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Review

South African *Salvia* species: A review of biological activities and phytochemistry

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ABSTRACT

The genus *Salvia* (sage) belongs to the Lamiaceae and encompasses 900 species worldwide of which *ca.* 26 indigenous species are found in southern Africa. *Salvia* is the largest genus in this family and constitutes almost one quarter of the Lamiaceae. In South Africa, the majority of *Salvia* species are distributed predominantly in the Cape region. *Salvia* species are used in many parts of the world to treat various conditions. Many sages, if not all, form an integral part of traditional healing in South Africa particularly in regions where they occur in abundance. Several species are used to treat microbial infections, cancer, malaria, inflammation, loss of memory and to disinfect homes after sickness. Despite the extensive traditional use and the general interest in phytoconstituents of *Salvia* it remains ironic that research on the South African counterparts has until recently been neglected. The review aims to collate recent research results on the phytochemistry and pharmacological properties of indigenous species. Bio-active compounds with antiplasmodial and antibacterial activities have been isolated and structurally elucidated from *Salvia chamelaegnea*, *Salvia radula* and *Salvia verbenaca*. The essential oil composition of *Salvia* showed the dominance of monoterpene hydrocarbons, oxygen-containing monoterpenes and oxygen-containing sesquiterpenes. *Salvia runcinata* is identified as an alternative source of natural α-bisabolol. Many pharmacological activities are summarised (anti-oxidant, antimicrobial, antiplasmodial, analgesic, antipyretic, anticancer, anti-inflammatory and antinociceptive) as a first attempt to provide scientific support for past and present local traditional uses.

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1. Introduction

Salvia species (sage) belong to the Lamiaceae family (formerly Labiatae). The genus name *Salvia* L. is derived from the Latin *salvare* meaning 'to heal or to be safe and unharmed' referring to the medicinal properties of some of the species (Blumenthal et al., 2000). It has been used for centuries, especially by the Chinese to promote longevity and in Roman ceremonies as a sacred herb. This name was translated to *sauge* (sage) in French and *sawge* in Old English. Sage encompasses about 900 species, widespread throughout the world and includes several ornamental, culinary and medicinal species (Codd, 1985; Paton, 1991; Gali-Muhtasib et al., 2000). The genus has a sub-cosmopolitan distribution, but is largely absent in the North and most of the low-lying tropical areas of the world such as the Amazon basin and central and west Africa (Paton, 1991). Although Mexico has the highest number of species (about 250), the center of origin of the genus is speculated to be Afghanistan and Soviet Central Asia where a larger range of primitive morphological types occur (Paton, 1991). In Africa, the greatest number of species are found in the north-west and the southern parts (Hedge, 1974). The genus is absent from most of western and central tropical Africa (Jäger and Van Staden, 2000). Southern Africa is home to more than 24,000 higher plant taxa (Arnold and De Wet, 1993) and a large proportion of these are endemic in character (Mulholland and Drewes, 2004). About 30 *Salvia* species have been identified in southern Africa (Codd, 1985; Paton, 1991) but species such as *Salvia coccinea*, *Salvia officinalis*, *Salvia reflexa*, *Salvia sclare* and *Salvia tillifolia* have been introduced and are thus not indigenous to the region. Most of the South African species are confined to the Cape region (Codd, 1985). In the past decade a new and unusual fynbos species, *Salvia thermarum*, (sometimes incorrectly termed *Salvia thermara*) was discovered growing on rocky slopes of quartzitic sandstones around the Citrusdal and Caledon District of the South-Western Cape (Van Jaarsveld, 1999; De la Cruz, 2005).

1.1. Morphological description

Salvia species are easily recognized by their square stems and opposite, simple pairs of leaves that are usually velvety or hairy. Leaves are often rugose, entire, toothed and lobed. Flowers are clustered in racemes, spike-like racemes, spikes and panicles are usually large and brightly coloured, depending on the species. Flowers and stems are key diagnostic characteristics for identification of the genus (Hedge, 1974; Codd, 1985). There are four stamens, but only two bear anthers. *Salvia* grows optimally in full sun and needs well-drained soil.

1.2. Traditional uses of Salvia species in South Africa

The positive benefits of *Salvia officinalis* (common sage) to health are reputed throughout Ancient Romans times and the Middle Ages. A quote such as: '*Cur moriatur homo cui Salvia crescit in horto?*'—'Why should a man die whilst sage grows in his garden?' epitomizes the impact of this sage on that society at the time. Apart from general scientific curiosity, understanding the chemistry of *Salvia* plants is important for several commercial industries because these plants are utilized for flavouring food, used in cosmetic formulations, aromatherapy and insecticides.

Most *Salvia* species are inherently linked to local traditional medicine systems in their country of origin (Codd, 1985). *Salvia* species are used to treat various conditions which are summarized in Table 1. Historically, European descendants in South Africa have utilized the species endemic to the Western Cape in a comparable fashion to *Salvia officinalis* for treating colds, coughs and bronchial infections. Amongst the African tribes, sages are used for a wide variety of ailments including ethnoveterinary purposes and as disinfectants. For instance, *Salvia africana–lutea* (beach or brown dune sage) which mainly grows along the coast, extending from Namaqualand to the Cape Peninsula and eastwards to the Eastern Cape Province in Port Alfred (Codd, 1985) is still an ethnoherbal product commonly found in informal markets in the Western Cape today, particularly in Cape Town.

