# Antioxidant, antiinflammatory activities and HPLC analysis of South African *Salvia* species

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A B S T R A C T

The antioxidant and antiinflammatory activities of the methanol:chloroform (1:1) extracts of 16 *Salvia* species indigenous to South Africa were evaluated. Antioxidant activity was measured using the 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assays and compared to the control values obtained with Trolox®. Nearly all the solvent extracts displayed antioxidant activity, with the $IC_{50}$ value ranging from 1.6 to 74.5 µg/ml using DPPH·, whilst the $IC_{50}$ values ranged from 11.9 to 69.3 µg/ml, when tested with ABTS·+. The extract of *Salvia schlechteri*, with an $IC_{50}$ value of 1.6 µg/ml, was three times more active than the reference compound, Trolox® ($IC_{50}$ value: 2.51 µg/ml). The antiinflammatory activity was evaluated using the 5-lipoxygenase assay. With the exception of *Salvia radula* ($IC_{50}$ value: 78.8 µg/ml), the extracts displayed poor inhibition of the 5-lipoxygenase enzyme, with all $IC_{50}$ values being greater than 100 µg/ml. The total phenolic content based on gallic acid equivalents (GAE) confirmed the presence of total soluble phenolics in the various extracts from 45 to 211 mg of GAE per g dry sample and showed strong association ($r^2 = 0.90$) with antioxidant activity. High-performance liquid chromatography (HPLC) was used to identify various compounds in the extracts. Betulafolientriol oxide and rosmarinic acid were detected in all the species investigated, and rosmarinic acid, carnosic acid, carnosol and oleanolic acid/ursolic acid were abundant in many species.

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## 1. Introduction

Recent developments in biomedical science emphasise the involvement of free radicals in many diseases. There is increasing evidence to suggest that many degenerative diseases, such as brain dysfunction, cancer, heart disease and immune system decline could be the result of cellular damage caused by free radicals and that antioxidants may play an important role in disease prevention (Aruoma, 1998). Phenolic compounds are known to exhibit a range of biological activities, including anticancer, antibacterial, antioxidant and antiinflammatory properties (Cuvelier, Berset, & Richard, 1994; Lu & Foo, 2002). In recent years, a large body of evidence has been accumulated, demonstrating that free radicals are important components of inflammation. The majority of the phenolic acids in *Salvia* species are exclusively those of caffeic acid derivatives. Caffeic acid plays a central role in the biochemistry of the Lamiaceae and occurs predominantly in dimer form as rosmarinic acid (Gerhardt & Schroeter, 1983). In many *Salvia* species, caffeic acid is the building block of a variety of plant metabolites, ranging from simple monomers to multiple condensation products that give rise to a variety of oligomers (Lu & Foo, 2002). The trimers and tetramers are also interesting from a therapeutic point of view as they have demonstrated various biological activities (Lu & Foo, 2002). Compounds, such as carnosic acid and ursolic acid, which have potent antioxidant activity, have also been shown to possess antiinflammatory activity (Baricevic et al., 2001; Sala et al., 2003).

The genus *Salvia* belongs to the family Lamiaceae and encompasses about 900 species worldwide of which 26 are found in southern Africa (Jäger & Van Staden, 2000). Members of the genus are extensively used in South Africa in healing rites, especially to treat infections. Although sage is a popular kitchen herb, which has been used in a variety of food preparations since ancient times (Durling et al., 2007), no report has shown the commercial use of indigenous species by the food industry, probably due to a lack of data. In South Africa a tea is prepared from *Salvia africana-lutea* to treat coughs, colds, bronchitis and female ailments (Watt & Breyer-Brandwijk, 1962). Many Lamiaceae extracts are of commercial interest to the food industry as a source of natural antioxidants (Thorsen & Hildebrandt, 2003). The quality of antioxidant activity is highly correlated with phenolic compounds (e.g., carnosic acid, rosmarinic acid and caffeic acid) (Thorsen & Hildebrandt, 2003).

