HOSTED BY
ELSEVIER
Contents lists available at ScienceDirect
Asian Pacific Journal of Tropical Medicine
journal homepage: http://ees.elsevier.com/apjtm

Original research http://dx.doi.org/10.1016/j.apjtm.2016.03.031

Anti-inflammatory activity of the essential oils of *Cymbopogon validus* (Stapf) Stapf ex Burtt Davy from Eastern Cape, South Africa

Pamela Rungqu[1], Opeoluwa Oyedeji[1*], Benedicta Nkeh-Chungag[2], Sandile Songca[3], Oluwatobi Oluwafemi[4], Adebola Oyedeji[3]

[1] *Department of Chemistry, Faculty of Science and Agriculture, University of Fort Hare, Alice 5700, South Africa*

[2] *Department of Biological and Environmental Sciences, Faculty of Natural Sciences, Walter Sisulu University, Mthatha 5099, South Africa*

[3] *Department of Chemical and Physical Sciences, Faculty of Natural Sciences, Walter Sisulu University, Mthatha 5099, South Africa*

[4] *Department of Applied Chemistry, Faculty of Sciences, University of Johannesburg, Doornfontein 2028, South Africa*

ARTICLE INFO

Article history:
Received 15 Jan 2016
Received in revised form 16 Feb 2016
Accepted 15 Mar 2016
Available online 23 Mar 2016

Keywords:
Cymbopogon validus
Essential oils
Anti-inflammation
Artemisia ketone
Linalool
Egg albumin-induced edema

ABSTRACT

Objective: To evaluate the essential oil composition and the anti-inflammatory activity of *Cymbopogon validus* (*C. validus*) leaves and flowers.

Methods: A total of 300 g of fresh or dry (leaves and flowers) of *C. validus* were cut into small pieces and subjected to hydro-distillation method for approximately 5 h using the Clevenger apparatus. The extracted essential oils were then used for testing the anti-inflammatory activity. The anti-inflammatory activity was evaluated by using egg albumin-induced paw edema.

Results: The extracted oils had the following yields 2.2% for fresh leaves, 2.0% for dry leaves and 2.4% v/w for dry flowers. GC–MS results revealed that the oils contained artemisia ketone (37.5%), linalool (3.2%–29.6%), northujane (4.4%–16.8%), verbenone (13.5%), naphthalene (1.7%–9.6%), δ-cadinene (0.5%–8.1%), hedycaryol (5.4%–7.6%) and α-eudesmol (6.5%–6.7%) as the major constituents. *C. validus* essential oils showed significant ($P < 0.05$) anti-inflammatory effects from the first 30 min after albumin injection compared to aspirin which had a later onset of effect.

Conclusions: The findings of this study show that the essential oil extracted from *C. validus* fresh or dry leaves and flowers have anti-inflammatory properties; that might be associated with the major components and the minor components found in the essential oils.

1. Introduction

Cymbopogon genus is one of the most important essential oil yielding genera of the Poaceae family [1]. It comprises of about 180 species, sub species, varieties as well as sub varieties [2]. *Cymbopogon* species are scattered all around the world and more than 52 species are said to be found in Africa, 45 in India, 6 in Australia and South America, 4 in Europe, 2 in North America and the remaining species are dispersed in South Asia [3]. The plants' essential oils are known for their pleasant aromas which are used to flavor foods and beverages; and as fragrances in cosmetic and pharmaceutical industries [4]. The oils are also used in household products and tobacco [5]. Numerous extracts and essential oils extracted from these plants have been tested for repellent and insecticidal properties using different types of arthropods; they have also been used to cure several infectious diseases that are engendered by bacteria, fungi, protozoa and virus. People from the jungle regions of Bolivia Amazon use *Cymbopogon* plants as repellents against mosquitoes [6]. Essential oils from *Cymbopogon* species like *Cymbopogon flexuous* (*C. flexuous*), *Cymbopogon citratus* (*C. citratus*), *Cymbopogon martini* (*C. martini*), *Cymbopogon winterianus* (*C. winterianus*), *Cymbopogon nardus* (*C. nardus*), *Cymbopogon giganteus* (*C. giganteus*), *Cymbopogon schoenanthus* (*C. schoenanthus*) *and Cymbopogon parkeri* (*C. parkeri*) contain monoterpenoid components such as geraniol and citronellol which are considered dominant in the oils; while the *C. flexuous* oils and that of *C. parkeri* shows the prevalence of sesquiterpenoids such as isontermedeol. The essential oil of *Cymbopogon* species also

*Corresponding author: Opeoluwa Oyedeji, Ph.D, Department of Chemistry, Faculty of Science and Agriculture, University of Fort Hare, Alice 5700, South Africa.
Tel: +27 764260280
E-mail: ooyedeji@ufh.ac.za

Peer review under responsibility of Hainan Medical College.
Foundation project: It was financially supported by Govan Mbeki Research and Development Centre (GMRDC) (UFH), Directorates of Research, Walter Sisulu University (WSU) and NRF-IKS (UID-82640).

reveals that the citral chemotype is common in *Cymbopogon pendulus* (*C. pendulus*), *C. flexuous* and *C. citratus* [7].

