doi: 10.18869/JHD.2017.59

Journal of Herbal Drug

journal homepage: www.jhd.iaushk.ac.ir

Chemical composition and anti-inflamatory, anti-nociceptive and antipyretic activity of rhizome essential oil of *Globba sessiliflora* Sims. collected from Garhwal region of Uttarakhand

Kumar Ravendra[1*], Prakash Om[1], Pant Anil K[1], Kumar Mahesh[2], Isidorov Valary A[3], Szczepaniak Lech[3]

[1]*Department of Chemistry, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture & Technology, Pantnagar, U.S. Nagar, Uttarakhand, India;*

Email:** ***ravichemistry.kumar@gmail.com

[2]*Department of Vet. Epidemiology and Preventive Medicine, College of Veterinary & Animal Sciences. G.B. Pant University of Agriculture & Technology, Pantnagar, U.S. Nagar, Uttarakhand, India;*

[3]*Institute of Chemistry, Białystok University, Division of Environmental Chemistry, UL. Hurtowa 1,15-399, Białystok, Poland;*

ARTICLE INFO

Type: *Original Research*
Topic: *Medicinal Plants*
Received *July 24th 2016*
Accepted *December 16th 2016*

Key words:
- ✓ *Globba sessiliflora* Sims
- ✓ *Essential oil*
- ✓ *Zingiberaceae*
- ✓ *β-eudesmol,*
- ✓ *Anti-inflamatory*
- ✓ *Anti-nociceptive*
- ✓ *Antipyretic activity*

ABSTRACT

Background & Aim: Family Zingiberaceae is worldwide in distribution. Plants of the zingiberaceae family are used in traditional herbal folk medicine besides their uses in spices, cosmetic, ornamental, food preservatives etc. In Uttarakhand the herbs grow from sub-tropical to temperate region. *Globba sessiliflora* Sims rhizomes were collected at maturity stage in November from Garhwal region of Uttarakhand, India. In present communication the medicinal use of various zingiberaceous herb provoked us to study the chemical diversity and pharmacological activity determination of this important traditional herb.

Experimental: The essential oil was extracted using hydrodistillation method and analyzed by GC-MS. Anti-inflamatory, anti-nociceptive and antipyretic activities of essential oil were experimently determined using mice model.

Results: The major compounds identified were β-eudesmol (27.6%), (*E*)-β-caryophyllene (24.3%), α-humulene (3.0%), (6*E*)-nerolidol (4.1%), caryophyllene oxide (9.7%), γ-eudesmol (6.4%) and τ-muurolol (8.3%) besides other minor constituents. Essential oil of *G. sessiliflora* rhizome showed good anti-inflamatory, anti-nociceptive and antipyretic activities at the dose level of 100 mg/kg body weight. The oral administration of the essential oil exhibited no toxicity at 400, 600 and 800 mg/kg b.wt. concentration. Ibuprofen, indomthacin and paracetamol were used as standard drugs for comparison.

Recommended applications/industries: *G. sessiliflora* essential oil can be used as herbal remedy for its nontoxicity anti-inflamatory, anti-nociceptive and antipyretic activities

1. Introduction

The complex biological response by harmful factors like pathogens, irritants and damaged cells results in inflammation. The common symptoms are redness of skin, edema, stiffness, pain and difficulty in of joint function which may be acute or chronic. Inflammation could result in a chronic stage which may indicate a factor of pathogenesis. A self defence mechanism may cause inflammation in primary phase which can be treated with chemotherapic agents to treat and eliminate symptoms and disease (Shailasree *et al.*, 2012).

The nociceptive pathway is described in three-neuron chain which transmits nociceptive information to the cerebral cortex from the periphery (Besson and Chaouch, 1987; Almeida *et al.*, 2004). The dorsal root ganglion has the first order neurons in their hair cell bodies. From here, two axons are projected to the peripheral tissues and the dorsal horn of the spinal cord. From the spinal cord, the second order neurons originate and ascend to the thalamus or the regions of the brainstem. From the thalamus, the third order neurons are ascended to the cerebrocortex (Besson and Chaouch, 1987; Abelson, 2005).

The secondary impact of infection is tissue damage, inflammation, graft rejection, malignancy or other diseased states which results in fever or pyrexia (Chattopadhyay *et al.*, 2005). The body's natural defense system generates in an environment for non survival of the infectious agent or damaged tissues. The enhanced formation of pro-inflammatory mediator's (cytokines like interleukin 1β, α, β and tumor necrosis factor-α) is normally caused by the infected or damaged tissues. The proinflammatory factors results the increase the synthesis of prostaglandin E2 (PGE2) near preoptic hypothalamus area and triggers the hypothalamus area to increase the body temperature (Saper and Breder, 1994).