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In this study, we investigated the *in vitro* antioxidant and anti-inflammatory activities and identified some compounds present in the solvent extracts of indigenous South African *Salvia* species.

## 2. Materials and methods

### 2.1. Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), Tween® 20, linoleic acid and potassium persulfate ($K_2S_2O_8$) were purchased from Fluka, whilst 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 3,4,5-trihydroxybenzoic acid (gallic acid), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®), caffeic acid, carnosic acid, kaempferol, oleanolic acid, rosmarinic acid and ursolic acid were obtained from Sigma (St. Louis, MO). Folin–Ciocalteu phenol reagent was purchased from Merck (Darmstadt, Germany). 5-Lipoxygenase and nordihydroguaiaretic acid (NDGA) were obtained from Cayman Chemical Company (Ann Arbor, MI). Carnosol and 7-*O*-methylepirosmanol were isolated from *Salvia chamelaeagnea* (Kamatou, Van Vuuren, Van Heerden, Seaman, & Viljoen, 2007), whilst betulafolientriol oxide and salvigenin were isolated from *Salvia radula* (Kamatou, 2006). All solvents used and other chemicals were of analytical grade and used without further purification.

### 2.2. Plant material and extraction

Based on traditional use, the aerial parts of 16 South African *Salvia* species were harvested from January to December 2004, during the growing season, at various localities in South Africa (Table 1). The plants were identified at the South African National Biodiversity Institute (Pretoria) and voucher specimens are housed in the Department of Pharmaceutical Sciences, Tshwane University of Technology (Pretoria). The plant materials were air dried at room temperature, powdered in a grinder, sieved (Mesh No. 52, aperture 300 microns) and extracted with methanol:chloroform (1:1) for 4 h at 37 °C. The extracts were obtained by removing methanol:chloroform with a rotary evaporator at 62–63 °C.

### 2.3. Evaluation of the antioxidant activity

#### 2.3.1. The ABTS method

The antioxidant activity was evaluated using the ABTS$^{\cdot+}$ method (Moolla, Van Vuuren, Van Zyl, & Viljoen, 2007). Stock solutions of the solvent extracts (10 mg/ml) and dilutions were prepared with DMSO. To a sample volume of 50 μl of each concentration, in a cuvette, 1 ml of the ABTS$^{\cdot+}$ was added and kept at 30 °C for 4 min in a water bath before the absorbance was recorded at 734 nm. The ABTS$^{\cdot+}$ stock solution (7 mM) was generated with $K_2S_2O_8$ as the oxidant agent. Each sample was tested in duplicate. The percentage of decolourisation was calculated from Eq. (1) using Microsoft Excel® software. Trolox® was used as positive control:

$$\% \text{ Decolourisation} = \left(1 - \frac{A_{\text{sample}}}{A_{\text{control}}}\right) \times 100 \tag{1}$$

where $A$ = absorbance at 734 nm.

#### 2.3.2. The DPPH method

A DPPH assay (Lourens, Reddy, Başer, Viljoen, & Van Vuuren, 2004) was also employed to investigate the antioxidant activity of different plant extracts. From the stock solution (10 mg/ml in DMSO), a 1:20 dilution was made with DMSO followed by two fold serial dilutions. Samples at various concentrations (50 μl) were plated out in triplicate in a 96 well-plate with the appropriate control. For the DPPH and methanol controls, 50 μl of DMSO were added into the wells. A volume of 200 μl of DPPH$^{\cdot}$ (96 μM in HPLC grade methanol) was added to the test samples, whilst 200 μl of HPLC grade methanol was added to the methanol control wells. The plate was then shaken at 960 rpm for 2 min in a microtitre plate reader (Labsystems Multiskan RC connected to a computer equipped with Genesis® version 3.03 software) and incubated in the dark, at room temperature, for 30 min. The absorbance was read after incubation using a spectrophotometer (Labsystems Multiskan RC) at a single wavelength of 550 nm and the percentage of decolourisation was determined using Microsoft Excel® software (Eq. (2)). Trolox® was used as positive control:

$$\% \text{ Decolourisation} = \frac{100 \times [(A_{\text{control}}) - (A_{\text{test}} + A_{\text{methanol}})]}{A_{\text{control}}} \tag{2}$$

where $A$ = absorbance at 550 nm, $A_{\text{control}}$ = average absorbance of DPPH − average absorbance of methanol, $A_{\text{test}}$ = average absorbance obtained in the wells containing DPPH and test sample, $A_{\text{methanol}}$ = average absorbance obtained in the wells containing methanol and test sample (no DPPH).