Salvia africana-lutea was used by early Europeans settlers in the Western Cape as an infusion to treat colds (Watt and Breyer-Brandwijk, 1962). Before the discovery of antibiotics, it was frequently prepared as a component of herbal tea mixtures, to treat tuberculosis and chronic bronchitis (Watt and Breyer-Brandwijk, 1962). The cultural use still continues today, for example, *Salvia africana–lutea* is collected fresh when needed or sold in dried or semi-dried bundles comprising mainly of leaves or occasionally flowers and fruits. The traditional healers in the Western Cape Province prescribe a decoction of *Salvia africana–lutea* to treat respiratory ailments, influenza, gynaecological complaints (Watt and Breyer-Brandwijk, 1962), fever, headaches and digestive disorders (Amabeoku et al., 2001). Leaves of *Salvia runcinata* are administered to infants as a purgative (Gerstner, 1941). Van Wyk and Wink (2004) reported that *Salvia africana–caerulea* and other *Salvia* species are used as cold and flu medicines. *Salvia repens* is added to a bath to treat sores on the body, while a decoction prepared from the roots is taken orally to treat stomachache and diarrhoea. The smoke obtained through burning the plant is used to disinfect a hut and also used as an insect repellent (Clebsch, 2003). A decoction of *Salvia runcinata* has been used by Europeans for the relief and the treatment of urticaria. The Zulu use a paste of crushed leaves as a purgative for infants, while the Xhosa administer extracts of the leaves to newly born babies (Watt and Breyer-Brandwijk, 1962).

So far only three species (*Salvia africana–caerulea, Salvia africana–lutea* and *Salvia chamelaeagnea*) have been documented in the South African Pharmacopeia Monographs Project (South Africa Health Info, 1999) which was recently established due to a lack of documentation of medicines used traditionally.

2. Chemical constituents of *Salvia* species

2.1. Volatile compounds (essential oils)

Limited work has been carried out on the volatile components of indigenous *Salvia* species. The essential oils of 11 species were isolated by hydrodistillation and analyzed by GC and GC–MS (Kamatou, 2006; Kamatou et al., 2006a,b, 2007b). The oil yield was relatively low compared to that reported in literature for exotic *Salvia* species. Bellomaria et al. (1992) reported the yield of *Salvia pomifera*, *Salvia willeana* and *Salvia fruticosa* to be 1.30, 2.80 and 5.30%, respectively. Pitarevic et al. (1984) found that the yield of *Salvia officinalis* collected at various seasons throughout the year varied from 1.80 to 3.10%. The relatively high yield obtained for

Table 1
Medicinal uses of South African *Salvia* species (Watt and Breyer-Brandwijk, 1962; Clebsch, 2003; Van Wyk and Wink, 2004; Amabeoku et al., 2001)

Species	Plant part used	Mode of use	Ailments
Salvia africana-caerulea	Twig	Infusion mixed with Epson salt and lemon	Colic, diarrhoea, indigestion, abdominal trouble
Salvia africana-lutea	Whole plant	Infusion, decoction	Colds, cough, flu
Salvia chamelaeagnea	Leaves	Decoction, infusion	Colds, cough, flu
Salvia triangularis	Whole plant	Decoction with *Helichrysum latifolium* and *Commelina africana*	Liver sickness, barrenness
Salvia repens	Roots, leaves, whole plant	Decoction, added to bath	Sores on the body, stomach problems, diarrhoea
Salvia runcinata	Whole plant, leaves and stems	Burned, decoction	Disinfect dwelling sickness, urticaria
Salvia scabra	Leaf	Decoction	Purgative
Salvia species (in general)	Roots, whole plants	Decoction	Biliousness, cold, febrile attacks
Salvia species (in general)	Whole plant	Infusion in combination with *Leonotis leonurus*	Tuberculosis, cough, influenza, bacterial infections, cold
Salvia stenophylla	Whole plant	Infusion, decoction	Disinfect dwelling sickness

Salvia muirii (0.50%) and *Salvia chamelaeagnea* (0.41%) may be attributed to the high density of glandular trichomes (Kamatou, 2006).

Ninety-three components were identified in 11 aromatic South African *Salvia* species (Kamatou, 2006). Quantitative and qualitative variations in oil composition were observed. The essential oils were dominated by monoterpene hydrocarbons in *Salvia muirii* (53.0%), *Salvia stenophylla* (41.5%), *Salvia africana–lutea* (35.6%) and *Salvia repens* (32.6%). Oxygen-containing monoterpenes dominated in *Salvia dolomitica* (71.8%) and *Salvia chamelaeagnea* (42.8%), while oxygen-containing sesquiterpenes were dominant in *Salvia africana–caerulea*, *Salvia albicaulis*, *Salvia lanceolata*, *Salvia radula* and *Salvia runcinata* samples. The essential oil components identified in high amounts (>10%) included α-pinene, 1,8-cineole, linalool, limonene, myrcene, β-caryophyllene, spathulenol, β-caryophyllene oxide, viridiflorol, δ-3-carene and α-bisabolol. α-Bisabolol was present in *Salvia runcinata* in high levels (>65%) and therefore this plant could be considered as a natural source of α-bisabolol. This compound is an important component of cosmetic products, making it highly valued in the cosmetics industry. Many components identified in the 11 essential oils have previously been recorded in exotic *Salvia* species (Pitarevic et al., 1984; Bellomaria et al., 1992; Ahmed et al., 1994; Carta et al., 1996; Foray et al., 1999; Kaya et al., 2003). However, high levels of geraniol and linalyl acetate detected in *Salvia dolomitica* (19.6 and 19.6% respectively) have rarely been identified in exotic *Salvia* species (Bellomaria et al., 1992; Farhat et al., 2001).

The essential oil of *Salvia stenophylla* collected on the "highveld" (high altitude grassland) of the Free State revealed the presence of oxygenated monoterpenoids (constituting 5% of the oil) and the sesquiterpene hydrocarbons (35.5%). *Salvia stenophylla* was found to contain very low amounts of 1,8-cineole and camphor, and lacked α- and β-thujone (Jequier et al., 1980). The amount of oxygen-containing monoterpenes and sesquiterpene hydrocarbons in the study carried out by Kamatou (2006) represented 11.4 and 6.7%, respectively.

