Research Article

CHEMICAL COMPOSITION OF RHIZOME OLEORESIN AND ANTI-INFLAMMATORY, ANTINOCICEPTIVE AND ANTIPYRETIC ACTIVITY OF OLEORESINS OF *Alpinia allughas* ROSCOE. FROM TARAI REGION OF UTTARAKHAND

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Submitted: 12-08-2016

Revised: 19-02-2017

Accepted: 25-09-2017

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ABSTRACT

The investigation of volatile constituents of the rhizome oleoresin of *Alpinia allughas* Roscoe. growing in tarai region of Kumaun hills, Uttarakhand, revealed the presence of 55 terpenoids. The major constituents identified in the rhizome oleoresin were α -eudesmol (21.3%), β -selinene (13.9%), valencene (9.6%), borneol (7.1%), α -humulene (5.3%) and 7-epi- α -selinene (5.2%). Other minor constituents identified were (*E*)- β -caryophyllene (3.8%), (*6 E*)-nerolidol (3.5%), (*E,E*)-farnesol (3.2%), caryophyllene oxide (2.5%), humulene oxide (2.7%), bornyl acetate (1.9%), coranarin-*E* (1.8%), linalool (1.8%) and α -terpineol (1.6%). The total identified constituents contribute 97.0% of the oleoresin. The rhizomes oleoresins exhibited significant antinociceptive activity with 34.79% inhibition at 50 mg/kg BW and 43.24% at 100mg/kg BW compared to standard drug ibuprofen (40mg/kg BW), it also showed antipyretic activity in dose dependent manner with temperature reduction $77.57 \pm 5.88\%$ at 50mg/kg BW and $98.95 \pm 3.95\%$ at 100mg/kg BW after 3h. Oleoresin also showed 29.23% inhibition of carrageenan-induced paw edema at 50mg/kg BW. and 39.92% inhibition at 100mg/kg BW in comparison to ibuprofen 40.06% at 40mg/kg BW.

Keywords: α -eudesmol; β -selinene; anti-inflammatory; antinociceptive; antipyretic activity

INTRODUCTION

The genus *Alpinia* (Zingiberaceae) comprises about 230 species originated mainly in the tropical Asian region and now also cultivated in many parts of the world (Loetschert and Beese, 1983; Lemmon and Sherman, 1964). Rhizome extracts of some zingiberaceous herbs are widely used in dietary intake and in the traditional herbal medicine (Chirangini *et al.*, 2004).

It has been reported that *Alpinia* species possess antioxidant (Shi *et al.*, 2006), anti-inflammatory (Israf *et al.*, 2007), anti-oxidant (Vankar *et al.*, 2006), anticancer (Ali *et al.*, 2001; Lee and Houghton, 2005), immunostimulating (Benjeddou *et al.*, 2003), hepatoprotective (Kadota *et al.*, 2003) and antinociceptive (Arambewela *et al.*, 2004) activities. Further, the uses of rhizomes of *Alpinia* species (*Alpinia*

galanga) in the digestive system, bronchitis, measles, rubella cholera and respiratory infections have been reported (Brown, 1995; Benjeddou *et al.*, 2003).

Some zingiberaceous herbs have been reported to possess antibacterial (George and Pandalai, 1949), antifungal (Pushpangadan and Atal, 1984), anthelmintic activity (Kaleysa, 1975), antimicrobial activity (Robinson *et al.*, 2009) in extracts and myorelaxant activity in essential oil (Panday *et al.*, 2007) of *Alpinia calcarata*, antimicrobial and anti-inflammatory activity by diarylheptanoid from *Alpinia officinarum* (Subramanian *et al.*, 2009) and antiplasmodial activity by 1'-acetoxychavicol acetate from *Alpinia galanga* (Latha *et al.*, 2009).

Essential oil of rhizome of *A. allughas* contained caryophyllene oxide (23.07%), geraniol (19.93%), citronellol (6.8%), β -

caryophyllene (5.45%), α -pinene (3.84%), linalool (2.86%), (-) α -phellandrene (1.6%) and geranyl acetate (0.16%) (Purohit and Devi, 1976). Hence, the present study analyzed the chemical composition of the oleoresin and evaluated its *in vitro* anti-inflammatory, antinociceptive and antipyretic activities.

MATERIALS AND METHODS

Plant material and extraction

The rhizomes of *Alpinia allughas* Roscoe were collected in the month of November 2009, from the forest near Pantnagar located in tarai region of Kumaun hills, Uttarakhand, India. The specimen has been deposited in the Department of Chemistry, G.B. Pant University of Agric. & Tech., Pantnagar, India. The identity of the plant was confirmed from Botany Division of Forest Research Institute, Dehradun, India where herbarium specimens Herbarium nos.: 9747 & 72265 dated 12 Jan. 2004 have been deposited.