### 2.4. Antiinflammatory activity: the 5-lipoxygenase assay

The 5-lipoxygenase assay was used as an indication of the anti-inflammatory activity (Baylac & Racine, 2003). An aliquot of 50 μl of the stock solution (10 mg/ml in DMSO and Tween® 20 mixture;

**Table 1**
Peak area (%) of various compounds in the solvent extracts of 16 indigenous *Salvia* species.

| Species | Caffeic acid | Rosmarinic acid | Kaempferol | 7-*O*-Methyl-epirosmanol | Salvigenin | Carnosol | Carnosic acid | Oleanolic/ursolic acid | Betulafolientriol oxide |
|---|---|---|---|---|---|---|---|---|---|
| *S. africana-caerulea* | + | ++ | – | – | – | – | ++ | + | + |
| *S. africana-lutea* | – | ++ | – | – | – | – | ++ | + | + |
| *S. albicaulis* | + | +++ | – | – | – | + | – | + | + |
| *S. aurita* | + | ++ | – | – | – | ++ | + | + | + |
| *S. chamelaeagnea* | + | ++ | – | + | – | ++ | ++ | + | + |
| *S. disermas* | + | + | – | – | ++ | – | – | ++ | + |
| *S. dolomitica* | + | ++ | – | – | – | – | – | + | + |
| *S. garipensis* | – | ++ | – | – | + | – | – | – | + |
| *S. lanceolata* | + | + | – | – | – | + | – | ++ | + |
| *S. muirii* | + | +++ | – | – | + | + | ++ | + | + |
| *S. namaensis* | + | ++ | – | + | – | +++ | ++ | + | + |
| *S. radula* | – | + | – | – | + | – | – | – | + |
| *S. repens* | + | ++ | – | – | – | ++ | ++ | + | + |
| *S. runcinata* | + | +++ | – | – | + | – | + | + | + |
| *S. schlechteri* | + | ++ | – | – | – | + | + | + | + |
| *S. stenophylla* | – | + | – | – | – | ++ | ++ | + | + |

–, not detected; +, area < 10.0%; ++, 10.1% < area < 20.0%; +++, area > 30%.

1:29, w/w) of each test sample was placed in a 3-ml cuvette, followed by 2.95 ml of pre-warmed 0.1 M potassium phosphate buffer (pH 6.3) and 48 µl of linoleic acid solution. Thereafter, 12 µl of the ice-cold buffer (potassium phosphate) were mixed with 100 U of the thawed enzyme. The mixture was then transferred to the cuvette and the contents of the cuvette was shaken and placed into the spectrophotometer (Specord Analytikjena-40 connected to Winaspect® software; Ananlytikjena UK, Wembley, UK), before the absorbance was recorded at 234 nm. It is important to note that, prior to testing the sample, two samples were prepared as mentioned above but only with DMSO and Tween® 20 mixture, to serve as controls (no enzyme inhibition). 5-Lipoxygenase is known to catalyse oxidation of unsaturated fatty acids containing 1-4 diene and the modification of linoleic acid (1-4-diene into 1-3-diene) can be detected at 234 nm. Further dilutions were prepared only for sample(s) inhibiting the enzyme activity by at least 80%. Nordihydroguaiaretic acid (NDGA) was used as positive control.