Brunke and Hammerschmidt (1985) also analyzed the essential oil of *Salvia stenophylla* and found that δ-3-carene (20.0%) and α-bisabolol (29.8%) were the dominant components. Gono-Bwalya (2003) studied the composition of *Salvia stenophylla* collected at 10 different locations in South Africa. The major compound obtained was α-bisabolol ranging from 1.80 to 46.50%.

In another investigation (Kamatou, 2006), the two components (δ-3-carene and α-bisabolol) represent 22.6 and 26.1%, respectively. The essential oil of six populations of *Salvia repens* collected at various areas of South Africa revealed that the predominant compounds include δ-3-carene and α-bisabolol (24.9–33.0%) (Gono-Bwalya, 2003). α-Bisabolol and (*E*)-nerolidol were found to be the major constituents in *Salvia runcinata* of the populations studied (Gono-Bwalya, 2003). The oil of *Salvia disermas* was dominated by linalyl acetate (34.5%) and revealed the presence of other compounds not detected in other species such as shyobunone (10.7%) and epi-isoshyobunone (6.2%) (Fisher, 2005). A study by Fisher (2005) showed that *Salvia namaensis* is rich in camphor (33.5%), camphene (14.7%) and α-pinene (9.3%).

2.2. Non-volatile compounds (phenolic compounds)

The majority of the phenolic acids in *Salvia* species are almost exclusively those of caffeic acid derivatives. Caffeic acid plays a central role in the biochemistry of Lamiaceae and occurs predominantly in the dimer form as rosmarinic acid (Gerhardt and Schroeter, 1983). In many *Salvia* species, caffeic acid is the building block of a variety of plant metabolites, ranging from the simple monomers to multiple condensation products that give rise to a variety of oligomers (Lu and Foo, 2002). The trimers and tetramers are also interesting from a therapeutic point of view as they have demonstrated various biological activities such as anti-oxidant, antimicrobial and anticancer (Lu and Foo, 2002). The monomers that are frequently present in *Salvia* species are represented by caffeic acid (Qian and Li, 1992) and other monomeric derivatives including ferulic acid (Cuvelier et al., 1996) and isoferulic acid (Ai and Li, 1992).

High performance liquid chromatography, UV and mass spectroscopy were used to detect the presence of caffeic acid, carnosic acid, kaempferol, oleanolic acid, rosmarinic acid and ursolic acid in 17 methanol:chloroform extracts of indigenous *Salvia* species. Compounds isolated from *Salvia chamelaeagnea* (7-*O*-methylepirosmanol, oleanolic acid, ursolic acid and carnosol) (Kamatou et al., 2007a) and *Salvia radula* (betulafolientriol oxide and salvigenin) (Kamatou et al., 2008) were also included to verify their presence in other indigenous *Salvia* species. Betulafolientriol oxide was the only compound identified in all 17 species tested, but at relatively low levels. Rosmarinic acid, carnosic acid, carnosol and ursolic acid were present in the majority of the extracts in relatively high levels. *Salvia albicaulis, Salvia runcinata* and *Salvia muirii* were particularly rich in rosmarinic acid and *Salvia verbenaca* was the only species devoid of rosmarinic acid (Kamatou, 2006). Carnosol and carnosic acid were abundant in *Salvia aurita*, *Salvia chamelaeagnea*, *Salvia namaensis* and *Salvia stenophylla* (area >20%). Salvigenin and oleanolic acid/ursolic acid were abundant in *Salvia disermas* (Kamatou, 2006).

Caffeic acid was also present in the solvent extracts but in relatively low levels. However, it was identified in its dimer form (rosmarinic acid) in most of the species investigated. Before the chemical structure of rosmarinic acid was elucidated, rosmarinic acid and similar compounds have been known as "Labiatengerbstoffe", a type of tannin known from the Lamiaceae (Petersen and

Table 2
Geographical distribution of known *Salvia* species in southern Africa and pharmacological actions tested on wild collected leaf samples

