
The IC_{50} values in μg/ml (mean ± S.D.) of antimalarial, anti-oxidant, anti-inflammatory activities and toxicity profile of three S*alvia* species and controls

Species		Antimalarial activity ($n = 3$)	Toxicity profile ($n = 3$)	Anti-oxidant activity ($n = 3$)	Anti-inflammatory	
					5-LOX	COX-2 (% inhibition by 1% solution of EO
Salvia stenophylla	Extract	17.03 ± 3.21	21.67 ± 3.87	15.30 ± 2.90	>100	n.d.
	EO	4.38 ± 1.07	6.68 ± 0.27	>100	26.48 ± 0.70	35.27
Salvia runcinata	Extract	29.0 ± 4.63	>100	6.09 ± 0.86	>100	n.d.
	EO	1.23 ± 0.31	2.54 ± 0.73	>100	22.81 ± 0.01	15.93
Salvia repens	Extract	78.90 ± 6.10	>100	8.52 ± 0.55	>100	n.d.
	EO	1.68 ± 0.26	6.66 ± 0.90	>100	28.04 ± 4.50	60.21
Controls		0.13 ± 0.04[a]	>80[b]	2.93 ± 0.42[c]	5.0 ± 0.5[d]	52.0[e]

n.d: Not determined EO: essential oil.
[a] Quinine.
[b] Quinine.
[c] Vitamin C.
[d] Nordihydroguaiaretic acid (NDGA).
[e] Indomethacin (200 μM).

3.3. Antimalarial activity

The antimalarial activity varied greatly between volatile (essential oil) and non-volatile (methanol extracts) components (Table 1). The essential oils showed good antimalarial activity. Although essential oil of *Salvia stenophylla* differs statistically from the others ($P < 0.002$), there is no significant difference between *Salvia runcinata* and *Salvia repens* ($P < 1$).

The methanol extracts exhibited moderate antimalarial activity compared with essential oils (Table 1). The IC_{50} values were variable for all three taxa ($F_{2,6} = 140.55$, $P < 0.001$) with the extract of *Salvia stenophylla* displaying the most favorable activity which contrasts that of the essential oil.

3.4. Toxicity profile

In general the solvent extract showed a low toxicity profile except for *Salvia stenophylla* (Table 1). However, the essential oils displayed a more toxic profile with *Salvia runcinata* exhibiting the highest toxicity. No significant difference was observed ($P < 1$) between the essential oil of *Salvia repens* and *Salvia stenophylla*.

3.5. Antimicrobial activity

All the essential oils screened against Gram-negative bacteria, yeasts and moulds displayed poor activity compared to their respective controls (data not shown). The methanol extracts also exhibited poor activity against Gram-negative bacteria. In contrast, the methanol extracts inhibited the growth of all Gram-positive bacteria, with *Salvia stenophylla* exhibiting the highest activity against *Staphylococcus aureus* and *Bacillus cereus*, while *Salvia runcinata* was the least active (Table 2). The Gram-positive bacteria, *Staphylococcus aureus* (ATCC 25923) was the most susceptible strain to the methanol extracts while *Staphylococcus epidermidis* was the least susceptible.

3.6. GC–MS and HPLC-UV profiles

The volatile oils showed a variation both in yield and chemical composition (Table 3). A total of 55 constituents were identified in *Salvia repens* consisting mostly of β-phellandrene (22.2%), β-caryophyllene (12.4%), limonene (9.8%) and camphor (6.9%) while 73 constituents were characterised for *Salvia runcinata*

Table 2
Antimicrobial activity of the solvent extracts of three related taxa of *Salvia* determined by the disc diffusion assay (mm) and micro-dilution assay (in μg/ml) for Gram-positive organisms

Species	*Staphylococcus aureus* (ATCC 25923)		*Staphylococcus epidermidis* (ATCC 2223)		*Bacillus cereus* (ATCC 11778)		*Bacillus subtilis* (ATCC 6051)	
	MIC	IZ	MIC	IZ	MIC	IZ	MIC	IZ
Salvia stenophylla	98.0	5.0	3130.0	5.0	98.0	6.0	390.0	6.50
Salvia runcinata	313.0	1.0	3130.0	1.0	3130.0	3.0	1560.0	3.0
Salvia repens	390.0	2.0	3130.0	2.0	390.0	5.0	625.0	4.0
Controls	0.31	6.0	0.63	6.0	0.63	6.0	0.04	5.0

IZ: Inhibition zone, Neomycin (30 μg/disc), Ciprofloxacin (0.01 mg/ml).