### 2.5. Determination of total phenolic content

The total phenolic content (TPC) of the solvent extracts was determined by the method using Folin–Ciocalteu reagent and gallic acid as standard to produce the calibration curve (Slinkard & Singleton, 1977). A 2-ml volume test sample (4 mg/4 ml; in DMSO) was mixed with 10 ml of Folin–Ciocalteu reagent, followed by the addition of 8 ml of anhydrous sodium carbonate. After incubation at room temperature in the dark for 2 h, the absorbance of the reaction mixture was read twice at 765 nm against a sample containing only DMSO, using a double beam spectrophotometer (Hitachi). TPC was expressed as mg gallic acid equivalents (GAE) per gram of dry mass. Duplicate samples were prepared for the analysis.

### 2.6. High-performance liquid chromatography

Certain compounds present in the extracts were identified using a Waters 2695 HPLC system (Waters Corporation, Milford, MA) equipped with both a 2996 photodiode array (PDA) detector and a Thermabeam electron impact (EI) mass selective detector (TMD). The TMD detector was operated in EI mode with the ioniser at 70 eV (fixed) with a gain of 10 and scanning mass range of 50–550 amu. Analysis was performed on a Phenomenex Aqua C18 column (250 × 2.1 mm, 5 µm; Phenomenex, Torrance, CA) thermostated at 40 °C. The flow rate of the HPLC was 0.2 ml/min and the gas flow through the nebuliser 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 ion mode with no flow splitting, thereby utilising the full HPLC eluent at a flow rate of 0.2 ml/min. The mobile phase started with 10% acetonitrile, 90% water containing 10 mM formic acid. The solvent ratio was changed through a linear gradient to 90% acetonitrile, 10% water (with 10 mM formic acid) at 40 min. This ratio was maintained for 10 min where after the solvent ratio was changed back to the initial conditions. Standards of caffeic acid, carnosic acid, kaempferol, oleanolic acid, rosmarinic acid, ursolic acid, carnosol, 7-O-methylepirosmanol, betulafolientriol oxide and salvigenin were injected separately and co-injected with samples (10 µl, 50 mg/ml). Comparison of the MS spectra of standards after fragmentation of the parent ion together with retention time (Rt) and ultra-violet (UV) spectra were used to confirm the presence/absence of these compounds in the solvent extracts using Empower 1® software.

### 2.7. Data analysis

The $IC_{50}$ values (concentration at which 50% of decolourisation is obtained) were determined from the log sigmoid dose–response curves generated by Enzfitter® Version 1.05 software. All results of the antioxidant activity were obtained from three independent experiments and given as mean ± standard deviation (SD). Experimental data were analysed with ANOVA followed by the t-tests using Statistica Version 5.0 and Prism® Version 3.0 software (Statsoft Inc., Tulsa, OK). Throughout the analysis, $p < 0.05$ was considered significant. The correlation between the antioxidant activity and total phenolic content was determined using the linear regression equation.

## 3. Results and discussion

### 3.1. HPLC analysis of the solvent extracts

The HPLC analysis revealed the presence of various compounds in Salvia species (Table 1). Betulafolientriol oxide and rosmarinic acid were the two compounds identified in all 16 extracts (Table 1). Rosmarinic acid, carnosic acid, carnosol and ursolic acid were present in most of the extracts at relatively high levels. The extracts of Salvia albicaulis, S. runcinata and S. muirii were particularly rich in rosmarinic acid (Table 1). Carnosol was abundant in Salvia namaensis. Salvigenin and oleanolic acid/ursolic acid were higher in Salvia disermas (Table 1). 7-O-Methylepirosmanol was detected only in S. namaensis and S. chamelaeagnea. Kaempferol reported in exotic Salvia species was absent in all the 16 Salvia species investigated (Table 1). S. albicaulis, S. muirii and S. runcinata may therefore be considered as an alternative commercial source of natural rosmarinic acid, whilst S. namaensis can be consider as a natural source of carnosol.

The majority of the phenolic acids in Salvia species are exclusively caffeic acid derivatives (Gerhardt & Schroeter, 1983). Caffeic acid plays a central role in the biochemistry of Lamiaceae and occurs predominantly in the dimer form as rosmarinic acid. The monomers that are frequently present in Salvia species are represented by caffeic acid (Qian & Li, 1992) and other monomeric derivatives, including ferulic acid (Cuvelier, Richard, & Berset, 1996) and isoferulic acid (Ai & Li, 1992). Caffeic acid was present in 75% of the extracts investigated (Table 1). It was also identified in a dimer form (rosmarinic acid) in all the species investigated.

