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CHEMICAL ANALYSIS AND BIOLOGICAL POTENTIAL OF *VALERIAN ROOT* AS USED BY HERBAL PRACTITIONERS IN THE EASTERN CAPE PROVINCE, SOUTH AFRICA

Nelisa Assistance Dyayiya[1], Idris Ajayi Oyemitan[1, 2], Reuben Matewu[3], Opeoluwa Oyehan Oyedeji[4], Samuel Oluwatobi Oluwafemi[5], Benedicta N Nkeh-Chungag[6], Sandile Phindile Songca[7], and Adebola Omowunmi Oyedeji[1,*]

[1]Department of Chemical & Physical Sciences, Faculty of Natural Sciences, Walter Sisulu University, Mthatha, Republic of South Africa, [2]Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria, [3]1124 Buchanan Street, Ginsburg, King Williams Town, Eastern Cape, South Africa, [4]Department of Chemistry, Faculty of Science & Agriculture, University of Fort Hare, Alice, Republic of South Africa, [5]Department of Applied Chemistry, Faculty of Science, University of Johannesburg, Republic of South Africa, [6]Department of Biological and Environmental Sciences, Faculty of Natural Sciences, Walter Sisulu University, Mthatha, Republic of South Africa, [7]Academic and Research Division, Office of the Deputy Vice Chancellor (Academics), Walter Sisulu University, Mthatha, Republic of South Africa
*Correspondence: aoyedeji@wsu.ac.za

Abstract

Background: Herbal practitioners in the Eastern Cape of South Africa use valerian root (*Valeriana capensis*, Valerianaceae) to manage pains, arthritis and inflammation. The herb prepared from this plant was studied to determine the chemical composition of its essential oil, carried out phytochemical screening and biological activities on its infusion extract as used by the herbal practitioner.
Materials and Methods: Essential oil of *Valerian* root was obtained by hydrodistillation and subjected to chemical analyses. Infusion extract of the *Valerian* root was screened to determine its secondary metabolites and the relative abundance of some major metabolites. The infusion extract was further evaluated for acute toxicity (LD_{50}), anti-inflammatory and analgesic activities in rodents.
Results: The yield of the essential oil was 0.18% w/w. The GC/MS analysis indicated the presence of 42 compounds with major ones being caryophyllene oxide (18.11%), viridiflorol (9.37%) and bornyl acetate (8.84%). Phytochemicals found in the infusion extract were alkaloids, saponins, tannins and flavonoids while quantitative screenings showed saponins and flavonoids accounted for 6.39% and 7.40% respectively. The LD_{50} of the extract was found to be 3808 mg/kg per oral. The infusion extract of the root (250-500 mg/kg, *p.o.*) caused significant ($p<0.01$) activity in the carrageenan-induced rat paw oedema model comparable to aspirin, indicating anti-inflammatory activity; but lacked analgesic activity on the acetic acid-induced writhing test.
Conclusion: The infusion extract possessed significant anti-inflammatory but lacked analgesic activity; the present data justify the use of this herbal agent by the herbal practitioners from the Eastern Cape region of South Africa.

Key words: *Valeriana capensis*, essential oil, caryophyllene oxide, infusion extract, anti-inflammatory, analgesic

Introduction

Medicinal plants have been in use for centuries in South Africa, especially in the rural communities throughout the Eastern Cape Province where many people consult traditional healers who use medicinal plants as major sources of their medication (Cocks and Moller, 2002). *Valerian* root (Family: Valerianaceae) is a perennial flowering plant with over 350 species and several more subspecies (Bardakci *et al.*, 2012). *Valerian* is native to Europe and Asia, although it has been naturalized in Eastern North America and other parts of the world including South Africa (Van Wyk *et al*., 1997). The part of the plant used medicinally is the root or rhizome which is light grayish brown, about the size of a finger joint, bearing many rootlets. The mature plant is about 50-150 cm tall with pinnate leaves and the stem is upright and without branches (Fleming, 1998). Valerian fresh root has no odour, while the dried root possesses distinctly unpleasant smell similar to old dirty socks (Schulz *et al.*, 1998).