The clinical treatment of inflammatory diseases is dependent on drugs which belong either to the non-steroidal or steroidal chemical therapeutic agents. The early steps in the biosynthetic pathway of prostaglandins by inhibition of COX enzymes are affected by the nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin, indomethacin and ibuprofen and reduce the untoward consequences of inflammation (Albert *et al.*, 2002). However NSAIDs cause side effects like gastric injury and ulceration, renal damage and bronchospasm due to their non-selective inhibition of both isoforms of the COX enzymes (Tapiero *et al.*, 2002). The controversy of the use of steroidal drugs as anti-inflammatory agents is because of their multiple side effects (Vanden Worm *et al.*, 2001). Therefore, a demand has arisen for the search of newer anti-inflammatory drugs of natural origin with potential activity and with minor side effects as substitutes for chemical therapeutic agents. Some Indian medicinal plants and their active principles which have anti-inflammatory activity are documented by Chatterjee and Pal (1984).

The available analgesic drugs in the market are often associated with several adverse effects and are either too potent or too weak. Opiods are known to cause side effects which includes; sedation, respiratory depression, potential for addiction and tolerance whereas NSAIDS are known to cause gastric irritation that may lead to gastric bleeding. There is need for new analgesic compounds (Mattison *et al.*, 1998). Plants of medicinal value in ethnopharmacology are an important source of natural products with potential therapeutic effects (Blumenthal, 2000; Bisset, 2001). Study of plant species that are used in traditional herbal medicine as pain killers, therefore, form a logical search strategy for new analgesic drugs (Farnsworth, 1989; Mattison *et al.*, 1998).

High selectivity of irreversible inhibition of COX-2 expression by antipyretic drugs is the cause of reduction of increase body temperature by possible inhibition of biosynthesis of PGE2. The natural COX-2 inhibitors have lower selectivity with less side effects and less toxic to hepatic cells, glomeruli, cortex of brain and heart muscles. To combat the pro-inflammatory mediators, a number of plant extracts have been screened those inhibit leukotriene and prostaglandins synthesis modulating cyclooxygenase pathway by inhibition of COX-1 and COX-2 enzymes (Oj *et al*., 2007, Alberto *et al.*, 2009).

The members of family Zingiberaceae are currently of interest to phytochemist and pharmacologist all the world-over and work on their chemistry and pharmacology has been intensified recently. The research in family Zingiberaceae has evolked much attention after patent issue on haldi (*Curcuma longa* L.). Several species of ginger like *Zingiber officinale* Rosc. *Z. capitatum* Roxb and *Z. roseum* Rosc are known to grow in Kumaun and Garhwal region of Uttaranchal (Strachey, 1979). Numbers of plants from

this family are used in traditional system of medicine (Hussain *et al.*, 1992). Plants of this family have been attributed to have anti-inflammatory, antiulcer, antioxidant and antimicrobial properties (Jaganath *et al.*, 2000).

Globba (Zingiberaceae-Zingiberales) consists of 100 species of small perennial herbs from East Asia and Malaysia. Flowers of *Globba* are strinking orange, yellow, purple or white, contrasting with the often green inflorescence bracts (Endress, 1994; Takano and Okada, 2003). *Globba* L. genus is represented by 17 species in India mainly distributed in the Himalaya, South India and Andman-Nicobar Islands (Karthikeyan *et al.*, 1989). In Uttarakhand the genus is represented by three species *G. orixensis* Roxb, *G. racemosa* Smith and *G. sessiliflora* Sims (Uniyal *et al.*, 2007). *G. sessiliflora* is erect, pemrennial herbs, with a creeping short rhizome which emits fleshy roots (Babu 1977).

We have earlier studied the mono and sesquiterpene composition of the rhizomes and aerial parts of *G. sessiliflora* Sims at flowering stage. β-caryophyllene, α-cadinol, selin-11-en-4α-ol, caryophyllene oxide, γ-eudesmol and myrcene were the major constituents in the rhizome volatile oil. The oil from aerial part revealed the presence of myrcene, β-caryophyllene, selin-11-en-4α-ol, β-longipinene, manool, germacrene D and β-eudesmol as major constituents. The oils showed significant myorelaxant effect on isolated duodenum smooth muscles of Wistar rats. The antioxidant activity of the oils were evaluated using reducing power assay, 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and the chelating effect on Fe^{2+}. Both rhizome and aerial part oils exhibited good to moderate antioxidant and myorelaxant activity in a dose-dependent manner (Kumar *et al.*, 2012).

In present study we worked on the chemical composition of essential oil of mature rhizomes of *G. sessiliflora* after litter fall collected from Garhwal region of Uttarakhand and anti-inflammatory, antinociceptive and antipyretic activity of rhizome essential oil of *G. sessiliflora*.

2. Materials and Methods

2.1. Plant material

G. sessiliflora rhizomes were collected in November, from the Dehradun- Mussoorie highway, Garhwal region of Uttarakhand, India. The identification and authentication of the plant was made by Dr. D. S. Rawat, Assistant Professor and plant taxonomist, Department of Biological Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar. The voucher specimen has been deposited in the Department of Biological Sciences, GBPUAT, Pantnagar for future reference.

2.2. Isolation of essential oil

Essential oil from rhizomes was isolated by hydrodistillation in a Clevenger-type apparatus for 8 hours. The oil was extracted with CH_2Cl_2 and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and the oil was stored at 4°C for further analysis.

