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ORIGINAL ARTICLE

Volatile constituents and biological activities of the leaf and root of *Echinacea* species from South Africa

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KEYWORDS

Echinacea purpurea;
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Abstract *Echinacea* is used ethnomedicinally for the treatment of various diseases such as cough, respiratory infections, and bronchitis among other uses in Eastern Cape region of South Africa. This study evaluated the volatile components of the essential oil of the plant, its toxicity, anti-inflammatory and analgesic activities in rodents.

Dried leaf and root of the plant were separately processed by hydrodistillation for 4 h and their essential oils (EOs) were collected. Extracted oils were subjected to GC/GC–MS analysis. The essential oil was further evaluated for acute toxicity, anti-inflammatory and analgesic activities. The toxicity profile of the essential oil was evaluated in mice through the oral route (p.o.), and anti-inflammatory activity was evaluated on the carrageenan-induced edema model in rats at the doses of 100–200 mg/kg, while its analgesic effect was evaluated on the acetic acid-induced writhings model in mice at doses of 100–200 mg/kg.

GC/GC–MS analysis of EOs showed that a number of compounds identified in the leaf and root oils were 25 and 31 respectively. The chemical compositions of the oils varied and the major compounds identified in the oils include germacrene D, naphthalene, caryophyllene oxide, α-phellandrene and α-cadinol. The essential root oil did not cause mortality at the highest dose of 5000 mg/kg; hence, its LD_{50} was estimated to be $\geqslant$5000 mg/kg, p.o. The anti-inflammatory test

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results showed that the essential root oil caused significant ($p < 0.05$–0.01) reduction in edema size compared to the negative control group on the carrageenan-induced edema and the results for the analgesic test showed that the essential root oil caused significant ($p < 0.05$) reduction in number of writhings at 1000 mg/kg compared to the negative control group.

It is concluded that root and leaf of this *Echinacea* species contain volatile oils which varied in their yield and chemical compositions. The essential root oil is non-toxic orally and it demonstrated significant anti-inflammatory and analgesic activities in laboratory animals.

1. Introduction

Medicinal plants are plants that are assumed to have healing properties and often used to treat some illnesses. These medicinal plants have been identified and used throughout the human history, making an impact on both world health and international trade for treating various diseases such as diarrhea, wounds, and dry cough (Van Wyk et al., 1997).

Echinacea also known as purple coneflower is a medicinal plant that belongs to the Asteraceae family. The plant consists of more than 19 species with three species of medicinal interest known as *Echinacea purpurea, Echinacea angustifolia* and *Echinacea pallida*. *Echinacea* has been used for centuries by Native Americans for the treatment of various diseases (Turner et al., 2000). Currently, *Echinacea* is being used to combat bacterial, viral, protozoan and fungal infections, and as an anti-inflammatory agent and as a possible chemo preventative agent (Barnes et al., 2005).

All the three species of medicinal interest of *Echinacea* contain varying amounts of essential oils in root, leaf, flower and aerial parts (Mazza et al., 1999). Essential oil of *E. purpurea* contains caryophyllene, caryophyllene epoxide, germacrene D and borneol while that of *E. angustifolia* and *E. pallida* contains ketoalkynes and ketoalkenes (Mazza et al., 1999; Hudaib et al., 2002).

Essential oils have been recognized as therapeutic agents and widely utilized as potent natural medicinal components of plants (Djilani and Dicko, 2012). Essential oils are complex mixtures of volatile constituents frequently containing 20–60 or more individual compounds (Miguel, 2010). Chemical composition of a particular species can vary greatly and some factors that have been identified to be responsible include genetic, geographical location, climatic changes, growing conditions, seasonal variation, time of collection etc. among other factors (Andrade et al., 2011). Common classes of compounds found in essential oils include hydrocarbons, esters, oxides, lactones, alcohols, phenols, aldehydes and ketones. These components have been reported to be responsible for several bio-activities ascribed to essential oils (De Sousa et al., 2011). In this study the essential oils of root and leaf of *Echinacea* were evaluated for chemical composition and biological activities in order to determine its contribution to the reported bioactivities of the plant and to validate its traditional uses in treating some diseases including infections, respiratory diseases and pain-related ailments.