Species	Geographical distribution	Voucher number	Bioactivity investigated	Extract type	Reference
Salvia africana–caerulea L.	Cape	AV 875	Antimicrobial, anti-oxidant, anti-inflammatory, antiplasmodial, cytotoxicity, antituberculosis	LE and/or EO	Fisher (2005); Kamatou (2006)
Salvia africana–lutea L.	Cape	AV 873	Antimicrobial, anti-oxidant, anti-inflammatory, antiplasmodial, cytotoxicity, antipyretic, antianalgesis, antituberculosis	LE and/or EO	Amabeoku et al. (2001); Fisher (2005); Kamatou (2006)
Salvia albicaulis Benth.	Cape	AV 894	Antimicrobial, anti-oxidant, anti-inflammatory, antiplasmodial, cytotoxicity, antituberculosis	LE and/or EO	Kamatou (2006)
Salvia aurita L.f.	Cape, Gauteng, Limpopo	AV 1066	Antimicrobial, anti-oxidant, anti-inflammatory, antiplasmodial, cytotoxicity, antituberculosis	LE	Kamatou (2006)
Salvia chamelaeagnea Berg.	South Western Cape	AV 848	Antimicrobial, anti-oxidant, anti-inflammatory, antiplasmodial, cytotoxicity, antituberculosis	LE and/or EO	Kamatou (2006)
Salvia disermas L.	Cape, Free Sate Gauteng	AV 1194	Antimicrobial, anti-oxidant, anti-inflammatory, antiplasmodial, cytotoxicity, antituberculosis	LE	Fisher (2005); Kamatou (2006)
Salvia dolomitica Codd	Cape, Gauteng, Limpopo	AV 838	Antimicrobial, anti-oxidant, anti-inflammatory, antiplasmodial, cytotoxicity, antituberculosis	LE and/or EO	Fisher (2005); Kamatou (2006)
Salvia garipensis E. Mey.	Cape	AV 1193	Antimicrobial, anti-oxidant, anti-inflammatory, antiplasmodial, cytotoxicity, antituberculosis	LE	Kamatou (2006)
Salvia lanceolata Lam.	Cape	AV 877	Antimicrobial, anti-oxidant, anti-inflammatory, antiplasmodial, cytotoxicity, antituberculosis	LE and/or EO	Fisher (2005); Kamatou (2006)
Salvia muirii L. Bol.	South Western Cape	AV 874	Antimicrobial, anti-oxidant, anti-inflammatory, antiplasmodial, cytotoxicity, antituberculosis	LE and/or EO	Kamatou (2006)
Salvia namaensis Schinz	Cape, Free State	AV 497	Antimicrobial, anti-oxidant, anti-inflammatory, antiplasmodial, cytotoxicity, antituberculosis	LE	Fisher (2005); Kamatou (2006)
Salvia radula Benth.	Gauteng, Limpopo	AV 880	Antimicrobial, anti-oxidant, anti-inflammatory, antiplasmodial, cytotoxicity, antituberculosis	LE and/or EO	Kamatou (2006)
Salvia repens Burch. Ex Benth	Eastern Cape, KwaZulu-Natal	AV 615	Antimicrobial, anti-oxidant, anti-inflammatory, antiplasmodial, cytotoxicity, antituberculosis	LE and/or EO	Gono-Bwalya (2003); Kamatou (2006)
Salvia runcinata L.f.	Cape, Free State, KwaZulu-Natal	AV 679	Antimicrobial, anti-oxidant, anti-inflammatory, antiplasmodial, cytotoxicity, antituberculosis	LE and/or EO	Gono-Bwalya (2003); Fisher (2005); Kamatou (2006)
Salvia schlechteri Briq.	Cape	AV 1068	Antimicrobial, anti-oxidant, anti-inflammatory, antiplasmodial, cytotoxicity, antituberculosis	LE	Kamatou (2006)
Salvia stenophylla Burch. Ex. Benth	Cape, Free State, KwaZulu-Natal	AV 893	Antimicrobial, anti-oxidant, anti-inflammatory, antiplasmodial, cytotoxicity, antituberculosis	LE and/or EO	Gono-Bwalya (2003); Kamatou (2006)
Salvia verbenaca L.	Cape, Free State, Gauteng, Limpopo	AV 631	Antimicrobial, anti-oxidant, anti-inflammatory, antiplasmodial, cytotoxicity, antituberculosis	LE	Fisher (2005); Kamatou (2006)

Simmonds, 2003). Rosmarinic acid occurs throughout the Boraginaceae whereas within the Lamiaceae it is restricted to the sub-family Nepetoideae (Janicsák and Máthe, 1997). Rosmarinic acid is the most abundant caffeic acid dimer in *Salvia* species (Cuvelier et al., 1994; Lu and Foo, 1999) and has been reported to be the major phenolic compound of several other *Salvia* samples (Cuvelier et al., 1996).

The variation in levels of caffeic acid and rosmarinic acid in the same plant has been investigated in 96 Lamiaceae taxa (Janicsák and Máthe, 1997). The results indicated that concentrations of caffeic acid were always much lower than those of rosmarinic acid. In the South African species studied where the two compounds were detected, the same pattern was observed (Kamatou, 2006). The majority of the compounds detected in southern African species have previously been identified in exotic *Salvia* species (Cuvelier et al., 1996; Baricevic et al., 2001; Liu, 2005).

A solvent extract of *Salvia stenophylla* collected in the Eastern Cape was investigated for the presence of flavonoids. Apigenin-7-methyl ether, luteolin and 6-hydroxyluteolin-6,7-dimethyl ether were identified (Wollenweber et al., 1992). Flavonoid aglycones such as luteolin and apigenin 7,4′-dimethyl ether were identified in *Salvia stenophylla*, while cirsiliol was identified in *Salvia verbenaca* (Wollenweber et al., 1992). The total phenolic content determined for South African *Salvia* species on the methanol:chloroform extracts based on gallic acid equivalents (GAE) confirmed the presence of total soluble phenolics in the various extracts from 45 to 211 mg/g of GAE dry sample and as expected showed strong association with anti-oxidant activity (Kamatou, 2006). Some exotic *Salvia* species contain alkaloids and the southern African *Salvia* species such as (*Salvia chamelaeagnea*, *Salvia namaensis* and *Salvia runcinata*) tested positive for alkaloids (Raffauf, 1996).

3. Biological activities

Although *Salvia* species are well known to be used in traditional medicine in South Africa, experimental studies to support the traditional use is scarce. This section assembles the published *in vitro* pharmacological data for several species to provide a scientific rationale for traditional use and to encourage further research on species exhibiting promising *in vitro* activity. The localities where some of the species studied were collected are presented in Table 2.