2.3. GC-MS analysis of essential oil

10% solution of essential oil was prepared in CH_2Cl_2 and filtered to obtain clear solution. GC-MS analysis of oleoresin solution (0.2 µl) was performed using Autosystem XL (Perkin-Elmer, USA) fitted with Elite-5MS fused silica column (30 m × 0.25 mm; 0.25µm film thickness), with split-splitless injector. Helium flow rate through the column was 1 ml/min in constant flow mode. The initial column temperature was 40ºC rising 325ºC at a rate 3ºC/min. The MS detector temperature was 280ºC. The constituents were detected in the full scan mode from *m/z* 41 to 620.

A hexane solution of C_8-C_{28} *n*-alkanes was previously separated under the above conditions, and their retention times were determined. Linear temperature programmed retention indices (LTPRI) were calculated from the results of the separation of the oleoresin and *n*-alkanes according to eq.:

$$LTPRI = 100(t_x - t_n)/ (t_{n+1} - t_n) + 100n,$$

Where t_x, t_n and t_{n+1} are the retention times of component *x*, and *n*-alkanes with the number of carbon atoms in the molecule *n* and *n*+1, respectively. After integration the fraction of each component in the total ion current (TIC) was calculated.a

Components of essential oil were identified with the help of NIST II, mass spectra library. Identification was considered reliable if the calculated values of LTPRI confirmed the results of library search at mass spectra library ($LTPRI^{Exp} - LTPRI^{Lit} \leq 10$ index units) (Adams, 2007).

2.4. Analysis of anti-inflammatory, antinociceptive and antipyretic activity of Essential oil

The investigations of different biological activities of the essential oil were carried out on Swiss strain albino mice weighing 18-23 g and purchased from the Indian Veterinary Research Institute(IVRI), Izatnagar, Bareilly, India. The mice were divided into different experimental groups. Each group consisted of six mice. All the groups were maintained under standard laboratory conditions of food and water. Prior to conduct the experiment, all the mice in each group were weighed individually to calculate the dose of drugs for each group. The animals were maintained in laboratory environment for two weeks to get them acclimatized. All experiments were conducted between 900, 1700 hours. All the animals were sustained under observation for one week, after the completion of experiments to observe acute or sub acute toxicity, if any [Experiments protocols were approved by the institutional animal ethics committee, CPCSEA, New Delhi (Ref: IAEC/Chem/CBSH/118)].

2.5. Animal model for anti-inflammatory, antinociceptive and antipyretic activities

Total four different groups of mice with six in each group were taken for conducting various pharmacological activities. The experiments were conducted using two different doses (50 mg/kg and 100 mg/kg b. wt.). These concentrations were selected as per IC_{50} calculations. 50 and 100 mg of oleoresins were separately triturated by the addition of small amount of Tween-20 and saline water was poured to make the final volume of 10 ml. Paracetamol, Ibuprofen and indomethacin were used as positive and saline water as negative control respectively. The essential oil, standard drug and saline water were given at the dose rate of 0.1 mL/10 gm weight of the experimental animals.

2.6. Anti-inflammatory assay

2.6.1. Acute Anti-inflammatory assay

Anti-inflammatory activity was determined by carrageenan induced hind paw edema method (Winter *et al.*, 1962). Acute inflammation was produced by subplantar injection of 0.1 mL of 1% suspension of carrageenan. The measurement of paw volume was made by plethysmometer (UGO Basile, Italy) at intervals of 1, 3 and 24 h after the injection of carrageenan. Standard anti-inflammatory drug, ibuprofen suspension was used orally at a dose of 40 mg/kg b.wt. The anti-inflammatory effect was measured as the reduction of volume displacement by hind foot in comparison to control.

2.6.2. Sub-acute Anti-inflammatory assay

The formaldehyde induced arthritis method was used to determine the subacute activity of the rhizome essential oil (Selye, 1949). 0.1 ml formaldehyde (1%) solution was injected in the right hind paw of the mice on first day for the experiment. Doses of the oleoresins (50 and 100 mg/kg b. wt.) were administered orally daily in the morning till the end of the experiment study period for 10 days. Whereas control group received only saline water. Ibuprofen at a dose of 10 mg/ kg b.wt. was orally given used as standard anti-inflammatory drug. Paw volume of all the mice were measured plethysmometrically in the evening hours for 10 days till the end of the experiment.

2.7. Antinociceptive assay

2.7.1. Writhing effect

The pain sensation in experimental mice was created by administering Glacial acetic acid intraperitoneally. Initially 0.2 mL of essential oil, standard drug (Ibuprofen – 40 mg/kg body wt) and control (saline water) were administered orally to each group of mice. Glacial acetic acid (1% at dose of 0.1 mL/10 g body weight) was administered intraperitoneally after 40 min to each mice. The numbers of writhing per minute were counted for every mice. The inhibition of writhing and percentage of pain protection in mice was calculated as per following formula:

$$\% \text{ writhing} = (T/C) \times 100$$

$$\% \text{ inhibition} = \frac{C-T \times 100}{C}$$

Where:

T = treatments (group II-III); C= control saline group (I)

2.7.2. Hot plate method

The standard hot-plate test was used to determine the analgesic response latencies (Langerman *et al.*, 1995). The mice were placed into Perspex cylinder on the heated surface of the hot-plate (maintained at 55.0 ± 0.5 ^{0}C). The time (sec) to discomfort reaction (licking paws or jumping) was recorded to record response latency. The measurements were recorded initially and after 30, 60, 120, and 150 min of oral administration of the essential oil at the

dose rate of 50 and 100 mg/kg b.wt. For positive control, standard drug indomethacin (5 mg/kg b. wt.) was used. Saline water (0.2 mL) given orally as a negative control. A latency period of 20 sec. was marketed as complete analgesia and the measurements were stopped if it exceeded the latency period to avoid injury.