Table 3
Percentage composition of the essential oil of *Salvia stenophylla*, *Salvia runcinata* and *Salvia repens* with their relative retention times (RRI)

RRI	Compounds	*Salvia stenophylla*	*Salvia runcinata*	*Salvia repens*
1000	Decane	–	0.1	–
1014	Tricyclene	0.1	tr	0.2
1032	α-Pinene	2.7	1.8	6.6
1035	α-Thujene	tr	–	–
1072	α-Fenchene	tr	–	–
1076	Camphene	3.3	0.6	4.0
1100	Undecane	–	tr	–
1118	β-Pinene	0.7	0.8	3.0
1132	Sabinene	0.1	tr	0.2
1145	Ethylbenzene	–	tr	–
1146	δ-2-Carene	tr	–	–
1159	δ-3-Carene	18.4	0.3	0.1
1174	Myrcene	1.7	0.2	2.3
1176	α-Phellandrene	tr	–	–
1187	*o*-Cymene	0.1	-	–
1188	α-Terpinene	0.3	tr	0.2
1203	Limonene	5.3	0.6	9.8
1205	Sylvestrene	1.3	–	–
1213	1,8-Cineole	–	2.0	–
1218	β-Phellandrene	2.9	tr	22.2
1244	Amylfuran	–	tr	–
1246	(*Z*)-β-Ocimene	tr	0.4	2.7
1255	γ-Terpinene	0.5	0.1	0.5
1265	5-Methyl-1,3-heptanone	–	tr	–
1266	(*E*)-β-Ocimene	–	0.8	1.5
1278	*m*-Cymene	0.2	–	–
1280	*p*-Cymene	tr	0.2	0.7
1282	*cis*-Allo ocimene	–	–	0.1
1286	Isoterpinolene	0.8	–	–
1290	Terpinolene	0.5	0.1	0.3
1327	(*Z*)-3-hexenylacetate	–	–	0.1
1348	6-Methyl-5-hepten-2-one	–	tr	–
1360	Hexanol	–	tr	–
1382	*cis*-Allo ocimene	–	tr	–
1393	3-Octanol	–	0.1	0.1
1400	Nonanal	tr	tr	–
1443	2,5-Dimethylstyrene	0.1	–	–
1450	*trans*-Linalool-oxide	-	tr	–
1452	1-Octen-3-ol	0.2	0.4	0.5
1467	6-Methyl-1,5-hepten-2-ol	–	–	0.1
1474	*trans*-Sabinene hydrate	0.1	0.1	0.3
1478	*cis*-Linalool oxide	–	0.1	–
1497	α-Copaene	–	0.1	–
1532	Camphor	6.0	2.0	6.9
1544	α-Gurjunene	–	–	0.2
1553	Linalool	0.1	0.3	0.2
1556	*cis*-Sabinene hydrate	–	–	0.2
1562	Octanol	–	tr	–
1568	1-Methyl-1,4-acetyl-cyclo-hex-1-ene	–	0.2	–
1571	*trans*-*p*-menth-2-en-1-ol	0.2	–	0.2
1586	Pinocarvone	–	–	0.1
1589	*iso*-Caryophyllene	–	0.6	–
1594	*trans*-β-Bergamotene	–	0.6	–
1597	Bornyl acetate	–	–	0.5
1612	β-Caryophyllene	7.3	11.4	12.4
1628	Aromadendrene	1.2	–	0.9
1638	*cis*-*p*-menth-2-en-1-ol	–	–	0.2
1638	β-Cyclocitral	–	0.1	–
1650	γ-Elemene	–	–	0.5
1661	Alloaromadendrene	0.1		0.5
1668	(*Z*)-β-Farnesene	tr	tr	–
1687	α-Humulene	1.7	2.7	3.2
1695	(*E*)-β-Farnesene	–	0.2	–