In the Eastern Cape region of South Africa, *Echinacea* is a popular herbal medicine used by the traditional healers to manage various diseases including wound healing, respiratory infections, and pain among several others. The dried leaf and root of the *E. purpurea* species used in this study was supplied by the Indigenous Knowledge System holder (Mr. Reuben Matewu) and was among the several medicinal plants submitted for chemical and biological studies for the purpose of verifying their medicinal efficacies in laboratory animals by our research group.

2. Materials and methods

2.1. Drugs, chemicals and reagents

Acetic acid (BDH Chemical Ltd., Poole, England), diclofenac potassium (Diclogesic® Supreme), aspirin. All chemicals and reagents were obtained from Sigma–Aldrich Chemical Co. (St Louis, MO, USA). All the chemicals used including the solvents, were of analytical grade.

2.2. Animals

Mice and rats were obtained from the South African Vaccine Initiative, Johannesburg, and kept at the Animal Holding Facility, Zoology Department, WSU. Male and female Wistar rats (200–300 g) were randomly selected ($n = 6$), and were used for the anti-inflammatory test. Male Swiss mice (25–35 g; $n = 6$) were also used for the acute toxicity and the analgesic tests. The animals were kept under standard conditions of temperature and humidity and had free access to rat chow and water. Food was however withheld overnight prior to experiments while water was provided ad libitum. This study was approved by the Department of Higher Education, WSU, and Ethical Clearance Approval obtained, Walter Sisulu University Ethics Committee Reference No. DVC (AA&R) DRD/SREC: Reference No: 31.

2.3. Plant collection

Dried *Echinacea* root and leaf was supplied by the Herbal Practitioner (IKS holder) at King William's town, Eastern Cape on the 3rd of July, 2014.

2.4. Extraction of the essential oil

Essential oil of the dried leaf (342 g) and root (368 g) of the plant were obtained by hydrodistillation using the Clevenger-type apparatus for 4 h (British Pharmacopoeia, 1980). The oils were separately collected in airtight glass vial containers, and stored at 4 °C (Oyedeji et al., 2006) before analysis. The yields

of the oils were 0.82 g (0.24% w/w) for the leaf and 1.05 g (0.29% w/w) for the root.

2.5. GC/GC–MS analysis

Analysis of the oils was performed on an Agilent 5973 N Gas Chromatography - mass spectrometer system operating in EI mode at 70 eV, equipped with a HP-5 MS fused silica capillary system with 5% phenylmethylsiloxane stationary phase, and capillary column parameter was 30 m by 0.25 mm, film thickness 0.25 μm. The initial temperature of the column was 70 °C and was heated to final temperature of 250 °C at a rate of 5 °C/min. Helium was used as the carrier gas at a flow rate of 1 ml/min. The split ratio was 100:1. Scan time was 78 min with a scanning range of 35–450 amu. One microliter (1 μl) of the diluted oil (in hexane) was injected for analysis. n-Alkane of C8 to C30 were run under the same condition of Kovat indices determination. The constituents were identified by GC using retention indices compared with those of literature. The retention indices were determined in relation to a homologous series of alkanes under the same operating conditions. The components of the oils were identified by matching their spectra and retention indices (Kovat Index) with those of the authentic samples and literature values (ESO, 2000; Adams, 1989; Joulain, 1988).

3. Biological studies

3.1. Acute toxicity

Essential oil obtained from the root was used for the biological assessment because its yield was more and it contains more compounds (from the GC/MS analysis). The essential root oil was emulsified with Tween 80 to obtain a maximum concentration of 5%v/v before administration to the animals. The acute toxicity of the root oil of the plant was accessed according to Lorke's method (Lorke, 1983). This procedure was divided into 2 phases. The first phase of the test consists of three sub-groups ($n = 3$) for each dose level of 10, 100 and 1000 mg/kg. The second phase employed 4 subgroups ($n = 1$) per dose level of 1000, 1600, 2900 and 5000 mg/kg respectively. Immediately after the treatment, each mouse was placed inside the Plexiglas cage and observed for immediate effects up to 30 min and thereafter for 24 h for lethal effects culminating into death. The LD_{50} of the essential root oil was estimated as the geometric mean of the lowest dose causing death and the highest dose causing no death according to the formula below:

$$LD_{50} = \sqrt{((A) \times B)}$$

where A is the maximum dose producing 0% death and B is the dose that produces 100% death (Lorke, 1983). From the result of LD_{50} the working doses will be determined according to the equation below:

$$\text{Working Doses} \leqslant 1/2(LD_{50})$$

3.2. Anti-inflammatory test

Rats were used for the acute inflammation study using carrageenan as the phlogistic agent. Animals were divided into four (4) experimental groups of six rats each (Nkeh-Chungag et al., 2010). Group I was negative control group (5% Tween 80, 10 ml/kg), groups II and III were *Echinacea* essential root oil (100 and 200 mg/kg respectively), while group IV was the positive control group (aspirin, 100 mg/kg). All treatments were by oral route (p.o.) one hour prior to injection of 0.1 ml of 2% carrageenan into the sub-plantar surface of the left hind paw of the rat. Baseline paw was measured prior to and after 1, 2, 3 and 4 h post injection of the carrageenan using Vernier Calipers (Yato).

3.3. Analgesic test

Acetic acid-induced writhing test model was used where there are four groups of six mice.

Group I was negative control group (5% Tween 80, 10 ml/kg), and groups II and III were *Echinacea* essential root oil (100 and 200 mg/kg respectively), while group IV was the positive control group (diclofenac, 100 mg/kg). All treatments were by oral route (p.o.) one h prior to intraperitoneal injection of 0.6% acetic acid (10 ml/kg). Number of abdominal constrictions or writhings was counted 5 min post acetic acid injection for a period of 20 min inside the Plexiglas cages. The number of writhing displayed by each mouse was counted and recorded (Hajhashemi et al., 2003).

4. Results and discussion

This study determined the chemical composition of the root and leaf essential oils of *Echinacea,* and evaluated the root essential oil for acute toxicity profile, anti-inflammatory and analgesic activities in rodents. The results obtained showed that the essential oils obtained contain several compounds while the root oil was found to be non-toxic, and possess significant anti-inflammatory and analgesic activities in laboratory animals.

The yields of the essential oils obtained from this *Echinacea* species were 0.24 and 0.29% w/w for the leaf and root respectively. In Table 1, the chemical composition of the essential oil isolated from *Echinacea* is summarized; from the GC–MS results it is evident that *Echinacea* contains various compounds; and major compounds for both the leaf and the root were Germacrene D, Naphthalene, Caryophyllene oxide, Cedrol α-Phellandrene, and α-Cadinol. Previous studies reported that concentrations of dried material of *Echinacea* essential oil range from 0.1% to 1.25% w/w depending on the part of the plant material used (Mazza et al., 1999). Mistrikova and Vaverková (2006) reported that the essential oil of *E. purpurea* species obtained from Slovakia contains caryophyllene epoxide, caryophyllene and Germacrene D as the main constituents signifying that the *E. purpurea* species being reported here closely resemble that of Slovakia's. The results from GC–MS showed that these oils may be useful in cosmetic and pharmaceutical industries and this justifies the use of the plant for various ailments by traditional practitioners (Herbalists). The presence of these compounds indicates that the South African *Echinacea* species closely resemble the *E. purpurea* species from other regions of the world (Barnes et al., 2005; Bauer, 1999).

The acute toxicity test showed that the essential oil of this plant did not cause mortality at the highest dose of 5000 mg/kg,

Table 1 Essential oil composition of the leaf and root of *Echinacea*.