Valerian is a popular herbal product often used to treat insomnia, anxiety and related ailments (Murti *et al*., 2011). *Valerian* root extracts contain essential oils rich in sesquiterpenes such as valerenic acid and its derivatives. *Valerian* is most often used to treat insomnia though evidence to support this is inconclusive (Stevenson and Ernst, 2000) but it is still considered as an alternative treatment to hypnotic drugs (Dietz *et al.*, 2005). *Valeriana wallichii* is another species used in Europe as an antispasmodic, particularly for abdominal or uterine cramps and nervousness (Boon and Smith, 2004).

Analyses of *Valerian* have reported over 150 chemical constituents with various biological activities. Like other medicinal plants, there is substantial variation in the chemical constituents of this plant species from different regions due to climatic conditions, processing and storage conditions (Buckland, 1999). *Valeriana officinalis* root from India contains up to 2% volatile oil, of which bornyl acetate a monoterpene, is a major constituent (Murti *et al.*, 2011); while common compounds found in the oil include valerenic, valeric, isovaleric acid, monoterpenes and sesquiterpenes. Isovaleric acid has been shown to possess anticonvulsant (Eadie, 2004) and sedative properties (Murphy *et al*., 2010). Valerian also contains small amounts of phenolic acids and flavonoids, valerosidatum, chlorogenic acid, caffeic acid, choline, β-sitosterol, fatty acids, and various minerals. *Valeriana officinalis* extract contains 0.5-2% essential oil (Lacher *et al*., 2007), iridoid valepotriates: valtrates, isovaltrate, didrovaltrate, valerosidate and others (Murphy *et al*., 2010). Flavonoids with CNS activity, such as 6-methyl apigenin and 2S (-)-hesperidin have been isolated from *Valeriana wallichii* and have been proven to possess a benzodiazepine-like binding site ligand. These compounds have sedative, sleep-enhancing and anxiolytic properties (Marder *et al*., 2003). The large differences in the concentrations of these substances are undoubtedly responsible, at least in part, for the variations in the biochemical and clinical responses to this herbal agent (Hendriks *et al*., 1981).

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Biological studies on valerian have confirmed many activities including anti-inflammatory (Wang *et al*., 2010), anticonvulsant and antidepressant (Khuda *et al*., 2012), anxiolytic (Murphy *et al*., 2010) and sedative effects in preclinical (Fernandez *et al*., 2004) as well as in clinical studies (Balderer and Borbély, 1985; Leathwood *et al*., 1982). *Valerian*'s effect on the central nervous system has been well documented and attributed to the many active compounds such as iridoid, valerenic acid, valepotriates, valtrates, isovaltrate, didrovaltrate, valerosidate and other constituents in the essential oil (Morteza and Joorabloo, 2012).

In 2012, the Department of Science and Technology (DST) and the National Research Foundation (NRF) of South Africa inaugurated the Indigenous Knowledge System (IKS)/Medicinal Plant Based Indigenous Value Chains and Trade. One of the objectives was to support Herbal Practitioners in the Eastern Cape to develop medicinal products for public use. As a preliminary step in this direction, *V. capensis* Thum known in IXhosa language as *umvuthuza* (Dod and Cocks, 1999), one of the herbal medicines used by the herbal practitioner in our team, was studied by investigating the chemical composition of the essential oil; phytochemical screening of the infusion extract, determination of the acute toxicity profile and evaluating some biological activities of the infusion extract of the *valerian* root of this species found in this region of South Africa. To the best of our knowledge, this is the first study of its kind reporting chemical composition and biological activities of this particular species from the Eastern Cape. The results obtained here would be used to rationalize the traditional use of this plant and provide additional data to the scientific community on this natural product.

Materials and Methods

Plant Collection

Seven hundred g of dried grounded *Valerian* root was collected from the herbal practitioner, Mr. Reuben Matewu in Ginsburg, King William Town on the 3rd of July, 2014. The sample collected was further identified by Dr. K. Immelman, Herbarium Unit, Department of Botany, Walter Sisulu University, Mthatha, South Africa.

Extraction of Essential Oil

Essential oil of the dried *Valerian* root was extracted by hydro-distillation using the Clevenger-type apparatus. Three hundred and fifty g of the dried root was hydro-distilled for 4 h and the oil obtained stored in lightproof bottle until analysis.

