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SCIENCE DIRECT
Journal of Ethnopharmacology 102 (2005) 382–390
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

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

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Received 16 July 2004; received in revised form 1 June 2005; accepted 21 June 2005
Available online 15 August 2005

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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doi:10.1016/j.jep.2005.06.034

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*