Table 3 (*Continued*)

RRI	Compounds	*Salvia stenophylla*	*Salvia runcinata*	*Salvia repens*
1706	α-Terpineol	1.0	0.2	–
1719	Borneol	4.8	0.3	1.5
1741	β-Bisabolene	0.2	0.9	–
1755	Bicyclogermacrene	–	–	0.8
1755	Sesquicineole	–	0.5	–
1766	1-Decanol	–	tr	–
1773	δ-Cadinene	0.5	tr	0.3
1776	γ-Cadinene	–	tr	–
1783	β-Sesquiphellandrene	–	0.3	–
1804	Myrtenol	1.0	–	–
1805	α-Campholene-alcohol	–	–	2.3
1838	(*E*)-β-Damascenone	–	tr	–
1845	*trans*-Carveol	tr	tr	0.2
1853	*cis*-Calamenene	–	tr	–
1854	Germacrene B	–	–	0.6
1864	*p*-Cymen-8-ol	0.2	tr	–
1868	(*E*)-Geranylacetone	tr	tr	–
1900	*epi*-Cubelol	–	tr	–
1941	α-Calacorene-I	tr	tr	–
1949	*trans*-Jasmone	–	0.1	–
1969	*cis*-Jasmone	–	0.2	–
1984	α-Calacorene-II	–	tr	–
2008	Caryophyllene-oxide	1.2	6.7	2.0
2045	Humulene-epoxide I	tr	–	–
2050	(*E*)-Nerolidol	0.3	6.8	–
2057	Ledol	–	–	0.6
2071	Humulene-epoxide-II	0.4	1.4	0.5
2098	Globulol	–	–	0.2
2103	Guaiol	3.3	–	–
2104	Viridifloral	0.3	–	3.5
2131	Hexahydrofarnesyl acetone	–	0.1	–
2144	Spathulenol	0.3	–	1.0
2156	α-Bisabolol oxide B	–	1.7	–
2162	Bisabolol oxide	0.1	1.8	–
2185	γ-Eudesmol	–	0.1	–
2187	τ-Cadinol	0.3	–	0.2
2209	τ-Muurolol	–	–	0.1
2232	α-Bisabolol	8.2	41.1	0.7
2250	α-Eudesmol	0.8	–	0.1
2255	α-Cadinol	–	–	0.3
2256	*epi*-α-Bisabolol	–	2.1	–
2257	β-Eudesmol	0.9	–	–
2324	Caryophylladienol-II	0.7	1.4	0.3
2389	Caryophyllenol-I	–	0.8	0.9
2392	Caryophyllenol-II	0.7	1.1	0.1
2518	*cis*-Lanceol	3.6	1.5	0.3
2676	Manool	10.1	–	–
Total		94.8	96.0	98.0

tr: Traces: <0.1%.

with the major constituents being α-bisabolol (41.1%), β-caryophyllene (11.4%), caryophyllene-oxide (6.9%), *(E)*-nerolidol (6.8%) and α–humulene (2.7%). In contrast, 59 compounds were identified in *Salvia stenophylla* with δ-3-carene (18.4%), manool (10.1%), α-bisabolol (8.2%), β-caryophyllene (7.3%) and camphor (6.0%) being identified as the major constituents. Overall, the ketones and alcohols were the most abundant components with β-caryophyllene being the major component present in large amounts (7–12%) in all three taxa.

The HPLC-UV-MS profiles (Fig. 1) revealed the presence of rosmarinic acid in all three taxa, while carnosic acid was present in *Salvia stenophylla* and *Salvia repens*.

