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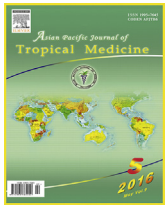

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Anti-inflammatory activity of the essential oils of *Cymbopogon validus* (Stapf) Stapf ex Burtt Davy from Eastern Cape, South Africa

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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

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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

120 min after albumin injection. Change in paw size was calculated as:

Δ paw size = paw size after albumin injection at pre-determined times − paw size before albumin injection.

2.7. Statistical analysis

One way of Analysis with Turkey-Kramer Multiple Comparisons Test was performed using GraphPadInstat (version 3.05 for Windows 95, GraphPad Software, San Diego California USA, www.graphPad.com) to determine the difference between each treatment group and control. The *P* value for the overall relationship was 0.001 and was considered significant. Results were expressed as mean ± standard error of the mean.

3. Results

3.1. Chemical composition of essential oils

The pale yellow oils extracted from *C. validus* had a pleasant odor. The percentage yields were as follows 2.2% v/w for fresh leaves, 2.0% v/w for dry leaves and 2.43% v/w for dry flowers. Table 1 presents the examined constituents of the essential oils as well as their percentage compositions. A total of 32 components were identified in the essential oil of *C. validus*; 17 components accounted for 85.2% in fresh leaves, 20 components for 91.3% in dry leaves and 16 components for 89.6% in dry flowers. The main components identified in the essential oil of *C. validus* fresh leaves were artemisia ketone (37.5%), verbenone (13.5%), naphthalene (9.6%) and northujane (4.4%); linalool (29.6%), northujane (12.3%), δ-cadinene (8.1%) and α-eudesmol (6.7%) were the major components in dry leaves; while in dry flowers linalool (28.0%), northujane (16.8%), hedycaryol (7.6%) and α-eudesmol (6.5%) were the major components. The GC–MS results also showed that the fresh leaves oil of *C. validus* contained 8.6% monoterpenes, 55.4% oxygenated monoterpenes, 5.4% sesquiterpenes, 1.8% oxygenated sesquiterpenes, 14% others in fresh leaves; while in dry leaves the oils were mostly composed of 1.9% monoterpenes, 31.0% oxygenated monoterpenes, 3.1% sesquiterpenes, 41.1% oxygenated sesquiterpenes, 14.2% other. *C. validus* dry flower's oil was mostly composed of 1.9% monoterpenes, 28.0% oxygenated monoterpenes, 12.0% sesquiterpenes and 29.6% oxygenated sesquiterpenes and 18.5% other (Table 2).

Table 2
Chemical classes of compounds found on different parts of *C. validus* essential oil (%).

Chemical classes of compounds	Fresh leaves	Dry leaves	Dry flowers
Monoterpenes	8.6	1.9	1.9
Oxygenated monoterpenes	55.4	31.0	28.0
Sesquiterpenes	5.4	3.1	11.9
Oxygenated sesquiterpenes	1.8	41.1	29.3
Others	14.0	14.2	18.5
Total identified	85.2	91.3	89.6

Table 1
Chemical constituents from different parts of *C. validus* essential oil extracts.

No	Constituents	RI[a]	RI[b]	Fresh leaves (%)	Dry leaves (%)	Dry flowers (%)	Identification method
1	Northujane	859	859	4.4	12.3	16.8	MS[c], RI[d]
2	Camphene	953	946	–	0.6	–	MS[c], RI[d]
3	Sabinene	976	969	1.5	–	–	MS[c], RI[d]
4	Myrcene	991	988	4.1	1.3	1.9	MS[c], RI[d]
5	α-Phellandrene	1005	1002	2.2	–	–	MS[c], RI[d]
6	Artemisia ketone	1062	1056	37.5	–	–	MS[c], RI[d]
7	cis-Linalool oxide	1074	1067	–	1.4	–	MS[c], RI[d]
8	m-Cymene	1082	1082	0.8	–	–	MS[c], RI[d]
9	Linalool	1098	1095	3.2	29.6	28.0	MS[c], RI[d]
10	Naphthalene	1179	1178	9.6	1.9	1.7	MS[c], RI[d]
11	α-Terpineol	1189	1186	0.5	–	–	MS[c], RI[d]
12	Verbenone	1204	1204	13.5	–	–	MS[c], RI[d]
13	Carvone	1242	1239	0.7	–	–	MS[c], RI[d]
14	α-Cubebene	1351	1345	0.7	2.7	–	MS[c], RI[d]
15	α-Longipinene	1351	1350	–	–	2.8	MS[c], RI[d]
16	Cyclosativene	1368	1369	–	0.9	–	MS[c], RI[d]
17	β-Elemene	1375	1389	–	1.2	1.5	MS[c], RI[d]
18	β-Cubebene	1390	1387	2.3	–	–	MS[c], RI[d]
19	β-Caryophyllene	1418	1418	1.9			MS[c], RI[d]
20	α-Muurolene	1499	1500	–	1.0	1.2	MS[c], RI[d]
21	β-Dihydroagarofuran	1504	1499	–	2.4	6.4	MS[c], RI[d]
22	Germacrene D-4-ol	1511	1574	0.6	–	–	MS[c], RI[d]
23	δ-Cadinene	1524	1522	0.5	8.1	–	MS[c], RI[d]
24	Hedycaryol	1530	1546	–	5.4	7.6	MS[c], RI[d]
25	Elemol	1547	1548	–	2.2	1.8	MS[c], RI[d]
26	Globulol	1576	1590	–	–	1.7	MS[c], RI[d]
27	Caryophyllene oxide	1581	1582	1.2	1.9	–	MS[c], RI[d]
28	Guaiol	1595	1600	–	2.3	1.5	MS[c], RI[d]
29	γ-Eudesmol	1630	1630	–	4.0	4.5	MS[c], RI[d]
30	Cubenol	1642	1645	–	3.5	4.1	MS[c], RI[d]
31	β-Eudesmol	1649	1649	–	1.9	1.6	MS[c], RI[d]
32	α-Eudesmol	1652	1652	–	6.7	6.5	MS[c], RI[d]