3.1. Antimicrobial activity

3.1.1. Antimicrobial activity of the crude extracts and essential oils

Micro-organisms are involved in the pathogenesis of many diseases and cause deterioration of a variety of products. Despite the progress in understanding the life cycle and control of many pathogens, nearly all the diseases affecting millions of people in developing countries are still caused by micro-organisms. *Salvia* species are commonly used in traditional medicine to treat various microbial infections. Eleven essential oils and 17 solvent extracts of South African *Salvia* species were investigated for their antibacterial and antimycobacterial activities using the micro-dilution and BACTEC™ 460 radiometric methods, respectively. The micro-organisms included Gram-positive (*Staphylococcus aureus* and *Bacillus cereus*), Gram-negative (*Escherichia coli* and *Klebsiella pneumoniae*) bacterial strains, as well as the organism responsible

for tuberculosis, *Mycobacterium tuberculosis*. With the exception of *Salvia radula*, all the solvent extracts displayed moderate to good activity against all the Gram-positive and Gram-negative bacterial strains with the MIC values ranging from 0.03 to 8.00 mg/ml (Kamatou et al., 2007a). The essential oils and solvents extracts were less active than the positive control ciprofloxacin (MIC value <0.31 μg/ml). In general, *Salvia chamelaeagnea* exhibited the most favourable activity. The solvent extracts also exhibited promising activity against *Mycobacterium tuberculosis* with the MIC values ranging between 0.10 and 0.50 mg/ml with *Salvia dolomitica*, *Salvia radula* and *Salvia verbenaca* displaying the best activity (MIC value 0.10 mg/ml). Seaman (2005) found that the *Salvia africana–lutea* extract exhibited promising activity against *Mycobacterium aurum* and *Mycobacterium tuberculosis* (MIC values: 2 and 1 mg/ml, respectively) along with moderate activity against *Mycobacterium smegmatis* (MIC value 8 mg/ml). However, all the extracts were less active than rifampicin used as a positive control (MIC value < 2 μg/ml).

The essential oils of *Salvia* species however displayed moderate activity against Gram-positive bacteria *Bacillus cereus* and *Salvia aureus* (MIC values between 2.3 and 8.0 mg/ml) while negligible activity was obtained against Gram-negative bacteria *Klebsiella pneumoniae* and *Escherichia coli*, (MIC value >8 mg/ml) (Kamatou et al., 2006a,b, 2007b).

Gono-Bwalya (2003) demonstrated that methanol extracts and essential oils of *Salvia stenophylla*, *Salvia repens* and *Salvia runcinata* exhibited poor activity against Gram-negative bacteria, yeasts and moulds. However, the methanol extracts of the three species collected at various localities inhibited the growth of Gram-positive bacteria with the inhibition zone ranging from 1 to 7 mm. Using the microdilution method, the MIC values of *Salvia stenophylla* and *Salvia repens* against Gram-positive bacteria (*Salvia aureus*, *Salvia epidermidis*, *Bacillus cereus* and *Bacillus subtilis*) ranged from 0.09 to 6.3 mg/ml, while the MIC values of *Salvia runcinata* was greater than 1.6 mg/ml.

Fisher (2005) using the disc diffusion method also found that the methanol extracts of *Salvia africana–lutea*, *Salvia africana–caerulea* and their respective oils inhibited the growth of Gram-positive bacteria (*Salvia aureus* and *Bacillus cereus*) (inhibition zone ranging from 1 to 7 mm), while they were resistant to Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae* and *Yersinia enterolitica*). The antibacterial activity of exotic *Salvia* species has been reported in the literature (Lee et al., 1999; Tepe et al., 2004).

In many studies, antibacterial activity of solvent extracts is generally high compared to that of essential oils (Lourens et al., 2004; Njenga et al., 2005; Van Vuuren et al., 2006). However, Kamatou (2006) found that the activity of the essential oil of *Salvia africana–caerulea* against *Bacillus cereus* was higher than that of the solvent extract (MIC value 0.75 and 6.0 mg/ml, respectively).

Salvia species are used in traditional medicine in combination with *Leonotis leonurus* to treat microbial infections. Therefore the *in vitro* antibacterial activity of *Salvia chamelaeagnea* and *Leonotis leonurus* combined in various ratios was investigated (Kamatou et al., 2006c). The isobolograms of *Salvia chamelaeagnea* and *Leonotis leonurus* at various ratios against four pathogens showed that synergistic interactions were obtained against Gram-positive bacteria for nearly all ratios, while mostly antagonistic or additive interactions were observed with Gram-negative bacteria.

3.1.2. Antimicrobial compounds isolated from Salvia chamelaeagnea

In order to determine the active compounds responsible for the antimicrobial activity of *Salvia chamelaeagnea*, the antibacterial bio-assay guided fractionation resulted in the isolation, identification and characterization of four compounds namely carnosol, 7-*O*-methylepirosmanol, oleanolic acid and its isomer ursolic acid as the active compounds against *Salvia aureus* (Kamatou et al., 2007a). These active compounds from *Salvia chamelaeagnea* have previously been detected in many species of the Lamiaceae. Carnosol has been reported to display antibacterial activity against various micro-organisms including *Staphylococcus aureus*, *Bacillus subtillus*, *Escherichia coli* and *Candida albicans* (Collins and Charles, 1987; Dimayuga et al., 1991). It is a common compound in *Salvia* species and was first isolated from *Salvia carnosa* (Dougl.) (White and Jenkins, 1942). Since then, this compound has been detected in *Rosmarinus officinalis* and many other species belonging to the Lamiaceae (Wu et al., 1982).

Medicinal plants containing ursolic acid have been used in folk medicine before it was known which constituents were responsible for their therapeutic effectiveness (Liu, 1995). Contemporary scientific research that led to the isolation and identification of ursolic acid revealed and confirmed that several pharmacological properties such as antitumour, hepatoprotective, anti-inflammatory, anti-ulcer, antimicrobial, antihyperlipidemic and antiviral activity can be attributed to this compound (Liu, 1995). Ursolic acid was also identified as one of the active principles in *Rosmarinus officinalis*.