4. Discussion

Essential oils/extracts of all three taxa showed potent anti-malarial, anti-oxidant, anti-inflammatory and antibacterial activities. The essential oils from aromatic and medicinal

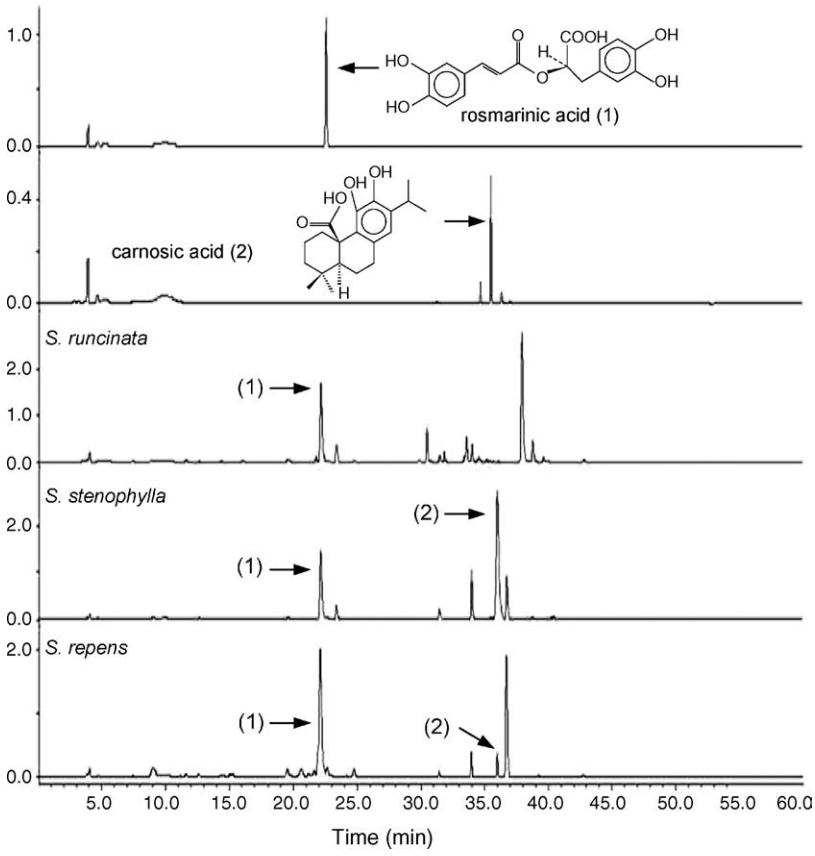

Fig. 1. HPLC, chromatograms of *Salvia runcinata, Salvia stenophylla* and *Salvia repens* and standards of rosmarinic and carnosic acids.

plants such as sage (Dorman et al., 1995) and other Lamiaceae species have been known since antiquity to possess biological activity notably anti-oxidant properties (Baratta et al., 1998).

All the methanol extracts for *Salvia runcinata*, *Salvia stenophylla* and *Salvia repens* displayed anti-oxidant activity (Table 1) as previously reported for other *Salvia* species including *Salvia officinalis* (Cuvelier et al., 1994). Rosmarinic acid, a well-known anti-oxidant was found as a major compound in all the methanol extracts (Fig. 1) and it is a common constituent of the Lamiaceae family and in particular the subfamily Nepetoideae (Janicsák et al., 1999). The anti-oxidant activity of the methanol extract may partly be associated with the presence of compounds with known anti-oxidant activity such as rosmarinic acid, carnosic acid, isocarnosol and carnosol (Cuvelier et al., 1996; Masuda et al., 2002).

In our study, essential oils showed very poor anti-oxidant activity ($IC_{50} > 100\ \mu g/ml$) which contrasts the good anti-oxidant activity ($IC_{50} = 17.8\ \mu g/ml$) previously reported for *Salvia* species including *Salvia multicaulis*. This could be accounted by the fact that in our study, we used a stable free radical in the form of DPPH; while in other studies the unstable radicals generated by either lipid peroxidation or by modifying thiobarbituric acid were used (Tepe et al., 2004). Furthermore, many compounds such as thymol, carvacrol, *p*-cymene, linalool and eugenol have been demonstrated to possess in vitro anti-oxidant properties (Dorman et al., 1995) and these compounds were present in low amounts or totally absent in the essential oils studied (Table 3).