2.8. Antipyretic assay

To measure the antipyretic activity, pyrexia was induced using yeast as per the method given by Rao *et al.* (1997) generally being practiced. To record the basal body temperature a thermometer was inserted in to the rectum after restraining the mice. All the experimental groups were given a subcutaneous injection of 10 ml/kg b.wt. of 20% suspension of Brewer's yeast *(Sacchromyces cerevisiae)* except in control group. The mice were allowed to remain quiet in the cage for 18 hour, to rise in their body temperature. At nineteenth hour, again the rectal temperature was recorded. Immediately, 0.1 ml/10 gm body wt. of the oleoresins in the calculated doses of 50 mg/kg and 100 mg/kg b. wt. and paracetamol (33 mg/kg b. wt.) were given orally. Control group received 0.2 ml normal saline water only. The rectal temperature was recorded at hourly intervals in all the groups up to 3 hours. The percentage reduction in rectal temperature was calculated by considering the total fall in temperature to normal level as 100%.

% Reduction = {(B-Cn/B-A) × 100}

Where:

A=normal temp B= Pyrexia temp C= temp at hourly interval

2.9. Assessment of toxicity

The acute toxicity was performed according to OECD guidelines **(**423, OECD/OCDE, 2001**).** The Lethality of essential oil of *G. sessiliflora* was checked by oral administration of essential oil at 400, 600 and 800 mg/kg b.wt. Behavioral changes were recorded for 24 hr. The numbers of casualties were recorded after upto 48 hr.

2.10. Statistical analysis

Data were expressed as mean±S.E. Results were analyzed using programme 43 (software) one way ANOVA and $p<0.05$ was considered to be statically significant.

3. Results and discussion

3.1. Essential oil composition

The rhizomes were collected in the month of October-November after litter fall and the essential oil analysed by GC and GC-MS representing 98.1% of the oil. Table 1 shows the chemical composition of the oil studied. The oil was very rich in oxygenated sesquiterpenes (65.8%), major component of the oil was β-eudesmol (27.6%). The other major oxygenated sesquiterpenes components in oil were caryophyllene oxide (9.7%), τ-muurolol (8.3%), γ-eudesmol (6.4%), (6*E*)-nerolidol (4.1%), (*E*)-sequisabinene hydrate (2.4%), 1-*epi*-cubenol (2.6%) and β-bisabolenol (2.2%). Other oxygenated sesquiterpenoids were found to be less than 1%. Sesquiterpene hydrocarbons contribute to 29.2% of the oil. Among sesquiterpene hydrocarbons, (*E*)-β-caryophyllene (24.3%) was found in highest amount. Other hydrocarbons identified were α-humulene (3.0%), (*E*)-β-elemene (0.4%), β-selinene (1.0%) and α-selinene (0.5%). (*E*)- α- bergamotene, (*E*, *E*)- α- farnesene and γ-cadinene were found in trace amounts. The oil had low amount of oxygenated monoterpenoids (1,8-cineole, linalool, borneol, 4-terpineol, α-terpineol, bornyl acetate), contributing to 1.4% of the oil. Monoterpene hydrocarbons (sabinine, β-pinene, p-cymene, limonene) were found in trace amount in the oil.

It is interesting to note that the essential oil of mature rhizome of *G. sessiliflora* collected from garhwal region has different chemical makeup, collected from kumaon region (Kumar *et al.*, 2012). In the essential oil collected from both the regions have sabinene, p-cymene, linalool, α-terpineol, β-caryophyllene, α-humulene, caryophyllene oxide and 1-*epi*-cubenol present in their essential oils. however γ-eudesmol (6.4%) and β-eudesmol (27.6%) were present in essential oil collected from Garhwal region were as γ-eudesmol (3.0%) and β-eudesmol (4.1%) in the essential oil collected from kumaun region. The eesential oil of *Globba sessiliflora* from Kumaun region has the presence of selin-11-en-4α-ol (12%), Manool (1.0%) and Sandaracopimara-8(14)-diene (1.5%) which were not present in the oil collected from Garhwal region.

doi: 10.18869/JHD.2017.59

Table 1. Chemical Composition of rhizome essential oil of *G. sessiliflora* Sims.