The oleoresin was extracted from *Alpinia allughas* rhizomes with the help of cold percolation method using ethanol, 1kg of powdered rhizome were extracted for three times, the extracts were combined to evaporated under vacuum. The yield obtained was 112g oleoresin. The oleoresin was stored in a refrigerator at 4°C for further use.

GC-MS analysis of oleoresin

On 10% solution of oleoresin was prepared in CH₂Cl₂ and filtered to obtain a clear solution. GC-MS analysis of oleoresin solution (0.2 μ L) was performed using auto system XL (Perkin-Elmer, USA) fitted with Elite-5MS fused silica column (30m $\times$ 0.25mm; 0.25 μ m film thickness), with the split-splitless injector. Helium flow rate through the column was 1mL/min in constant flow mode. The initial column temperature was 40°C rising 325°C at a rate 3°C/min. The MS detector temperature was 280°C. The constituents were detected in the full scan mode from m/z 41 to 620.

A hexane solution of C₈-C₂₈*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.:

$$\text{LTPRI} = \frac{100(t_x - t_n)}{(t_{n+1} - t_n)} + 100n$$

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.

Components of oleoresin 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 ($\text{LTPRI}^{\text{Exp}} - \text{LTPRI}^{\text{Lit}} \leq 10$ index units) (Adams, 2007).

Drugs and chemicals

Carrageenan, acetic acid and formaldehyde were procured from Sigma Aldrich, Mumbai, India and Brewer yeast provided by India Glycols Limited, Kashipur, India. The reference drugs Ibuprofen, indomethacin and paracetamol were commercially purchased.

Experimental animals

The investigation of different biological activities of the extracts was carried out on Swiss strain albino mice weighing 18-23g 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 9 and 17h. 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)].

Animals and drugs model

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 (50mg/kg and 100mg/kg BW.). These concentrations were selected as per IC₅₀ calculations. 50 and 100mg 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 10mL. Ibuprofen, indomethacin and paracetamol were used as positive and saline water was as negative control respectively. The oleoresin, standard drug and saline water were given at the dose rate of the 0.1mL/10gm weight of the experimental animal.

Anti-inflammatory activity

Carrageenan produced hind paw edema

On 0.1mL of 1% suspension of carrageenan was injected to produces acute inflammation in the right hind paw of mice. The paw volume was measured by plethysmometer (UGO Basile, Italy) at 1, 3 and 24h after the carrageenan injection. Ibuprofen solution at a dose of 80 mg/kg was given orally. The percentage reduction of edema was calculated in comparison to the control group (Winter *et al.*, 1962; Vogel *et al.*, 2007; Saini and Singhal, 2012).

Formaldehyde induced arthritis

The formaldehyde induced arthritis oleoresin was determined by following the method used by Selye, 1949. In brief 0.1mL formaldehyde (1%) solution was injected in the right hind paw of the mice on the first day for the experiment. Doses of the oleoresins (50 and 100mg/kg BW) were administered orally daily in the morning till the end of the experiment study period for 10 days. Ibuprofen at a dose of 10mg/kg BW was orally given used as positive control while saline water was used as negative control. Finally, the paw volumes of all the experimental animals were recorded plethysmometrically in the evening hours for 10 days.

Antinociceptive activity

Writhing effect

The pain sensation in mice was induced using an intraperitoneal injection of glacial

acetic acid. At zero hour a calculated dose of 0.2mL of oleoresins, 40mg/kg BW of Ibuprofen was given orally. After 40min, glacial acetic acid (1% at a dose of 0.1mL/10gm BW) was injected intraperitoneally to each animal. The number of writhings was counted in each animal for one minute including positive control. Finally, the percentage of pain inhibition was calculated as under

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

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

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

Hot plate method

The test was performed to measure analgesic response latencies as reported earlier by Langerman *et al.*, 1995. In brief the hot-plate was sustained at 55.0±0.5°C and the mice were put into the perspex cylinder on the heated surface. The time in seconds to discomfort reaction was recorded as response latency, prior to and 30, 60, 120, and 150min of 50 and 100mg/kg BW oral administration of oleoresins. As a positive control, indomethacin (50mg/kg BW) and for negative control saline water (0.2mL) was given orally. A latency period of the 20s. was remarkeded as complete analgesia and the measurements were stopped if it exceeded the latency period to avoid injury.

Brewer's yeast induced pyrexia

To measure the antipyretic activity, pyrexia was induced using yeast as per the method is given by Rao *et al.*, 1997 and 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 10mL/kg BW of 20% suspension of Brewer's yeast (*Saccharomyces cerevisiae*) except control group. The mice were allowed to remain quiet in the cage for 18h, to rise in the body temperature. At the nineteenth hour again, the rectal temperature was recorded. Immediately, 0.1mL/ 10gm BW of the oleoresins in the calculated doses of 50mg/kg and 100mg/kg BW and paracetamol (33mg/kg BW) were given orally. Control group received 0.2mL normal saline water only.