RI[a]: retention indices on HP-5MS capillary column.
RI[b]: literature retention indices [18].
MS[c]: identification based on mass spectral data.
RI[d]: identification on the basis of NIST11 library and comparison with literature data.

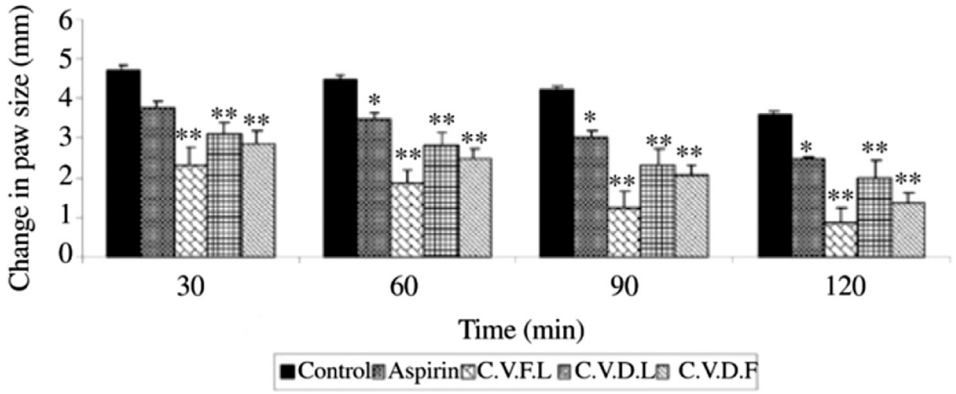

Figure 1. Anti-inflammatory effects of *C. validus* oil (fresh and dry leaves and dry flowers) on albumin-induced paw edema, at different time of inhibition. $^{*}P < 0.05$, $^{**}P < 0.01$ compared to control animals.

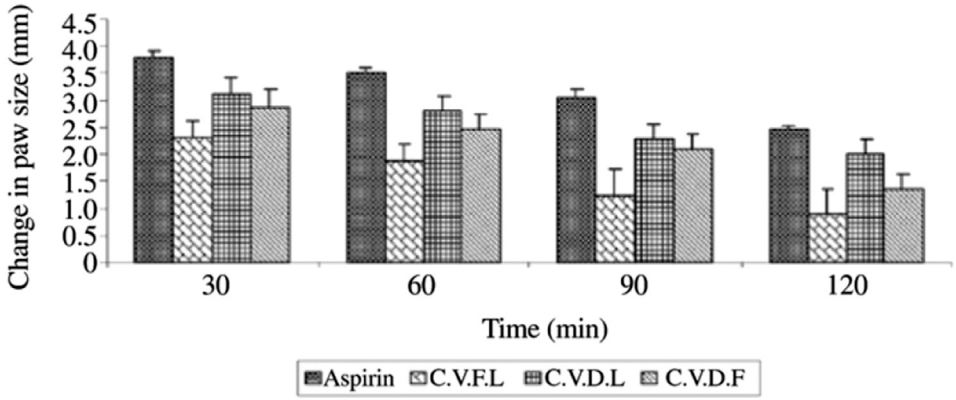

Figure 2. Anti-inflammatory effects of *C. validus* oil (fresh and dried leaves; and fresh buds and dried buds) on fresh egg albumin-induced paw edema, at different time of inhibition.
$^{*}P < 0.05$, $^{**}P < 0.01$ compared to aspirin treated animals.