3.2. Anti-oxidant activity of the crude extracts and essential oils

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 anti-oxidants may play an important role in disease prevention (Aruoma, 1998). Studies have also shown that phenolic compounds are potent scavengers of free radicals and as such, are potentially useful in the prevention of a number of diseases (Zainol et al., 2003). The commonly used anti-oxidants, butylated hydroxyanisol and butylated hydroxytolune are synthetic chemicals and the possible toxicity of these anti-oxidants has resulted in their reduced usage (Ito et al., 1985). Due to health concerns, natural anti-oxidants have been extensively employed in recent years (Yen et al., 2003). The solvent extracts of indigenous *Salvia* species were found to display anti-oxidant activity with IC_{50} values ranging from 1.61 to 74.50 μg/ml using the $DPPH^{\bullet}$, while the IC_{50} values ranged from 11.88 to 69.26 μg/ml with the $ABTS^{\bullet+}$ (Kamatou, 2006). The solvent extract of *Salvia schlechteri* was three times more active than vitamin C used as the reference compound. In contrast, all the essential oils were poorly active (IC_{50} values >100 μg/ml) in both assays. *Salvia dolomitica* and *Salvia radula* demonstrated weak anti-oxidant activity against the $DPPH^{\bullet}$ and moderate activity against the $ABTS^{\bullet+}$ (Kamatou, 2006). *Salvia* species are known to be good anti-oxidants. Kelen and Tepe (2008) showed that *Salvia namaensis* and *Salvia aurita* exhibited good anti-oxidant using the DPPH method (IC_{50} values <19 μg/ml).

3.3. Anti-inflammatory activity of the crude extracts and essential oils

Salvia species are reported to have anti-inflammatory properties and their local use as medicinal herbs includes the treatment of body wounds. The *in vitro* anti-inflammatory activity of essential oils and solvent extracts of *Salvia* species was evaluated using the 5-lipoxygenase assay (Kamatou et al., 2005; Kamatou, 2006). Essential oils exhibited better anti-inflammatory activity when compared to the solvent extracts with the IC_{50} values ranging between 22.81 and 77.32 μg/ml. However, this activity was very low in comparison to the nordihydroguaiaretic acid (IC_{50} value 4.9 μg/ml). With the exception of *Salvia radula*, solvent extracts

displayed poor ability to inhibit the enzyme with all IC_{50} values being greater than 100 μg/ml. It was interesting to note that in contrast to the poor activity recorded for the essential oil of *Salvia radula*, the solvent extract was fractionally more potent (Kamatou, 2006).

Baylac and Racine (2003) found that the essential oil of *Salvia stenophylla* has the potential to inhibit the *in vitro* 5-lipoxygenase enzyme ($10 < IC_{50}$ value < 30 in μg/ml). Kamatou (2006) also found that *Salvia stenophylla* displayed anti-inflammatory activity (IC_{50} value 49 μg/ml). The difference found between the two values reported, although the same method was used, may be attributed to variation in chemical composition of the two essential oils. α-Bisabolol, which is widely reported to have a skin soothing action, strongly inhibited the 5-lipoxygenase enzyme *in vitro* (Baylac and Racine, 2003). It is therefore expected that essential oils containing high levels of this sesquiterpenic alcohol like that of *Salvia runcinata* (65%) and *Salvia stenophylla* (26%) (Kamatou, 2006), could be expected to inhibit the 5-lipoxygenase enzyme as well.

Peana et al. (2002) demonstrated that linalool and its ester linalyl acetate exhibited anti-inflammatory activity. These two compounds were found in *Salvia dolomitica* (16.6 and 19.6%, respectively) (Kamatou et al., 2006c) and may have partly contributed to the anti-inflammatory activity.

Cyclo-oxygenase-1 (COX-1) and cyclo-oxygenase-2 (COX-2) are two key enzymes in the inflammation process. Therefore substances inhibiting these enzymes may be considered to have anti-inflammatory activity. The essential oils of three *Salvia* species from different locations showed inhibition of the COX-2 enzyme by the essential oils of *Salvia repens*, *Salvia runcinata* and *Salvia stenophylla* when tested at a concentration of 1% (Gono-Bwalya, 2003). The percentage inhibition of this enzyme using the essential oils of the three species collected at different locations ranged from 5 to 73%, with *Salvia stenophylla* exhibiting the highest inhibition. Urticaria is associated with inflammation and the traditional use of *Salvia* species to treat this condition is supported through the above-mentioned *in vitro* studies.

3.4. Antiplasmodial activity

3.4.1. Antiplasmodial activity of the crude extracts and essential oils

Not considering the future implications of the AIDS pandemic, malaria is currently one of the most important human diseases in developing countries and is still unconquered. It is the world's leading killer among the infectious diseases (Wanyoike et al., 2004). Most of the lethal cases are caused by *Plasmodium falciparum*, the most virulent of the four *Plasmodium* species that infect humans and which is distributed in tropical Africa, Asia and Latin America (Karou et al., 2003). Despite the extensive control efforts, the incidence of the disease is not decreasing, especially in developing countries where malaria remains a major public health problem (Karou et al., 2003).

Although occurrence in South Africa *per se* is limited, malaria is a significant problem in areas where it occurs with neighbouring countries such as Botswana reporting a 40% frequency at times. Secondly, influx of migrants from neighbouring countries does occur and migrants presenting with malaria are likely to be treated using local traditional knowledge and South African plants. Although malaria is seasonal (December to June) with highest incidence during the summer months in KwaZulu-Natal reports have documented about 45 000 cases such as in 2000 (Malaria in Southern Africa, 2005). Research in this area in South Africa is also driven by national goals where research centres are encouraged to engage in malaria research such as the Medical Research Council (http://www.mrc.ac.za/malaria/malaria.htm).