Analysis of The Essential Oil
GC and GC/MS Analyses

The oil was analysed by GC and GC/MS. GC analysis was carried out on a Perkin-Elmer 8500 gas chromatograph with a FID detector with a SGE BP X5 column that is 30 m in length with a film thickness of 0.25 µm and diameter 0.25 mm ID. The operating conditions were as follows: carrier gas, nitrogen with a flow rate of 3.0 ml/min; column temperature, 60-275 °C at 4 °C/min; injector and detector temperature, 280°C- ; volume injected 0.1 µl of the oil; split ratio, 1:50. GC/MS of the essential oil was carried out using an Agilent Gas Chromatography (890 equipped with a capillary column (Agilent 190915 30 m × 250 µm × 0.25 µm calibrated) attached with an Agilent mass spectrometer system (5975C VL MSD with Triple Axis Detector). The oven temperature was programmed from 50 °C – 310 °C. Helium was used as the carrier gas at a flow rate of 5 ml /min with a split ratio of 1:200. The essential oil (1 µl) was diluted in hexane and 0.5 µl of the solution was manually injected into the GC/MS. The chemical compositions of the essential oil of the dried Valerian root were determined according to their retention time and spectrometric electronic libraries (WILEY NIST). The identity of the constituents of the oils was established using GC retention indices (RI) and comparing their mass spectra with those reported in literature (Joulain and Koenig, 1998; Adams, 2007). Library search was carried out using the NIST and WILEY GC/MS spectral database.

Preparation of Infusion Extract

The infusion extract was obtained as per recipe provided by method used by the herbal practitioner. Briefly, the dried valerian root was weighed and put inside a glass flask (1000 ml) and boiling water was poured into the flask until it completely covered the herbal material. The mixture was kept with regular shaking for 24 h. Thereafter, it was filtered with Whatman filter paper 1. A portion of the filtrate was kept for phytochemical screening while the remaining portion was dried in the oven at 35 ^{0}C and the resultant infusion extract kept until needed for biological studies.

Phytochemical Screening of the Infusion Extract
Qualitative Analysis

Several phytochemical tests were carried out to detect the presence of phytochemical components in the infusion extract of the *valerian* root (Mir et al., 2012). Secondary metabolites screened include tannins, saponins, flavonoids, terpenoids, alkaloids, phenols, phytosterols, glycosides, anthraquinones, phylobotannins and proteins/amino acids. All the chemicals used in this study for qualitative and quantitative phytochemical screening were of analytical grade.

Quantitative Analysis of Secondary Metabolites in the Valerian Root

Saponins: Twenty g of dried, ground valerian root was put in a conical flask and 100 ml of 20% aqueous ethanol were added. The mixture was then heated on a hot water bath (55 ^{0}C) for 4 hours with continuous stirring, after which the mixture was filtered and the residue re-extracted with a further 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at 60 ^{0}C. The solution was then transferred into a 250 ml

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separatory funnel and 20 ml of dried ether was added and shaken vigorously. The ether layer was discarded and the aqueous extract recovered. The purification process was repeated three times. Sixty ml of n-butanol was added, the extract was then washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated on a water bath to evaporate. The remaining solution was dried in an oven (35°C) to a constant weight, the saponins content was calculated as a percentage of the starting material.

Flavonoids: Ten g of the valerian root was extracted repeatedly with 100 ml of 80% aqueous methanol, at room temperature. The solution was filtered; the filtrate transferred into a crucible and evaporated into dryness over a water bath, the dried extract was then weighed.

Tannins: Five hundred mg of valerian root was weighed into a 50 ml bottle, 50 ml of distilled water was added and the bottle was shaken for 1 h on a mechanical shaker. The solution was filtered into a 50 ml volumetric flask and filled up to the mark. Five ml of the filtrate was transferred into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1M HCl and 0,008 M potassium ferrocyanide. The absorbance was measured within 10 minutes. The Tannin content was calculated using a standard curve of Gallic acid.

Alkaloids: Five g of valerian root was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and the solution covered and allowed to stand for 4 h. The mixture was filtered and the filtrate concentrated on a water bath to one quarter of its original volume. Concentrated ammonia hydroxide was added drop-wise to the mixture until the precipitation was complete. The solution was allowed to settle and the precipitate was then collected and washed with dilute ammonium hydroxide and then filtered. The residue which is an alkaloid was dried and weighed.