Rosmarinic acid is the most abundant caffeic dimer in Salvia species (Ai & Li, 1988; Cuvelier et al., 1994). Several studies have reported on the detection and quantification of rosmarinic acid in various Salvia preparations (Janicsák, Máthe, Miklóssy-Vári, & Blunden, 1999). The variation in levels of caffeic acid and rosmarinic acid in the same plant has been shown. Janicsák, Máthe, Miklóssy-Vári, and Blunden (1999) demonstrated that concentrations of caffeic acid were always much lower than those of rosmarinic acid. In all the species studied where the two compounds were detected together, the same pattern was observed (Table 1).

### 3.2. Determination of the antioxidant activity

Several solvent extracts displayed potent activity against both ABTS·+ and DPPH· (Table 2). The $IC_{50}$ values ranged from 11.9 ± 1.52 to 69.3 ± 3.22 µg/ml and from 1.61 ± 0.03 to 74.2 ± 2.85 µg/ml for ABTS and DPPH assays, respectively. The solvent extract of Salvia schlechteri displayed the most favourable activity against DPPH·, and was significantly more active than the reference compound, Trolox® ($p < 0.05$). The extract of S. muirii was the most active against ABTS·+. The extracts of S. radula and S. dolomitica, which were poorly active ($IC_{50}$ values >100 µg/ml) against DPPH·, were moderately active against ABTS·+ ($IC_{50}$ values: 69.3 ± 3.22 and 49.9 ± 1.17 µg/ml, respectively; Table 2).

**Table 2**
Antioxidant, antiinflammatory activities and total phenolic content of the solvent extracts of 16 indigenous *Salvia* species (values followed by ± are SD of the mean, $n = 3$).

| Species | Locality/voucher | Antioxidant activity ($IC_{50}$ values in µg/ml) | | Antiinflammatory activity ($IC_{50}$ values in µg/ml) | Total phenolic content mg/g GAE |
|---|---|---|---|---|---|
| | | ABTS assay | DPPH assay | | |
| *S. africana-caerulea* L. | South Western Cape (AV 875) | 39.7 ± 1.76 | 33.4 ± 3.73 | >100 | 115 |
| *S. africana-lutea* L. | South Western Cape (AV 873) | 30.4 ± 2.97 | 47.6 ± 2.61 | >100 | 67.8 |
| *S. albicaulis* Benth. | KBG (AV 894) | 24.2 ± 1.28 | 19.9 ± 1.01 | >100 | 100 |
| *S. aurita* L.f | KBG (AV1066) | 22.9 ± 2.57 | 16.6 ± 0.22 | >100 | 119 |
| *S. chamelaeagnea* Berg. | KBG (AV 848) | 14.6 ± 2.25 | 12.8 ± 1.04 | >100 | 212 |
| *S. disermas* L. | KBG (AV1194) | 33.1 ± 2.86 | 55.1 ± 4.01 | >100 | 69.0 |
| *S. dolomitica* Codd | ex Manning (AV 838) | 49.9 ± 1.17 | >100 | >100 | 53.0 |
| *S. garipensis* E.Mey. ex Benth. | KBG (AV1193) | 41.7 ± 2.71 | 74.2 ± 2.85 | >100 | 45.6 |
| *S. lanceolata* Lam. | South Western Cape (AV 877) | 25.6 ± 0.66 | 68.1 ± 3.69 | >100 | 54.2 |
| *S. muirii* L. Bol. | KBG (AV 874) | 11.9 ± 1.52 | 11.1 ± 0.52 | >100 | 186 |
| *S. namaensis* Schinz | Swartberg (AV 497) | 16.6 ± 2.56 | 10.6 ± 0.75 | >100 | 191 |
| *S. radula* Benth. | Road to Derby (AV 880) | 69.3 ± 3.22 | >100 | 78.8 ± 5.89 | 55.7 |
| *S. repens* Burch. ex Benth. | KBG (AV 615) | 18.2 ± 1.87 | 15.5 ± 1.75 | >100 | 178 |
| *S. runcinata* L.f. | Klerkskraal Dam (AV 679) | 19.4 ± 2.78 | 19.3 ± 0.61 | >100 | 149 |
| *S. schlechteri* Briq. | KBG (AV 1068) | 17.5 ± 2.05 | 1.61 ± 0.03 [a] | >100 | 209 |
| *S. stenophylla* Burch. ex Benth. | East of Clarens (AV 893) | 20.8 ± 0.86 | 14.9 ± 0.93 | >100 | 161 |
| Controls | | 2.43 ± 0.07 [b] | 2.51 ± 0.41[b] | 4.95 ± 0.07[c] | – |