3.2. Anti-inflammatory of paw edema inhibition

Figure 1 illustrated the anti-inflammation effects of essential oils of *C. validus* on fresh egg albumin-induced inflammation: measured 30, 60, 90 and 120 min after injection of the phlogistic agent. All the essential oils showed significant ($P < 0.01$) anti-inflammatory effects from 30 to 120 min. Results obtained with essential oils were better than those obtained with aspirin during the experimental period. On the other hand Figure 2 showed a comparison of the anti-inflammatory effect of aspirin with oils from fresh; dry leaves and dry flowers of *C. validus*. The fresh leaves oil showed significantly ($P < 0.05$ and $P < 0.01$) greater anti-inflammatory effect compared to aspirin throughout the experimental period. Essential oil from the dry flowers also showed significantly ($P < 0.05$) greater effects than aspirin during the 60 and 120 min while the anti-inflammatory effects of oil from dry leaves was not different from results obtained with aspirin.

4. Discussion

4.1. Chemical composition of essential oils

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 components. The GC/MS results also revealed that oxygenated monoterpenes represented 5 of the 32 compounds corresponding to 55.4% of the fresh leaves oil, oxygenated sesquiterpenes represented 11 of the 32 compounds corresponding to 41.1% of the dry leaves, while the dry flower's oil represented 8 of the 32 compounds corresponding to 29.3%. The main constituents in our *C. validus* essential oil were quite different from the *C. validus* essential oil derived from Zimbabwe and Durban. For example, Chagonda *et al* reported that the essential oil of wild *C. validus* from Zimbabwe had the following major constituents 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%) while in cultivated *C. validus* essential oil 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%) as major constituents [7]. Naidoo revealed that the essential oil of *C. validus* from Durban had α-cubenene, camphene, citronellal, geraniol, limonene, palmitic acid and sabinene as major constituents [10]. When comparing our results to the essential oil of *C. validus* reported by Chagonda *et al* and Naidoo [7,10], it was observed that our *C. validus* essential oil showed a very distinct composition which was mainly characterized 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%). It was also observed that our *C. validus*

essential oil comprised of oxygenated monoterpenes as their major oil constituents, while the essential oil of *C. validus* from Durban consisted of more than 50% of sesquiterpene constituents [10]; Chagonda *et al* found that the oils from the wild and cultivated *C. validus* predominately comprised of monoterpenes [7]. Then it was concluded that the 3 plants from 2 different provinces (Eastern Cape and Kwazulu Natal) and one from Zimbabwe are 3 different chemotypes of the same plant species. The observed difference in chemical composition and content of the essential oil of *C. validus* could be due to climatic and soil variation, stage of vegetative cycle and seasonal variation [19], literature does support the identification of different chemotypes of plant species based on variation of their chemical constituents. Therefore, our results support the fact that plant species from the same genus can differ because of their geographical location.

4.2. Anti-inflammatory activity

The anti-inflammatory effect of *C. validus* essential oils in Wistar rats using the fresh egg albumin-induced rat paw edema model was analyzed. The essential oil from *C. validus* (*i.e.* fresh; dry leaves and dry flowers) showed anti-inflammatory action by reducing the paw volume significantly ($P < 0.05$ and $P < 0.01$). Fresh egg albumin-induced inflammation occurs in 3 phases. An initial phase during the first 1.5 h is caused by the release of histamine and serotonin [20]. The second phase involves the release of bradykinin from 1.5 h to 2.5 h, whilst the third phase involves the release of prostaglandins and that occurs from 2.5 h to 6.0 h after albumin injection [21]. The current study lasted for only 120 min due to rapid resolution of inflammation. This corresponds to the first phase of inflammation, indicating that the oil from the fresh; dry leaves and dry flowers of *C. validus* exert their anti-inflammatory properties by inhibiting the release of histamine and serotonin. *C. validus* essential oils exhibited significant ($P < 0.01$) anti-inflammatory activity as compared to the control group throughout the 2 h period of experimentation. Aspirin showed no effectiveness during the first 30 min, but it was effective ($P < 0.05$) at 60, 90 and 120 min. Linalool, a major constituent in the oil could have contributed to the observed anti-inflammatory effects. Indeed a few studies have revealed an effect of (−) – Linalool on chronic inflammation, which significantly reduced Complete Freund's Adjuvant induced paw edema at a dose of 200 mg/kg [22–25]. Artemisia ketone could also be attributed with the anti-inflammatory effect of the oil as other studies reveal that ketones can reduce pain and inflammation [26]. This inhibition may also be associated to the presence of α-eudesmol, naphthalene and δ-cadinene for example previous reports reveal that α-eudesmol was responsible for the inhibition of neurogenic inflammation in models of the electrical stimulation [27], while naphthalene derivatives are currently used for the treatment of inflammatory disorders [28]; Moreover, δ-cadinene which was one of the main components in the *Teucrium* essential oil exhibited anti-inflammatory activity [29]. Contents of essential oil from *C. validus* may exert their effects by inhibiting the release of mediators such as histamine and serotonin during the first phase. Thus, both fresh and dry parts of the plant proved to have significant anti-inflammatory properties.

Conflict of interest statement

The authors declare no conflict of interest.

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