Biological Studies

Chemicals and Drugs

The following drugs were used for biological studies: carrageenan (Sigma Chemical Co., MO, USA) and acetylsalicylic acid (Bristol-Mayers Squibb, USA), acetic acid (Sigma Chemical, USA).

Experimental 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) randomly selected (n=6), were used for the anti-inflammatory test. Swiss mice of both sexes (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, 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.

Acute Toxicity Test

Acute toxicity (LD_{50}) effect of the infusion extract of valerian root was assessed in mice (25-30 g) using the oral route (*p.o.*) according to Lorke's method (Lorke, 1983) using only thirteen animals on the whole for rapid and economic LD_{50} estimation. The procedure was divided into two phases. The first phase had three animals per group of 10, 100 or 1000 mg/kg. The second phase had four groups (n=1) for the dose levels of 1000, 1600, 2900 and 5000 mg/kg respectively. Immediately after treatment, each mouse was placed inside the Plexiglas cage and observed for immediate effects for up to 30 min and thereafter for 24 h for lethal effects culminating into death. The LD_{50} of the infusion extract 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).

Anti-Inflammatory Test

The anti-inflammatory activity of the infusion extract of valerian root was evaluated by carrageenan-induced rat paw oedema model (Winter *et al*., 1962) and as described by Olajide *et al.* (2000). In this test, four groups of rats (n=6) were used. Rats in the different groups were orally pre-treated with normal saline, two doses of the extract (250 and 500 mg/kg) and aspirin (100 mg/kg) 1 h prior to carrageenan injection (subplantarly with 0.1 ml of 2% carrageenan in normal saline). Baseline paw size was measured prior and after 1, 2, 3 and 4 h post injection of the carrageenan using Vernier Calipers (Joseph et al., 2005).

Analgesic Test: Acetic Acid-Induced Writhing Test

Four groups of mice (n=6) were randomly selected and orally pre-treated as follows: group 1 was given normal saline (10 ml/kg), groups 2-3 were administered 500 and 1000 mg/kg of the extract respectively, while group 4 received aspirin (100 mg/kg). 1 h after pre-treatment, each mouse was injected intraperitoneally with 10 ml/kg of 0.6% acetic acid and allowed 5 minutes delay before assessment for up to 20 min inside the Plexiglas cage. The number of writhings displayed by each mouse was counted and recorded (Hajhashemi et al., 2003).

Statistical Analysis

Results were expressed as Mean±SEM. Statistical analyses were carried out using one way analysis of variance (ANOVA) followed by Dunnett's post hoc test, and values were considered significant at $p<0.05$.

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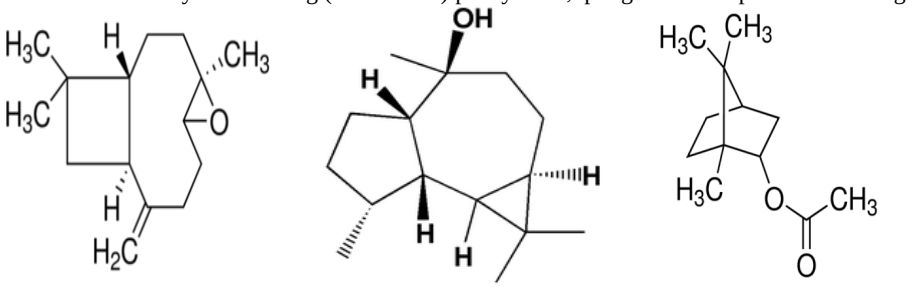
Results

Extraction of Essential Oil

Hydro-distillation of the valerian root yielded 0.63 g (0.18% w/w) pale yellow, pungent and unpleasent smelling essential oil.