S. N.	Compound	t_R, min	LTPRI^{exp}	LTPR^{Lit} (Adams,1995)	% contribution
1	sabinine	8.26	970	976	t
2	β-pinene	8.44	975	978	t
3	*p*-cymene	10.19	1021	1020	t
4	limonene	10.38	1028	1031	t
5	1,8-cineole	10.49	1033	1034	0.3
6	linalool	13.32	1098	1098	0.3
7	borneol	16.41	1168	1165	t
8	4-terpineol	16.81	1177	1177	t
9	α-terpineol	17.46	1192	1189	0.3
10	bornyl acetate	21.37	1281	1288	0.5
11	(*E*)-β-elemene	25.87	1386	1391	0.4
12	(*E*)-α-bergamotene	26.47	1400	-	t
13	(*E*)- β-caryophyllene	27.08	1415	1418	24.3
14	α-humulene	28.53	1451	1453	3.0
15	β-selinene	29.88	1484	1484	1.0
16	α-selinene	30.08	1488	-	0.5
17	(*E*,*E*)-α-farnesene	30.17	1491	1494	t
18	γ-cadinene	30.62	1502	1508	t
19	(6*E*)-nerolidol	32.79	1558	1561	4.1
20	caryophyllene oxide	33.48	1576	1575	9.7
21	(*E*)-sesquisabinene hydrate	33.63	1580	1584	2.4
22	humulene epoxide II	34.53	1603	1603	0.6
23	1-*epi*-cubenol	35.32	1625	1627	2.6
24	γ-eudesmol	35.40	1625	1633	6.4
25	(*E*)-Sesquilavandulol	35.57	1631	1633	0.6
26	β-eudesmol	36.23	1648	1639	**27.6**
27	τ-muurolol	36.35	1652	1647	8.3
28	6-hydroxy-caryophyllene	36.81	1665	1670	0.3
29	aromadendrene oxide	36.90	1667	1669	0.4
30	(6*R*,7*R*)-bisabolone	39.37	1735	1736	t
31	(*E*,*Z*)-farnesol	39.62	1742	1742	t
32	β-bisabolenol	41.31	1790	1786	2.2
33	occidol acetate	47.24	1971	1970	0.6
34	isokaurene	47.45	1978	1988	1.0
35	coranorin-*E*	51.57	2112	-	0.7
	Total				**98.1**
	Monoterpene hydrocarbons				0.0
	Oxygenated monoterpenes				1.4
	Sesquiterpene hydrocarbons				29.2
	Oxygenated Sesquiterpenes				65.8
	Diterpenes				1.7

t= trace <0.1%

Table 2: Acute anti-inflammatory activity of rhizome essential oil of *G. sessiliflora* (Mean±SE, n=6)

Group	Treatments	Dose (mg/kg) b.wt.	Change in paw thickness		% Inhibition
			0 hr	4 hr	
I	Control	0.2 mL	2.18±0.02	2.17±0.04	00.48
II	Ibuprofen	40	2.97±0.05	1.78±0.04[a]	40.06
III	GSREO	50	2.67±0.23	2.21±0.15[b]	17.22
IV	GSREO	100	2.86±0.12	1.90±0.42[a]	33.56

[a]= significant ($p<0.05$) as compared to control

[b] = significant ($p<0.05$) as compared to drug (Ibuprofen), GSREO: *G. sessiliflora* rhizome essential oil

Table 3. Effect of rhizomes essential oil of *G. sessiliflora* on formaldehede induced sub acute inflammation (Mean±SE, n=6)

Groups	Dose (mg/kg) b.wt.	Volume of inflammation(cm^3)										
		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Control (I)	0.2 mL	2.14 ± 0.06	2.20 ± 0.10	2.16 ± 0.13	2.25 ± 0.12	2.15 ± 0.07	2.18 ± 0.08	2.16 ± 0.15	2.29 ± 0.11	2.16 ± 0.12	2.19 ± 0.11	2.23 ± 0.13
Ibuprofen (II)	10	2.18 ± 0.08	2.35 ± 0.09[a]	2.28 ± 0.12	2.34 ± 0.03	2.31 ± 0.08[a]	2.31 ± 0.11[a]	2.29 ± 0.10	2.28 ± 0.10	2.26 ± 0.09	2.17 ± 0.O5	2.16 ± 0.06
GSREO (III)	50	2.09 ± 0.08	2.31 ± 0.10	2.38 ± 0.13[a]	2.35 ± 0.04	2.34 ± 2.26[a]	2.28 ± 0.07[a]	2.29 ± 0.06	2.26 ± 0.04	2.24 ± 0.13	2.11 ± 0.11	2.14 ± 0.09
GSREO (IV)	100	2.10 ± 0.06	2.39 ± 0.06[a]	2.31 ± 0.03[a]	2.32 ± 0.05	2.26 ± 0.02[a]	2.25 ± 0.03	2.17 ± 0.11[b]	2.12 ± 0.05[a,b]	2.11 ± 0.06	2.10 ± 0.06	2.10 ± 0.06

[a] = significant ($p<0.05$) as compared to control, [b] = significant ($p<0.05$) as compared to Ibuprofen GSERO: *G. sessiliflora* Rhizome Essential Oil

Table 4. Antinociceptive activity of rhizome essential oil of *G.sessiliflora* (Writhing effect) (Mean±SE, n=6)

Groups	Treatments	Dose (mg/kg. b.wt.)	No of Writhings	% Writhings	% Inhibition
I	Control	0.2 mL	217.00±1.26	100.0	-
II	Ibuprofen	40	123.5±3.44	56.91	43.08
III	GSREO	50	143.83±0.75	66.28	33.71[a]
IV	GSREO	100	129.16±0.75	59.52	40.47[a]

a = significant ($p<0.05$) as compared to control, b = significant ($p<0.05$) as compared to Ibuprofen

GSREO: *G. sessiliflora* Rhizome Essential Oil.