KBG, Kirstenbosch Botanical Garden.
[a] Activity of solvent extract greater than that of Trolox ($p < 0.05$).
[b] Trolox®.
[c] NDGA, $IC_{50} < 30$ µg/ml: good activity; $30 < IC_{50} < 80$ µg/ml: moderate activity; $IC_{50} > 80$ µg/ml: poor activity.

The results obtained in these assays are in agreement with previous investigations on *Salvia* species, although different solvents were used for extraction. Fisher (2005) demonstrated that most of the species studied displayed some degree of activity when plates prepared from methanol extracts were sprayed with DPPH reagent. Furthermore, Kamatou et al. (2005) also showed that methanol extracts of *S. runcinata*, *S. repens* and *S. stenophylla* exhibited good antioxidant activity ($IC_{50}$ values <16 µg/ml). However, as far as the literature survey has ascertained, there are very few quantitative data on the antioxidant activity of southern African *Salvia* species. Kamatou (2006) showed that isolated compounds from *S. radula* (betulafolientriol oxide and salvigenin) displayed antioxidant activity when sprayed with DPPH radical solution.

The primary antioxidant activity of sage is related to the presence of carnosic acid (Wenkert, Fuchs, & McChesney, 1964). It was also reported that carnosic acid and carnosol are responsible for 90% of antioxidant activity of rosemary (Cuvelier et al., 1996). Caffeic acid, rosmarinic acid, ursolic acid, oleanolic acid, carnosic acid and carnosol are common in many plants and all are strong radical scavengers (Cuvelier et al., 1996). Carnosol (isolated from *S. chamelaeagnea*), standards of caffeic acid and rosmarinic acid also exhibited good antioxidant activity using the DPPH assay with $IC_{50}$ values of 6.10 ± 0.6, 2.55 ± 0.17 and 2.48 ± 0.20 µg/ml, respectively (Kamatou, 2006). For example, *S. aurita*, *S. chamelaeagnea*, *S. muirii*, *S. repens*, *S. runcinata* and *S. schlechteri* containing rosmarinic acid, caffeic acid, carnosol and derivatives (carnosic acid) are good antioxidants, whilst those lacking these compounds exhibited poor activity (e.g., *S. dolomitica* and *S. radula*).

Nearly all the extracts exhibited poor antiinflammatory activity, except *S. radula*, which displayed moderate activity ($IC_{50}$ value: 78.8 ± 5.89 µg/ml). The $IC_{50}$ values of the solvent extracts were greater than 100 µg/ml against the 5-lipoxygenase enzyme (Table 2). Effective antioxidant treatment has also been found to be effective against inflammation (Sala et al., 2003). For instance, phenolic acids, such as carnosic and ursolic acids, which are good antioxidants, were reported to be good antiinflammatory compounds (Baricevic et al., 2001; Liu, 2005). However, ursolic acid showed poor ability to inhibit the key enzyme of leukotriene biosynthesis, namely 5-lipoxygenase (Safayhi, Rall, Sailer, & Ammon, 1997). Only the extract of *S. radula* exhibited some degree of activity, whilst