Caryophyllene oxide Viridiflorol Bornyl acetate

Figure 1: Some compounds identified in the root essential oil of *V. capensis*

Table 1: Chemical composition of the essential oil of the valerian root

Peak no.	Compound[a,b]	Elution time	KI[a]	% composition	
1	Isovaleric acid	3.556	877	1.28	
2	Alpha-pinene	5.205	937	1.20	
3	Camphene	5.515	951	2.80	
4	Beta-pinene	6.075	974	0.26	
5	p-cymene	7.030	1082	0.09	
6	Artemiseole	9.945	11.23	0.25	
7	Endo-borneol	10.765	1165	2.15	
8	Myrtenol	11.395	1192	0.59	
9	Piperitone	20.165	1252	3.04	
10	Bornyl acetate	13.720	1281	8.84	
11	Carvacrol	14.045	1299	0.17	
12	Myrtenyl acetate	14.725	1335	0.69	
13	Delta-elemene	15.090	1336	0.20	
14	Terpinenyl acetate	15.345	1340	0.36	
15	Unidentified	17.130	-	1.57	
16	Caryophyllene	17.335	1415	1.60	
17	Alpha-Gurjunene	18.095	1436	1.76	
18	alloaromadendrene	18.350	1461	0.83	
19	Unknown	18.625	-	1.73	
20	Gamma-cadinene	21.320	1473	2.28	
21	Valencene	22.735	1491	1.03	
22	Gamma-elemene	19.225	1492	0.11	
23	Beta-bisabolene	19.425	1504	0.17	
24	(E)-alpha-Bisabolene	19.660	1508	0.14	
25	Alpha-alaskene	23.220	1513	2.61	
26	Caryophyllene oxide	24.275	1577	18.11	
27	Unidentified	20.610	-	1.82	
28	Beta-Spathulenol	21.165	1572	3.24	
29	Viridiflorol	21.010	1590	9.37	
30	Ledol	21.635	1608	1.47	
31	beta-Eudesmol	21.835	1647	0.79	
32	Unidentified	22.330	-	10.93	
33	Alpha-Eudesmol	22.960	1647	3.03	
34	Patchouli alcohol	23.430	1659	3.32	
35	Alpha-bisabolol	23.615	1683	1.07	
36	Beyerene	26.025	1924	1.33	
37	Unidentified	26.175	-	1.01	
38	Unidentified	26.520	-	0.93	
39	Unidentified	27.370	-	2.60	
40	Unidentified	28.145	-	3.71	
41	Unidentified	30.990	-	0.97	
42	Ipsdienol	31.540	-	0.54	

[a](Adams, 2007); [b](ESO 2000,1990)

Chemical Composition of the Essential Oil

GC and GC/MS analyses of the essential oil of the valerian root reveals the presence of forty-two components (Table 1). The major compounds (>3%) identified in the oil included caryophyllene oxide (18.11%), viridiflorol (9.37%), bornyl acetate (8.84%), patchouli alcohol (3.32%), β-spathulenol (3.24%) and α-eudesmol (3.03%). The volatile oil was composed mainly of oxygenated sesquiterpenoids (40.94%) while the other components were oxygenated monoterpenes (17.32), sesquiterpene hydrocarbons (12.06%), monoterpene hydrocarbons (4.35%) and other unidentified compounds (25.28%). Chemical structures of the 3 most abundant compounds are shown in Figure 1.

Phytochemical Screening of the Infusion Extract of Valerian Root
Qualitative Screening

Phytochemical screening for secondary metabolites indicated the presence of alkaloids, tannins, saponins, phenols, steroids, flavonoids and terpenoids; but phytosterols, glycosides, proteins/amino acids, phlobatannins and carbohydrates were not detected in the infusion extract.

Quantitative Phytochemical Screening

The results of quantitative screening of infusion extract of Valerian root showed that the percentage composition of saponins and flavonoids were 6.39 and 7.4% respectively; while the alkaloid and tannin components were not recoverable.

Acute Toxicity Test

There was no mortality in the first phase. In the second phase, there was no mortality at doses up to 2900 mg/kg but there was mortality at 5000 mg/kg, hence the LD_{50} was calculated as follows:

$LD_{50} = \sqrt{A \times B} = \sqrt{2900 \times 5000} = 3808$ mg/kg, p.o.

Effect of the Infusion Extract of Valerian Root on the Carrageenan-Induced Rat Paw Oedema

The paws of all rats in the negative group were swollen after the carrageenan injection and remained swollen throughout the observation period. The infusion extract at 250 and 500 mg/kg significantly [$p<0.01$; $F_{(3,20)}=17$, 19.2 and 22.9] reduced carrageenan-induced oedema after 1, 2 and 4 h post-carrageenan injection respectively compared to the negative group. Aspirin also caused significant ($p<0.01$) reduction in oedema size throughout the observation period (Figure 2).