Table 5. Antinociceptive activity of rhizomes essential oil of *G. sessiliflora* (Hot Plate Method) (Mean±SE, N=6)

Groups	Dose (mg/kg) b.wt.	Hot plate reaction time (min)				
		0	30	60	120	150
I(Control)	0.02 mL	3.01±0.00	3.11±0.01	2.95±0.01	2.84±0.02	3.03±0.02
II(Indometha)	5	3.27±0.03	3.83±0.03[a]	4.93±0.04[a]	4.07±0.09[a]	3.84±0.03[a]
III(GSREO)	50	2.93±0.04	3.35±0.41[a,b]	3.12±0.03[a,b]	2.61±0.30[a,b]	2.66±0.01[a,b]
IV(GSREO)	100	3.37±0.19	3.37±0.04[a,b]	3.41±0.03[a,b]	3.14±0.03[a,b]	2.87±0.12[a,b]

[a]= significant ($p<0.05$) as compared to saline water, b = significant ($p<0.05$) as compared to Indomethacin
GSREO: *G. sessiliflora* Rhizome Essential Oil.

Table 6. Effect of rhizome essential oil of G. sessiliflora on yeast induced pyrexia in mice (Mean±SE, N=6

Groups	Treatment	Dose (mg/kg) b.wt	Body temp. (^{0}C)		Temp after administration of drug (^{0}C)		
			Before injection of Brewer yeast	After injection of Brewer yeast	1 hr	2 hr	3 hr
I	Control	0.2 mL	37.55±0.02	38.87±0.03	38.87±0.03 (2.36±1.07)	38.89±0.03 (3.77±5.46)	38.81±0.03 (6.05±3.70)
II	Paracetamol	33	37.53±0.06	38.82±0.04	37.63±0.04[a,b] (92.86±3.28)	37.54±0.02[a,b] (99.47±5.49)	37.49±0.01[a,b] (103.60±5.20)
III	GSREO	50	37.64±0.03	38.85±38.91	38.26±0.06[a,b] (50.66±10.04)	38.13±0.03[a,b] (52.23±22.96)	37.84±0.07[a,b] (83.40±8.09)
IV	GSREO	100	37.54±0.03	38.86±0.01	37.65±0.03[a] (91.90±3.28)	37.44±0.04[a,b] (108.08±3.78)	37.34±0.03[a,b] (115.05±2.89)

Percentage reduction in temperature is given within parenthesis, [a] = significant ($p<0.05$) as compared to control, [b] = significant ($p<0.05$) as compared to drug, GSREO: *G. sessiliflora* rhizome essential oil

However the oil of collected from Garhwal region shows the presence of two diterpines isokaurene (1.0%) and coranorin- E (0.7%). Nootkatone was not detected at maturity stage collected from Garhwal region. Rhizome and aerial part essential oils contain nootkatone which was less than 3% of the oil in earlier stage collection from Kumaun region. The variation in the makeup of essential oils may be because of climatic variations in the two different regions of Uttrakhand.

3.2. Anti-inflammatory Assay

3.2.1. Acute Anti-inflammatory Assay

The paw volume of carrageenan induced paw edema was remained unchanged in saline treated control. In comparison to the control, the essential oil showed some anti-inflammatory activity. Maximum inhibition (40.06%) was recorded in Ibuprofen treated group and it was almost parallel to essential oil at the dose level 100 mg/kg b. wt. (Table 2).

3.2.2. Sub-acute Anti-inflammatory Assay

In ibuprofen treated mice, significant decrease in the paw volume was noticed from day 3 and value came to normal by day 10. Essential oil (50 mg/kg b.wt.) showed decreased in the sub-acute inflammation from day 1 and 100 mg/kg b. wt. dose of essential oil showed a decrease in the sub-acute inflammation from day 4 and reverted to normal by day 10 (Table 3).

Carrageenan induces acute inflammation method is used for the detection of anti-inflammatory activity in the drugs (Di Rosa and Willoughby, 1971). It has been reported that the edema is developed to carrageenan injection is a biphasic event. In first phase histamine and serotonin are released while in phase two (3-5 hours) is said to be synergised by prostaglandins. The edema is maintained between first and second phase due to kinin. It has also been reported that the second phase edema is sensitive to clinically effective steroids and non steroids anti-inflammatory agents. A variety of clinically ineffective agents can reduce initial phase (Vinegar *et al.*, 1969).