Eleven essential oils and 17 solvent extracts of indigenous *Salvia* species were subjected to pharmacological testing in order to evaluate their potential to inhibit the *in vitro* growth of *Plasmodium falciparum* FCR-3 strain. The investigation was conducted using the [^{3}H]-hypoxanthine radiometric method (Kamatou, 2006). The outcome showed that both the essential oils and the solvent extracts displayed antiplasmodial activity. The IC_{50} values of the essential oils ranged from 1.20 to 13.50 μg/ml and displayed promising activity compared to the solvent extracts (IC_{50} values ranging from 3.91 to 26.01 μg/ml). These values are higher when compared to chloroquine disphosphate used as positive control (IC_{50} value 0.06 μg/ml). Clarkson et al. (2004) demonstrated that *Salvia repens* displayed antiplasmodial activity against *Plasmodium falciparum* (IC_{50} value 10.8 μg/ml) which was not significantly different to 8.25 ± 2.09 μg/ml obtained by Kamatou et al. (2008).

3.4.2. Antiplasmodial compounds isolated from Salvia radula

The bio-assay guided fractionation of the crude extract resulted in the isolation, identification and characterization of two compounds namely betulafolientriol oxide and salvigenin from *Salvia radula*, the most potent antiplasmodial extract (Kamatou et al., 2008). However, the two compounds displayed comparable or lower antiplasmodial activity (IC_{50} values 4.95 and 24.60 μg/ml, respectively) than the crude solvent extract.

The antimalarial activity of essential oils may be attributed to their high sesquiterpene content (Kamatou, 2006). Sesquiterpenoids and their derivatives are credited with numerous biological properties, including antiplasmodial activity. Van Zyl et al. (2006) evaluated the antimalarial activity of twenty individual commercial essential oil constituents (alcohols, aldehydes, esters, ketones, phenols and terpene hydrocarbons). Nerolidol, linalyl acetate, pulegone and α-pinene were amongst the most active constituents (IC_{50} values <0.3 μg/ml). α-Pinene was identified in the majority of essential oils of indigenous *Salvia* species (traces-22.3%), while linalyl acetate was only detected in *Salvia dolomitica* (19.6%) (Kamatou, 2006). These compounds may therefore be responsible for the antiplasmodial activity of the oils.

3.5. Cytotoxicity activity

3.5.1. Cytotoxicity of the solvent extracts against human cancer cells

It is reported that cancer causes 7 million deaths each year and results in 12.5% of deaths worldwide (WHO, 2006). Plants have played an important role as a source of effective anticancer agents and it is significant that over 60% of currently used anticancer agents are derived in one way or another from natural sources including plants, marine organisms and micro-organisms (Cragg et al., 1997; Valeriote et al., 2002). An experiment was conducted on three human cancer cell lines, including the breast adenocarcinoma (MCF-7), the colon adenocarcinoma (HT-29) and the glioblastoma (SF-268) cell lines, using the sulforhodamine B assay in order to determine the anticancer activity of indigenous *Salvia* species (Kamatou et al., 2008). The extracts inhibited cancer cells in a dose-dependent manner. The concentration required to inhibit 50% of cell growth (IC_{50} values) ranged between 9.69 and 43.65 μg/ml and between 8.72 and 59.12 μg/ml against the MCF-7 and SF-268 cell lines, respectively, with *Salvia radula* and *Salvia africana–caerulea* being the most active. IC_{50} values against the HT-29 cell line ranged from 17.05 to 57.00 μg/ml, with the extract from *Salvia lanceolata* being the most active. Cell line specificity was observed for *Salvia dolomitica* and *Salvia garipensis*, while *Salvia lanceolata*, *Salvia muirii*, *Salvia namaensis*, *Salvia repens*, *Salvia runcinata* and *Salvia verbenaca* showed some degree of cell-type selectivity. None of the extracts was as active as 5-fluorouracil, the anticancer drug used as

positive control (IC_{50} value <7 μg/ml). Many exotic *Salvia* species are known to exhibit cytotoxic effects on cancer cells. For instance, *Salvia hypargeia* was reported to inhibit a panel of cell lines with the IC_{50} lower than 20 μg/ml) (Ulubelen et al., 1999).

3.5.2. Cytotoxicity of the solvent extracts and essential oils against human epithelial cells

The *in vitro* toxicity profile to kidney epithelial cells of 28 samples (17 solvent extracts and 11 essential oils) was evaluated on human kidney epithelial cells using the 3-(4,5-dimethylthiazol-2-yl)-2,5 dimethyl tetrazolium bromide (MTT) colourimetric method (Kamatou, 2006). The samples displayed some degree of toxicity with IC_{50} values ranging from 1.79 ± 0.43 to 22.9 ± 5.28 μg/ml for the essential oils and from 12.12 ± 2.02 to 53.34 ± 3.90 μg/ml for the solvent extracts. The toxicity profile of the essential oils was significantly higher compared to the solvent extracts ($P < 0.05$). The toxicity of the essential oils is well reported in the literature (Van Zyl and Viljoen, 2003; Nyiligira, 2004; Van Vuuren et al., 2006). The essential oils and solvent extracts were always more toxic (lower IC_{50} values) than 5-fluorouracil, used as positive control (IC_{50} value greater than 136 μg/ml). The higher toxicity of the essential oil is attributed to the presence of highly hydrophobic essential oil components that have a low molecular weight. These compounds can easily cross and/or interact with the membrane causing a loss of structural integrity. This increases the permeability of protons and ions which eventually will cause cell death (Sikkema et al., 1995).

There are various types of toxic compounds in plants and these include tannins, lycosides and alkaloids. The identification of tannins in *Salvia* species has not yet been reported in the literature, but alkaloids and glycosides are generally found in the genus (Lu and Foo, 2002). Many alkaloids are poisonous to both animals and humans. Some of the species investigated, such as *Salvia chamelaeagnea*, *Salvia namaensis* and *Salvia runcinata,* tested positive for alkaloids (Raffauf, 1996).