Effect of the Infusion Extract of Valerian Root on Acetic Acid-Induced Writhings in Mice

The result obtained from the acetic acid-induced writhing test showed that there were no statistical differences in the number of writhes between the negative control and infusion extract treated groups. However, aspirin (ASA, 100 mg/kg, *p.o.*) treated group demonstrated significant ($p<0.01$) reduced abdominal constrictions compared to other groups (Figure 3).

Discussion

This study determined the chemical composition of the essential oil of the valerian root; qualitatively and quantitatively screened the infusion extract obtained from the valerian dried root for secondary metabolites; and evaluated the infusion extract for acute toxicity profile, analgesic and anti-inflammatory activities in laboratory animals. The results obtained indicate that the essential oil contains several compounds while its infusion extract also contain some important secondary metabolites. The infusion extract of this root showed no toxicity at 2900 mg/kg, p.o., demonstrated significant anti-inflammatory activity but lack analgesic potentials. The essential oil of *Valerian* root in this study was found to contain 42 compounds (Table 1) and major ones (>3%) identified include caryophyllene oxide (18.11%), viridiflorol (9.37%), bornyl acetate (8.84%), patchouli alcohol (3.32%), β-spathulenol (3.24%) and α-eudesmol (3.03%). Chemical studies on the essential oil of three different species of this plant from Iran showed the major components of the oil to include α-selinene (7.83%) in *V. sisymbriifol;* limonene (3.53%) in *V. alliariifolia* and spathulenol (13.33%); α-campholenal (11.48%), vulgarone B (8.38%) and valerenal (8.32%) in *V. officinalis*. However, their main common compounds were spathulenol, limonene, γ-terpinene, vulgarone B and p-cymene (Samaneh *et al.*, 2010). The main composition of essential oil *V. alliariifolia* from Turkey were isovaleric acid (28.60%), ð-guaene (7.20%), valeric acid (3.70%) and humulene epoxide (3.60%) (Bardakci *et al*., 2012); but that of *V. officinalis* from China reported patchouli alcohol (16.75%), Î±-pinene (14.81%), and Î²-humulene (8.19%) were the major compounds (Wang, 2010). Bhatt *et al.* (2012) reported in a study from India on *V. jatamansi*, that the major components of its oil were patchouli alcohol (36-52%), delta-guaine (10%), seychellene (4.8%) and α-humulene (3.96%) while in an Iranian *V. alliariifolia*, trans-caryophyllene (38.96%), β-pinene (12.06%), α-pinene (9.94%) and α-terpinene (9.49%) were the 4 major constituents identified (Taherpour *et al.*, 2010). Finally,

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Das *et al*. (2011) reported the 2 major constituents of Eastern Himalayan (India) Valarian root oil (*V. hardwickii*) to be methyl linoleate (21.1%) and valeracetate (11.6%). These data indicated a wide range of variation in the chemical composition of essential oils of this plant obtained from