Essential oil of *Globba sessiliflora* used in this study, showed anti inflammatory activity probably due to the inhibition of the cyclo-oxygenage pathway. It has been reported earlier that the essential oil show anti-inflammatory activity due to its interference with the production of prostaglandins (Smith and Willis, 1971; Seibert et al., 1994; Miguel, 2010). In the carrageenan induced paw edema, essential oil showed activity at higher dose level.

For studying sub acute anti-inflammatory activity, arthritis was induced by formaldehyde injection on the first day and the test samples were administered orally every day for 10 days. During present investigation, oleoresins exhibited significant anti-artritis effect. Essential oil had anti-inflammatory effect corresponding to standard drug ibuprofen.

3.3. Antinociceptive assay

3.3.1. Writhing Effect

Essential oil showed significant analgesic activity with 34.79% inhibition at 50 mg/kg and 43.24% inhibition at the dose level of 100 mg/kg b. wt. As compared to standard drug ibuprofen (43.08 %, inhibition) (Table 4).

3.3.2. Hot Plate Method

Perusal of **table 5** reveals that the discomfort reaction time in indomethacin treated mice was maximum at 60 minute while essential oil exhibited a dose dependent manner increase in the paw licking and jumping time.

The abdominal constitution response induced by glacial acetic acid is a sensitive procedure to establish peripherally acting analgesics. This response is thought to involve local peritoneal receptors. Essential oil, significantly attenuated the acetic acid induced squirm.

In this work, we also measured the central nociceptive reactivity to thermal stimuli in mice using the hot plate test. In central analgesic activity, essential oil revealed efficacy against thermal stimulus.

3.4. Antipyretic Assay

Globba sessiliflora essential oil produced significant antipyretic effect in a dose dependent manner. Negative control mice did not show any reduction in the body temperature on oral administration of saline. The maximum inhibition was obscured at 3 hr (Table 6).

An appreciable antipyretic effect was noticed by essential oil at dose level of 100 mg/kg b. wt. which was comparable to paracetamol. Previous studies already suggested that intensification of lipid per oxidation at increase body temperature is indicated by pyrexia and is associated by increase in oxidative stress. The use of antioxidants retards the process of lipid per oxidation (Brzezinska-Slebodziniska, 2001).

We have earlier reported that essential oil of *G. sessiliflora* exhibits antioxidant activity (Kumar *et al.*, 2012).

3.4. Toxicity

On oral administration of 400, 600, 800 mg/kg.b.wt. of *G. sessiliflora* essential oil, no behavioural or physiological change was observed for 24 hr and on subsequent day. None of the treated mice died. All reflexes (pedal and corneal) and rectal temperature as well heart, respiration rates were within normal physiological limits in the extract treated mice.

4. Conclusion

From the experimental results it can be concluded that the herb can be a good natural source of β-eudesmol, (*E*)-β-caryophyllene, α-humulene (6 *E*)-nerolidol, caryophyllene oxide, γ-eudesmol and τ-muurolol etc. The herb exhibited moderate to good pharmacological activity hence the results will be helpful to generate data base on this plant so that it can be exploited scientifically and judiciously for traditional system of medicine besides its academic importance.

5. References

Abelson, K., 2005. Acetylcholine in spinal pain modulation: An *in vivo* study in the rat. Digital Comprehensive Summaries of Upsulla Dissertations from the faculty of Medicine. 19. 55pp. Uppsula. ISBN 91-554-6178-6.

Adams, R.P., 1995. Identification of essential oil components by gas chromatography, mass Spectrometry. Allured Publishing Corporation, Carol Stream, Illinois, USA.

Albert, D., Z¨undorf, I., Dingermann, T., M¨uller, W. E., Steinhilber, D., Werz, O., 2002. Hyperforin is a dual inhibitor of cyclooxygenase-1 and 5-lipoxygenase. *Biochemical Pharmacology.* 64,1767–1775.

Alberto, M.R., Zampini, I.C., Isla, M.I.,2009. Inhibition of cyclooxygenase activity by standardized hydroalcoholic extracts of four Asteraceae species from the Argentine Puna. Braz J Med Biol Res. 42(9),787-90.

Almeida, T. I., Roizenblatl, S. and Tufik, S., 2004. Afferent pain pathways: a neuroanatomical review. *Brain Research*, 1000,40-56.

Babu, C.R., 1977. Herbaceous flora of Dehradun. CSIR, New Delhi, pp 502.

Besson, J. M., Chaouch, A., 1987. Peripheral and spinal mechanisms of Nociception. *Physiology Reviews*, 67,67-186.

Brzezinska-Slebodzinska, E., 2001. Fever induced oxidative stress. The effect on thyroid status and the 5'-mono deiodinase activity, protective role of selenium vitamin. Journal of Physiology and Pharmacology. 52,275-284.

Chattopadhyay, D., Arunachalam, G., Ghosh, L., 2005. Antipyretic activity of Alstonia macrophylla Wall ex A. DC: an ethnomedicine of Andaman Islands. J Pharm Phar Sci.8 (3), 558-64.

Chatterjee, G. K., Pal., S. P., 1984. Search for anti-inflammatory agents from Indian Medicinal Plants-A review. *Indian Drugs.* 21, 413.