Cymbopogon validus (*C. validus*) (Stapf) Stapf ex Burtt Davy is commonly known as a turpentine grass or the African Blue Grass in South Africa, and the Afrikaner people call it 'Reuse Terpentynegras'. This species belongs to the Poaceae family and has been described as a tufted perennial with culms up to 2.4 m tall. Normally *C. validus* is found in mountainous grasslands and also in the high-rainfall areas of South Africa where it is known to grow in wet sites, along roads and on the margins of tree communities [8]. It is widespread in the Eastern Cape and is often used as durable thatch [4,8]. *C. validus* oil is pure therapeutic quality aromatherapy essential oil that is produced by using wild-crafted plants and traditional methods from South Africa [9]. Essential oils from *C. validus* are used as astringents, skin toners and are also used in anti-aging preparation for men; these essential oils also have anti-fungal, antiseptic, as well as anti-viral properties. The oils are also popularly used as a soothing foot bath. *C. validus* essential oils and decoctions are used traditionally as anti-rodent, vermifuge, emetic, anti-infective, and anti-plasmodic; they also help in treating morning sickness [2,7]. Chagonda *et al* [7] reported that the major components from wild *C. validus* essential oils from Zimbabwe were myrcene (23.1%–35.6%), (*E*)-β-ocimene (10.3%–11.5%), geraniol (3.4%–8.3%), linalool (3.2%–3.7%) and camphene (5.2%–6.0%); in cultivated *C. validus* essential oils myrcene (11.6%–20.2%), (*E*)-β-ocimene (6.0%–12.2%), borneol (3.9%–9.5%), geraniol (1.7%–5.0%) and camphene (3.3%–8.3%) were the major components. Naidoo also revealed that *C. validus* essential oils from Durban contained α-cubenene, camphene, citronellal, geraniol, limonene, palmitic acid and sabinene as the major components [10]. The study was aimed at extracting essential oils from both (fresh and dry) parts of *C. validus* flowers and leaves, to determine their chemical profile, characterize the oils for medicinal and then evaluate the biological potential of the oils as anti-inflammatory agents.

2. Materials and methods

2.1. Plant material

C. validus was collected in the month of April 2013 at the Komga road, near King William's Town. The plants were taxonomically identified by Dr T. Dold and the voucher specimen was deposited in Selmar Schonland Herbarium Grahamstown (GRA) at Rhodes University and the collection number was PR/PL 02.

2.2. Extraction of essential oils

A total of 300 g of fresh or dry (leaves and flowers) of the plant material were cut into small pieces and subjected to hydro-distillation method for approximately 5 h using the Clevenger apparatus. The extracted essential oils were stored in sealed sample vials and stored in a refrigerator at 4 °C until the time of analysis and bioassays.

2.3. Analysis of essential oils

GC-FID was performed on a HP5890-II instrument, equipped with a DB-5MS (30 m × 0.25 mm; 0.25 μm film thickness) fused silica capillary column. Hydrogen was used as carrier gas adjusted to a linear velocity of 32 cm/s (measured at 100 °C). Split flow was adjusted to give a 20:1 ratio and septum sweep was a constant 10 mL/min. The oven was programmed as follows: 60 °C-240 °C at 3 °C/min. The samples were injected using the splitless technique using 2 μL of oil in hexane (2:1 000). Injector and detector were set at 250 °C. The GC was equipped with FID and connected with to an electronic integrator HP 5896 Series II. The percentage of the samples was computed from the GC peak areas without using correction for response factors.

GC–MS was performed on a Hewlett Packard-6890 system equipped with a HP-5MS fused capillary column (30 m × 0.25 mm; 0.25 μm film thickness), coupled to a selective mass detector Hewlett Packard-5973. Helium (1 mL/min) was used as carrier gas; oven temperature program: 60 °C–240 °C at 3 °C/min; splitless during 1.50 min; sample volume 2 μL of the oil solution in hexane (2:1 000). Injector and detector temperature was 250 °C. EIMS: electron energy, 70 eV; ion source temperature and connection parts: 180 °C.

2.4. Identification of compounds

Identification of compounds was done by matching their mass spectra and retention indices with those recorded in NIST11 library and by comparison of retention indices and mass spectra with literature values [11–13].

2.5. Bio-assay (anti-inflammation)

Both female and male Wistar rats weighing 195–240 g were used. The rats were obtained from the South African Vaccine Producers and were housed in the animal holding facility at the Zoology Department of Walter Sisulu University in Mthatha. Ethical clearance for the study was obtained from the Walter Sisulu University Research Ethics Committee DVC (AA&R)/DRD/SREC: Reference No: 31. The animals were kept under standard conditions with each cage housing 5 rats; room temperature was maintained at 24 °C and lighting was by daylight only. Animals had free access to food and water throughout except 8 h before experimentation when animals were only given only water. A total of 5 rats were randomly assigned to one of 5 groups, control group treated with 1 mL 0.09% NaCl, standard group treated with 100 mg/kg Aspirin, C.V.F.L group treated with 1 mL of 2% essential oil from fresh leaves of *C. validus*, C.V.D.L group treated with 1 mL of 2% essential oil from dry leaves of *C. validus* and C.V.D.F group treated with 1 mL of 2% essential oil from dry flowers of *C. validus*. All treatments were administered in 1 mL volumes.

Aspirin was procured from Reckitt Benckiser Pharmaceutical (PTY) LTD/(EDMS) BPK Elandsfontein – South Africa.

2.6. Anti-inflammatory activity: fresh egg albumin-induced right hind paw edema

Animals were randomly distributed to one of the 5 groups as indicated earlier. Baseline right hind paw diameter (paw size) was determined for each rat using a pair of YATO digital caliper [14–17]. Rats were administered with oral doses of drugs as per assigned group. A total of 30 min later the right hind paw of each rat was injected with 1 mL of 50% (v/v) fresh egg albumin. Paw sizes were again measured 30, 60, 90 and