other species were inactive at the starting concentration of 100 µg/ml (Table 2). In addition, the antiinflammatory activity of the extracts or isolated compounds of *Salvia* species is widely reported. For example, Baricevic et al. (2001) investigated the topical antiinflammatory activity of *S. officinalis* leaves and found that the solvent extracts inhibited ear oedema in mice in a dose-dependent manner, whilst aethiopinone, isolated from *S. aethiopis* roots, exhibited antiinflammatory activity (Hernandez-Perez, Rabanal, De la Torre, & Rodriguez, 1995). Although many pentacyclic triterpenes (e.g., ursolic acid) also bind to the 5-lipoxygenase enzyme (Safayhi, Sailer, & Ammon, 1995), the presence of an 11-keto group and a hydrophilic function in the pentacyclic ring system are crucial for potent inhibition of 5-lipoxygenase, and ursolic acid turned out to be non-inhibitory (Sailer et al., 1996). Although this compound was detected in the majority of species studied, the antiinflammatory activity of the extracts against the 5-lipoxygenase enzyme was very poor. This suggests that the components of the extract responsible for the antioxidant activity are not necessarily those responsible for the antiinflammatory activity. It is important to note that the 'quasi absence' of 5-lipoxygenase inhibitory activity in this study does not imply that the solvent extracts do not possess any antiinflammatory activity. The activity may be observed at higher concentrations. Furthermore, inflammation involves a complex cascade of events, involving not only the 5-lipoxygenase pathway, but also the 12- and 15-lipoxygenases, as well as cyclooxygenase-1, cyclooxygenase-2, cytochrome $P_{450}$ and epoxygenase pathways. Further investigation involving other inflammatory routes is required in order to determine whether the solvent extracts do exert antiinflammatory activity in other steps of the complex antiinflammatory cascade.

### 3.3. Total phenolic content and its relationship with the antioxidant activity

The phenolic content estimated in the organic extracts of the various species ranged from 45.6 to 212 mg GAE/g dry sample (Table 2). Species such as *S. chamelaeagnea*, *S. muirii*, *S. repens*, *S. runcinata*, *S. schlechteri* and *S. stenophylla* with high phenolic content (>150 mg of GAE) had the most promising antioxidant activity (Table 2).

A stronger correlation between the total phenolic content (TPC) and the antioxidant activity was observed for the DPPH assay compared to the ABTS assay ($r^2$ = 0.90 and 0.76, respectively). This implies that the phenolic compounds are partly, if not totally, responsible for the antioxidant activity observed in the species studied. The antioxidant activity of sage has been attributed to the phenolic compounds; Tepe, Daferera, Sokmen, Sokmen, and Polissiou (2005) demonstrated that S. tomentosa, which exhibited good antioxidant activity, also possessed a high phenolic content (200 μg/mg). Several reports have conclusively shown a close relationship between total phenolic content and antioxidant activity (Velioglu, Mazza, Gao, & Oomah, 1998). In the present study it was found that S. chamelaeagnea and S. schlechteri contained a substantial amount of phenolics that are at least partly, if not entirely, responsible for the antioxidant activity (Table 2). However, it can be observed from the present data that antioxidant activity does not necessarily correlate with high amounts of phenolics, as seen by the moderate correlation between the antioxidant activity using the ABTS method and the total phenolic content. It is known that different phenolic compounds have different responses in the Folin–Ciocalteu method. Liu et al. (2007) found that the TPC may not be highly correlated with DPPH· scavenging ability ($r^2$ = 0.27).

In conclusion, this study indicated that Salvia species indigenous to South Africa exhibit good antioxidant activity, whilst they displayed poor ability to inhibit the 5-lipoxygenase enzyme (one of the enzymes responsible for the inflammation process). Various phenolic compounds were detected, with rosmarinic acid and betulafolientriol oxide present in all species, whilst rosmarinic acid, carnosic acid, carnosol, ursolic acid/oleanolic acid were detected as major compounds in the chromatographic profiles.

## Acknowledgements

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