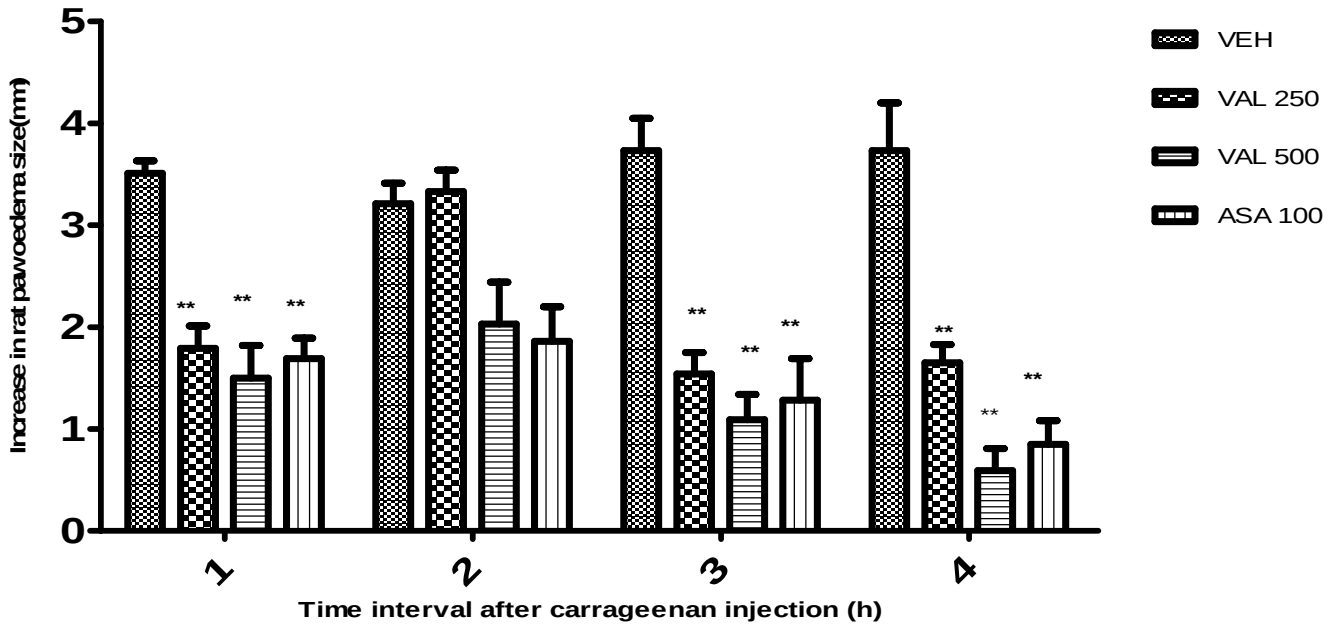

Figure 2: Effect of Valerian infusion extract on the carrageenan-induced rat paw oedema

VEH=Vehicle (Normal saline), VAL=Valerian root and ASA=Acetyl Salicylic Acid. Each histogram represents Mean±SEM, N=6.
**p<0.01, statistically different from negative control group (ANOVA, Dunnett's)

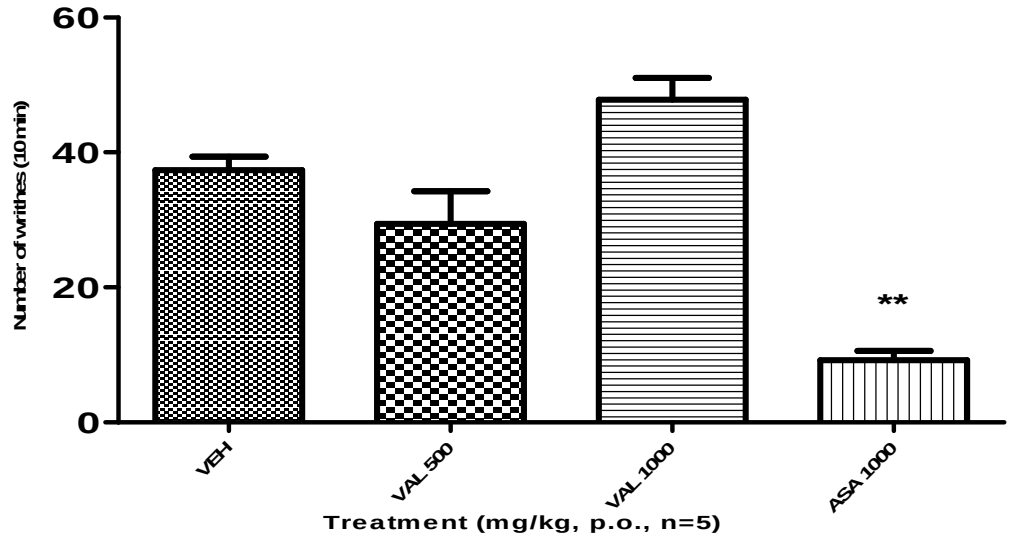

Figure 3: Effect of Valerian root infusion extracts on acetic acid-induced writhings
Results were expressed as Mean±SEM, n=5. VEH=Vehicle (Normal saline), VAL=Valerian root and ASA=aspirin.
**p<0.01; statistically different from other groups (ANOVA, Dunnett's)

different regions of the world (Bos et al., 1998). The implication of these variations would greatly influence the biological activities ascribable to thisherbal agent by the herbal practitioners from different regions. The results of the essential oil analysis obtained here provide additional data on the chemotypes of the valerian species found in South Africa. It could therefore be suggested here that the valerian species investigated in this study may be a different chemotype based on the variation in its major components compared to those of other regions of the world.