Di Rosa, M., Willoughby, D.A., 1971. Screens for anti-inflammatory drugs. Journal of Pharmacy and Pharmacology. 23,297-298.

Endress, P.K., 1994. Diversity and evolutionary biology of tropical flowers. Cambridge University Press, Cambridge.

Farnsworth, N. R. 1989. Screening plants for new medicines. In Wilson E.O. Editor, Biodiversity, Part II, National Academy Press, Washington, pp 83-97.

Hussain, A., Virmani, O.P., Popli, S.P., Misra, L.N., Gupta, M.M., 1992. Dictionary of Indian medicinal plants. Director, Central Institute of Medicinal and Aromatic Plants, Lucknow. 161-162.

Jaganath, I.B., Ng, L.T. 2000. Herbs: The green Pharmacy of Malaysia. Vinpress Sdn. Bhd. Malaysia Agriculture and Research and Development Institute. 95-99.

Karthikeyan, S., Jain, S.K., Nayar, M.P., Sanjappa, M., 1989. Floral Indicae Enumeratio: Monocotyledonae. Botanical survey of India, Calcutta, pp 293-294.

Kumar, R., Prakash, Om., Pant, A.K., Isidorov, Valery A., Mathela., C.S. 2012. Chemical composition, antioxidant and myorelaxant activity of essential oils of Globba sessiliflora Sims. 24(4),385-391.

Langerman, L., Zakowski, M.I., Piskoun, B., Grant, G.J., 1995. Hot plate versus tail-flick: Evaluation of acute tolerance to continuous morphine infusion in

rat model. Journal of Pharmacological and Toxicological Methods. 34,23-27.

Mattison, N., Trimple, A. G., Larsen, I., 1998. New drug development in the United States, 1963 through 1994. *Clinical Pharmacology Therapeutics Journal.* 43, 290-301.

Maria G. M., 2010. Antioxidant and Anti-Inflammatory Activities of Essential Oils: A Short Review. Molecules. 15, 9252-9287.

OJ, Oh., H. Y. Min, Lee, S.K.,2007. Inhibition of inducible prostaglandin E2 production and

cyclooxygenase-2 expression by curdione from Curcuma zedoaria. Arch Pharm Res. 30(10),1236-9.574.

423, OECD/OCDE, 2001. Acute oral toxicity-Acute toxic class method. Guideline for the testing of chemicals, guidance document on acute toxic class method.

Rao, R.R., Babu, R.M., Rao, M.R.V., Babu, M.G.V. 1997. Studies on antipyretic, analgesic and hypoglycaemic activities of root of *Gynandropsis gynandra* Linn. Indian Drugs. 34,12:690- 694.

Seibert, K., Zhang, Y., Leahy, K., Hauser, S., Masferrer, J., Perkins, W., 1994. Pharmacological and biochemical demonstration of the role of cyclooxygenase 2 in inflammation and pain. Proceedings of the National Academy of Sciences. 91,12013-12017.

Smith, J.B., Willis, A.L., 1971. Aspirin selectivity inhibition prostaglandin production in human platelets. Nature New Biology. 231,235-239.

Strachey, L., 1979. Catalogue of plants of Kumaun and of adjacent portion of Garhwal and Tibbet. Revised by Duthie JE pp 269, 1918, Reprint in 1974 periodicals publication Dehradun

Shailasree, S., Ruma, K., Kini, K.R., Niranjana, S.R., Prakash, H.S., 2012. Potential anti-inflammatory bioactives from medicinal plants of Western Ghats, India. Phcog Commn. 2,2-12.

Saper, C.B., Breder, C.D., 1994. The neurologic basis of fever. N Engl J Med, 330(26), 1880-1886.

Takano, A., Okada, H., 2003. Taxanomy of Globba (Zingiberaceae) in Sumatra, Indonesia. Syst Bot 28,524-546

Tapiero, H., Ba, G. N., Couvreur, P., Tew, K. D., 2002. Polyunsaturated fatty acids (PUFA) and eicosanoids in human health and pathologies. *Biomedicineand Pharmacotherapy.* 56,215–222.

Uniyal, B.P., Sharma, J.R., Chouddhery, U., Singh, D.K., 2007. Flowering plants of Uttarakhand (a checklist). Bishen Singh, Mahendra Pal Singh, Dehradun, pp 235.

Vanden Worm, E., Beukelman, C. J., Vanden Berg, A. J. J., Kores, B. H., Labadie, R. P., Van Dijk, H. 2001. Effects of methoxylation of apocynin and analogson the inhibition of reactive oxygen species production by stimulated human neutrophils. *European Journal of Pharmacology*. 433,225–230.

Vinegar, R., Schreiber, W., Hugo, R., 1969., Biphasic development of carrageenan edema in rates. Journal of Pharmacology and Experimental Therapeutics. 166,1:96-103.

Winter, C.A., Risley, E.A., Nuss, G.W., 1962. Carrageenan-induced edema in hind paws of the rats as an assay for antiinflammatory drugs. Proceedings of the Society for Experimental Biology and Medicine. 111,544-547.