The essential oil of *Salvia runcinata*, a strong inhibitor of 5-LOX was less active against COX-2 demonstrating variable activity in the two inflammation pathways. The sesquiterpenes such as α-bisabolol appear to be good 5-LOX inhibitors and have been widely reported to have a skin soothing action which strongly inhibits 5-LOX in vitro and essential oils containing higher proportion of this sesquiterpene alcohol like *Salvia runcinata* are thus expected to inhibit the enzyme (Baylac and Racine, 2003).

The methanol extracts displayed low antimalarial activity compared with essential oils which exhibited high antimalarial activity. Given the fact that, there was not significant difference between the antimalarial activity of *Salvia runcinata* and *Salvia repens*, these plants could be used interchangeably if their toxicity profiles were acceptable.

The potent antimalarial activity of the essential oil might be attributed to high sesquiterpene content. Sesquiterpenoids and their derivatives are credited with numerous biological properties including antimalarial activity. Recently, nerolidol (an acyclic oxygenated sesquiterpene) from leaves of *Virola surinamensis* (Rol.) Warb. was identified as an active component of an essential oil that displayed antimalarial activity

(Lopes et al., 1999). Moreover, parasites treated with nerolidol showed decreased ability to synthesize co-enzyme Q in all intra-erythrocytic stages (Boyom et al., 2003). In a study evaluating the antimalarial activity of some essential oil constituents, nerolidol was found to possess antimalarial activity (van Zyl and Viljoen, 2003). In our study, nerolidol was identified in the essential oil of *Salvia stenophylla* and *Salvia runcinata* and therefore might contribute to the observed antimalarial activity. In addition to the toxicity of essential oils, the physical properties (low density) and ability to readily diffuse across cell membranes might enhance targeting to intracellular malarial parasites (Boyom et al., 2003).

Camphor is known to be highly toxic if used in prolonged treatment or when ingested in small amounts. Nerolidol has also proven to have toxic properties (van Zyl and Viljoen, 2003). The toxicity associated with the *Salvia* essential oil is possibly due to the presence of camphor and nerolidol. Thus, given the potential toxicity of the essential oil constituents, the essential oil should be used with caution and extracts seem to be more safe in traditional medicine when taken as decoction.

The essential oils displayed poor antimicrobial activity which contrasted with the antibacterial activity of the methanol extracts (Table 2). Essential oils are very complex mixtures which constitute mainly monoterpenes and sesquiterpenes. These constituents have been shown to have antimicrobial activity (Shane et al., 1999). Evaluation of the antimicrobial activity of essential oils is quite problematic due to many factors such as the composition and solubility of the oil, the source of the test organism used, and the quantity of the active compound in the essential oil.

The essential oil rich in compounds such as carvacrol, thymol, α-and β-thujene possessed high levels of antimicrobial activity (Jalšenjak et al., 1987) and the essential oil of the *Salvia stenophylla* species complex contain more monoterpenes such as α-pinene, β-pinene, γ-terpene, limonene, δ-3-carene and *p*-cymene which are reported to exhibit little activity (Shane et al., 1999).

The test method used for assessing the antimicrobial activity is often the disc diffusion method, which is highly dependent on water solubility and the ability of test components to diffuse through the agar. Thus, it would be expected that hydrophobic compounds such as the essential oils would show less activity.

In contrast with the essential oils, the methanol extracts displayed promising antibacterial activity. This is consistent with the pattern of in vitro inhibition emerging from other studies (Izzo et al., 1995) in which it was found that plant extracts and essential oils readily inhibit Gram-positive rather than Gram-negative bacteria. The antibacterial properties of the methanol extracts in this study could be attributed to the presence of carnosol derivatives that are reported to have strong antibacterial activity as well as anti-oxidant properties. For example, in their study on *Salvia miltiorrhiza*, Lee et al. (1999) found that the ability of the compounds to generate superoxide radicals also inhibited bacterial growth by non-selective inhibitory action on DNA, RNA and protein synthesis.

In conclusion, the collective results on the pharmacological activity of these three species provide an in vitro scientific support for the use of these species in traditional herbal preparations and it seems likely that these plants may act at various therapeutic levels.

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