Phytochemical screening indicates the presence of some important secondary metabolites such as alkaloids, tannins, saponins, phenols, steroids, flavonoids and terpenoids. These phyto-constituents have been shown to exhibit various biological activities but there is no consensus on their specific bioactivities (Houghton, 1999) and effects observed may be due to the synergy of its various constituents (Blumenthal *et al*., 2000). The

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result of the quantitative screening showed that flavonoids was found relatively abundant (7.4% w/w) in the root, which means that this class of compound could contribute significantly to the bioactivities reported for this root as flavonoids have been variously implicated in anti-inflammatory activities of plants (Zanoli *et al.*, 2000). Furthermore, valerenic acid (non-volatile flavonoids) has been implicated in sedative and anti-convulsant activities of this plant in previous studies (Murphy *et al*., 2010) and linarin (a flavonoid glycoside) was also confirmed to exhibit sedative and sleep-enhancing properties in mice (Fernandez *et al.*, 2004). The mechanism(s) of actions of valerian root extract were reported to be mainly through enhanced GABA and adenosine neurotransmission (Muller et al., 2002) and also through 5-HT_{5a} receptor (Dietz *et al*., 2005). The data presented here is inadequate to propose possible mechanism of action of the extract, but the presence of flavonoids and saponins in high concentration suggest many mechanisms may be involved, especially, the extract may inhibit the release of chemical mediators such as cytokines, chemokines and lipid mediators normally released during inflammatory responses which can sensitize or stimulate peripheral synaptic targets or centrally, termed nuero-inflammatory mediators (Ellis and Bennett, 2013; Xanthos and Sandkühler, 2014).

In this study, we found the LD_{50} of *Valerian* root infusion extract to be 3808 mg/kg, indicating low toxicity (Rodricks, 1992; Hodge and Sterner, 1949). According to UN report (2011), LD_{50} of 2000-5000 mg/kg, p.o., is classified under Category 5, which are substances with low acute toxicity hazard, hence the extract could be declared to possess low acute toxicity profile and caution is therefore recommended for its continuous use. The root infusion extract demonstrated significant ($p<0.01$) anti-inflammatory activity which was comparable to aspirin, a standard anti-inflammatory drug (Figure 2). The most abundant secondary metabolite found in this root was flavonoids (7.4%) and may contribute to a greater extent to the observed anti-inflammatory effect. β-caryophyllene (a bicyclic sesquiterpene) was shown to exert significant anti-inflammatory effects in mice (Gertsch *et al*., 2008) and in this study, it constituted the most abundant compound in the root oil. Carrageenan is a potent chemical used for the release of inflammatory and pro-inflammatory mediators, e.g. prostaglandins and histamine, which mediates acute inflammatory processes (Muhammad *et al*., 2012). Hence, it can be suggested here that this valerian root extract may be effective in the inhibition of the arachidonic-prostaglandins pathway of inflammation similarly to NSAIDS (Jothimanivannan *et al*., 2010). The infusion extract of this root demonstrated potent anti-inflammatory activity on the carrageenan-induced inflammatory model similarly to aspirin.

The analgesic test showed that the infusion extract of this valerian root lacks significant effect against acetic acid-induced writhings. This model is regarded as a sensitive test for peripherally acting analgesics probably mediated by peritoneal mast cells (Ribeiro *et al*., 2000). The failure of this valerian extract to inhibit the acetic acid writhings suggests that it lacks peripheral analgesic activity (Uddin *et al*., 2014). The present results, to our knowledge, are the first report on the antiinflammatory activity of this South African species and current data support the use of Valerian root in managing arthritis and other inflammatory conditions by herbal practitioners in the Eastern Cape region.

Conclusion

This study showed that this *valerian* root species demonstrates low acute toxicity orally and contains a number of secondary metabolites that contribute to its bioactivities. Furthermore, its infusion extract possesses anti-inflammatory activity, thus supporting its traditional use in the management of arthritis and rheumatism.

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