3.6. Other points not discussed

The alcoholic extract of the aerial parts of *Salvia verbenaca* has been reported to potentiate smooth muscle contractions induced by acetylcholine, histamine, BaCl2, and serotonin (Todorov et al., 1984). Furthermore, diterpenes verbenacine and salvinine were isolated from the 95% alcohol extract (Ahmed et al., 2004).

A water extract of *Salvia africana–lutea*, was investigated for analgesic and antipyretic activities using acetic acid writhing and hot plate tests, and lipopolysaccharide (LP)-induced pyrexia test in mice and rats, respectively. Results indicated that *Salvia africana–lutea* significantly inhibited acetic acid-induced writhing and also significantly delayed the time of reaction of mice to thermal stimulation produced by the hot plate. The plant also significantly reduced fever induced by LP. Therefore, the *in vivo* analgesic and antipyretic potential of *Salvia africana–lutea* could be demonstrated (Amabeoku et al., 2001).

4. Sustainable use: *in vitro* cultivation

The conservation status of *Salvia* in Africa and many other ethnobotanicals still remains unknown. Three *Salvia* species (*Salvia repens*, *Salvia schlechteri* and *Salvia obtusata*) are listed among the Red Data List of the South African plant species (Interim Red Data List of South African Plant Taxa, 2007). Application of biotechnology offers an alternative source of important secondary metabolites thereby potentially alleviating pressure on wild populations which are over-utilized due to their popularity as traditional medicines. Other threats to biodiversity include the encroachment of urban development on natural habitats and/or selective destruction of flora due to bioprospecting activities.

There has been a considerable effort in the use of plant cell cultures as an alternative for production of pharmaceutically active compounds unique to plants (Dörnenburg and Knorr, 1995) to assist with biodiversity conservation. Some noteworthy sage examples include the exotic species: *Salvia milthiorrhiza* and *Salvia officinalis.* Studies of this nature on the South African species are in their infancy. The micropropagation of *Salvia chamelaeagnea* and *Salvia africana–lutea* was successful with the establishment of both callus and plantlet regeneration (Huang and Van Staden, 2002; Makunga and Van Staden, 2008, respectively). *In vitro* plantlets of *Salvia africana–lutea* were tested for their pharmacological activity and the extracts were most limiting to *Bacillus subtilis* growth (390 μg/ml) (Ramogola et al., 2008).

Using conventional *Agrobacterium rhizogenes* transformation several hairy root clones of *Salvia africana–lutea* were successfully established (Makunga et al., 2007) and subsequently tested for their pharmacological actions. *Fusarium verticilliodes* (a plant pathogen) was most sensitive to the effects of methanol:dichloromethane (1:1, v/v) extracts for all clones tested (MIC range of 20–320 μg/ml) (Ramogola et al., 2008). Differences in activity amongst transgenic clones were noted with *Agrobacterium rhizogenes* A4T-transformants being highly active against *Fusarium verticilliodes*. One of the hairy root clones exhibited activity which was similar to *ex vitro* leaf material (20 μg/ml) against *Fusarium verticilliodes*. This activity was lower than the standards amphotericin B (640 μg/ml) and cantus (160 μg/ml). Out of the clones tested, the clone coined A4T-3 exhibited better activity than the *ex vitro* leaf extracts and the standards for *Fusarium verticilliodes*. The MIC value of 20 μg/ml was recorded after 4 days for the A4T-3 clone against *Fusarium proliferatum*. This was higher than the activity noted for leaf extracts (MIC value 640 μg/ml) (Ramogola et al., 2008). Extracts from hairy root cultures had compounds which were not present in normal cultures. Metabolite profiles of hairy root clones, analysed via GC–MS and LC–MS, indicated an increased accumulation of mainly fatty acids and plant sterols plus caffeic acid derivatives. Differences in the biological activity of different clones may thus be attributed to compounds unique to hairy root clones. This needs further investigation and studies to identify active component(s) are currently underway. The biotechnological approach may provide an alternative supply of secondary compounds of *Salvia africana–lutea* in the future. Furthermore, the micropropagation systems established for *Salvia chameleagnea* and *Salvia africana–lutea* may be adopted for rare South African sages, meeting aims of *in vitro* conservation.

5. Conclusions

In South Africa, *Salvia* species are widely used in traditional medicine. These indigenous species are rich in phenolic compounds which in most of cases are responsible for the pharmacological properties of these plants. Some bio-active compounds have been isolated and various acids have been identified in South African *Salvia* species including caffeic acid, oleanolic acid and ursolic acid. Some species tested positive for alkaloids. The essential oil composition of indigenous *Salvia* species is highly variable qualitatively and quantitatively. *Salvia runcinata* was particularly rich in α-bisabolol and may therefore be considered as a natural source of this commercially important sesquiterpene. The collective results obtained for the *in vitro* pharmacology activity of *Salvia* species provide scientific support for the use of these plants in traditional medicine. Exploitation of the South African species for their medicinal properties concomitant with a drive to study medicinal flora is driving ethnopharmacological research in the country. This is hav-

ing significant positive impacts on our knowledge on South African plants. With respect to the sages, only nine of the endemic species are used medicinally. Even so, exploring the other species for phytoactives is important in order to gain a better understanding of local biodiversity. This is evidenced by recent ethnopharmacological efforts in the South African sages. Even though this group was previously ignored, we now have a better understanding of their general biology. The research input is likely to continue in search of new natural products for application in the pharmaceutical, food and cosmetics industries. It is important to note that most of the research done on *Salvia* employs *in vitro*-based studies and *in vivo* tests should be encouraged.

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