RT	Compounds	% Composition of compound		KI
		Leaf oil	Root oil	
3.24	2-Hexenal	0.9	0.3	853
4.83	α-Pinene	2.8	3.7	937
4.92	α–Fenchene	–	0.7	949
5.07	Camphene	–	2.0	951
6.15	β-Pinene	1.6	1.2	974
6.90	α-Phellandrene	6.9	6.6	1004
7.74	π-Cymene	3.7	2.9	1029
8.31	Limonene	2.3	1.7	1082
10.07	Linalool	1.5	2.7	1089
11.08	Camphor	–	3.4	1143
11.94	Isomenthone	0.1	0.5	1159
12.23	Borneol	–	2.1	1166
12.93	Lavandulol	–	0.5	1168
15.02	p-Cymen-8-ol	–	1.2	1183
13.76	α-Terpineol	–	0.2	1189
14.24	Naphthalene	7.8	6.4	1192
16.66	Estragole	2.2	3.3	1199
17.24	Trans-Carveol	4.8	2.6	1217
17.99	Cis-Carveol	1.0	1.0	1227
18.50	Carvone	1.0	3.8	1242
18.69	Peperitone	1.8	-	1253
18.98	Thymol	–	0.4	1290
19.72	α-Cubebene	1.4	0.9	1351
20.60	β-Cubebene	0.8	0.4	1387
21.16	Methyl eugenol	–	0.6	1402
22.03	Caryophyllene	4.5	4.0	1415
22.33	β-Humulene	0.1	-	1442
22.72	Geranyl acetone	–	0.2	1452
22.91	α-Humulene	0.6	-	1453
23.08	γ-Muurolene	1.2	-	1476
23.47	Germacrene D	18.1	20.3	1482
26.11	Caryophyllene oxide	11.3	12.2	1577
26.28	Viridiflorol	2.9	-	1590
27.25	Cedrol	7.2	10.5	1600
28.95	Ledol	–	3.1	1608
29.25	α-Cadinol	9.1	5.9	1653
% Total		93.6	94.1	

Table 2 Acute oral toxicity profile of the essential root oil of *Echinacea* in mice.

Dose (mg/kg, p.o.)	Death patterns after 24 h
Phase 1 (n = 3)	
10	0/3
100	0/3
1000	0/3
Phase 2 (n = 1)	
1000	0/1
1600	0/1
2900	0/1
5000	0/1
LD_{50}	$LD_{50} = \geqslant 5000$ mg/kg, p.o.

p.o. (Table 2), suggesting that it is non-toxic orally and can be used to explain the widespread and longtime use of this plant in traditional medicine. Other studies reported that the side effects and toxicological risks of *Echinacea* preparations are very low and the reports showed that *Echinacea* extracts are found to possess the acute toxicity in an extremely low level (Coeugniet et al., 1986).

The essential root oil of *Echinacea* (100–200 mg/kg) dose-dependently and significantly ($p < 0.05$–0.01) reduced the carrageenan–induced rat paw edema throughout the 4 h assessment period compared to the negative control group signifying anti-inflammatory activity (Adzu et al., 2003; Muhammad et al., 2015). Similarly, aspirin (standard drug) also caused significant ($p < 0.05$–0.01) reduction in edema size throughout the observation period of 4 h (Fig. 2). Previous antiinflammatory test of the flower essential oil of *E. purpurea* obtained from China showed positive result on the egg albumin-induced rat paw edema (Yu et al., 2013). Thus the results obtained here corroborated antiinflammatory potentials of *Echinacea* essential oil previously reported. *Echinacea* has wide application in folkloric medicine in managing various pain-related ailments including rheumatism and wound healing (Borchers et al., 2000); hence, the present results justify the use of this plant in treating these diseases. Although the

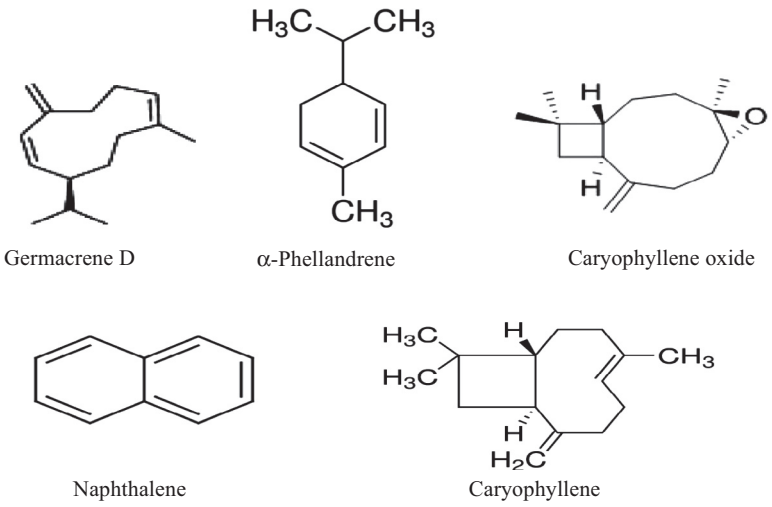

Figure 1 Some major compounds identified in the essential oil of *Echinacea* leaf and root.

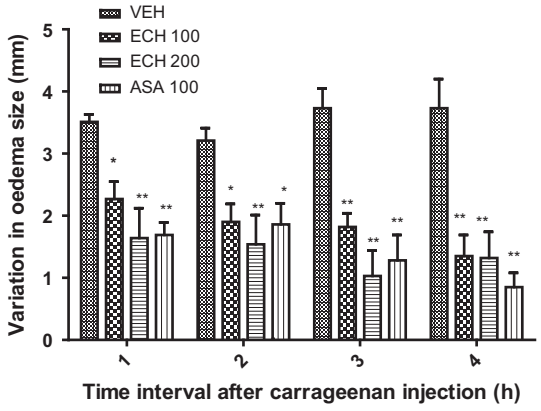

Figure 2 Effect of *Echinacea* essential root oil on the carrageenan-induced rat paw edema. Results are expressed as Mean ± SEM. VEH, ECH and ASA represent vehicle (5% Tween 80), *Echinacea* root essential oil and aspirin respectively. $^{*}p < 0.05$, $^{**}p < 0.01$, statistically different from negative control group (ANOVA, Dunnett's).

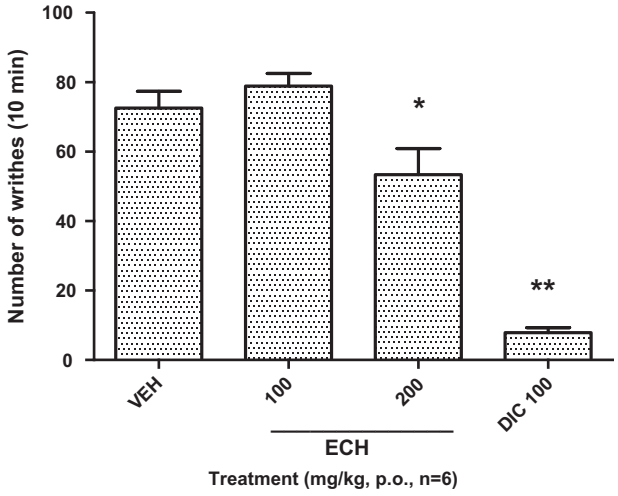

Figure 3 Effect of essential oil of *Echinacea* root on acetic acid-induced writhing. Results are expressed as Mean ± SEM. VEH, ECH and DIC represent vehicle (5% Tween 80), *Echinacea* root essential oil and diclofenac respectively. $^{*}p < 0.05$, $^{**}p < 0.01$; statistically different from vehicle group (ANOVA, Dunnett's).

traditional method of administering this plant to patients made use of the infusion extract which is not the pure essential oil, the present results implied that the volatile component of this plant contributes majorly to its anti-inflammatory activity. Future research can focus on isolating and testing the individual components identified in the essential oil (see Fig. 1).

Acetic acid-induced writhing test is a sensitive model for peripheral nociception in rodents. The results obtained here showed that the essential oil caused significant ($p < 0.05$) reduction in writhings caused by the acetic acid compared to the negative group (Fig. 3) suggesting analgesic potential (Yonglin et al., 2009; Olorunfemi et al., 2012). These results indicate that this plant may be more effective as an inflammatory than as an analgesic agent that can be used to further justify its use in inflammatory diseases such as wound healing and rheumatism.

5. Conclusion

The leaf and root oils of *Echinacea* are composed of similar compounds with seven of their major compounds being the same. *Echinacea* root oil is non-toxic and it demonstrated significant anti-inflammatory and analgesic activities, thus supporting the use of traditional application of this plant by Herbalists in the Eastern Cape of South Africa to treat some ailments.

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