The rectal temperature was recorded at hourly intervals in all the groups up to 3h. The percentage reduction in rectal temperature was calculated by considering the total fall in temperature to a normal level as 100%.

$$\% \text{ Temperature reduction} = \frac{B-Cn}{B-A} \times 100$$

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

Toxicity

The acute toxicity was performed according to OECD (The Organisation for Economic Co-operation and Development) guidelines (423, OECD/OCDE, 2001). Mortality was determined by giving oral doses of 400, 600 and 800mg/kg BW of oleoresin. The change in the behaviour of animals was observed for two days and the numbers of deaths were recorded after up to four days.

Statistical analysis

Data were expressed as Mean $\pm$ S.E. Results were analysed using one-way ANOVA and $p < 0.05$ was statically significant.

RESULTS AND DISCUSSION

Chemical composition of oleoresin

The GC and GC-MS analysis of the oleoresin of *A. allughas* revealed more than 70 components out of which 63 compounds were identified. The total identified compound contributes to 96.6% of the oleoresin (Table I). The analysis of oleoresin from the rhizome of *A. allughas* was found to be rich in sesquiterpenes. The major constituents identified were (*E*)- β -caryophyllene (3.8%), α -humulene (5.3%), β -selinene (13.9%), valencene (9.6%), α -selinene (1.1%), 7-*epi*- α -selinene (5.2%), (6 *E*)-nerolidol (3.5%), caryophyllene oxide (2.5%), humulene oxide (2.7%), α -eudesmol (21.3%), (*E,E*)-farnesol (3.2%) and coranarin- *E* (1.8%). The other constituents were in minor quantity, less than 1% (Table I). The major oxygenated monoterpenes identified included linalool (1.8%), borneol (7.1%), α -terpineol (1.6%) and bornyl acetate (1.9%).

Prakash *et al.*, 2007 from our laboratory have reported β -pinene (55.5%), α -pinene (9.7%), 7-*epi*- α -eudesmol (4.1%), β -selinene (3.2%), terpinene-4-ol (2.2%), 1, 8-cineole (2.1%), α -selinene (1.7%), α -humulene (1.5%),

β -caryophyllene (1.1%) as the major constituents in the essential oil of *A. allughas*.

The present investigation revealed that the oleoresin of *Alpinia allughas* analysed at the stage of maturity has different chemical make up than its essential oil isolated at an early stage before maturity. The monoterpenes content was lower at maturity while sesquiterpenes content increases.

Anti-inflammatory assay

Carrageenan induced hind paw edema

Perusals of table II indicating that the oleoresin exhibited anti-inflammatory effect against the carrageenan induced paw edema in dose dependent manners. In carrageenan induced rat paw edema, administration of oleoresin reduced inflammation significantly from 4h onwards compared to control group. The anti-inflammation at the dose level of 100mg/kg BW was observed almost like ibuprofen.

Formaldehyde induced arthritis

In ibuprofen treated mice, a significant decrease in the paw volume was noticed from day 3 and value came to normal by day 10. Oleoresin (50mg/kg BW) showed a decrease in the sub-acute inflammation from day 5. The decrease in the formaldehyde induced arthritis from day 4 was observed at a dose level of 100mg/kg BW of oleoresins. The inflammation reverted to normal after day 10 (Table III).

Carrageenan induces acute inflammation is essential 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 (3-5h) 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 (Vinegar *et al.*, 1969; Ramachandran *et al.*, 2011)

Alpinia allughas oleoresin used in this study, revealed anti-inflammatory activity probably due to the inhibition of the cyclo-oxygenase pathway. It has been reported that

Table I. Comparative chemical composition (%) of *Alpinia allughas* Roscoe. rhizome oleoresin and essential oil.

S.N.	Compounds	LTPRI ^{Exp}	Oleoresin (%)	Essential oil (%) †
1	(Z)-2-hexen-1-ol	859	t	-
2	2-methyl-2-heptanone	921	0.1	-
3	α -thujene	931	-	0.2
4	α -pinene	939	-	9.7
5	camphene	953	-	0.5
6	acetylcyclohexane	962	0.1	-
7	sabinene	970	t	t
8	β -pinene	975	t	55.3
9	myrcene	991	-	0.4
10	<i>p</i> -cymene	1022	t	-
11	limonene	1027	t	t
12	1,8-cineole	1029	0.3	2.1
13	γ -terpinene	1056	-	1.0
14	<i>cis</i> -sabinene hydrate	1067	0.1	-
15	terpinolene	1088	-	0.2
16	linalool	1098	1.8	0.5
17	nonanal	1103	0.2	-
18	<i>cis-p</i> -menth-2-ene-1-ol	1122	0.1	-
19	nopinone	1135	0.1	-
20	<i>cis</i> -sabinol	1137	0.1	-
21	camphor	1143	0.2	-
22	pinocarvone	1159	0.1	-
23	borneol	1168	7.1	-
24	terpinen-4-ol	1177	3.7	2.2
25	<i>p</i> -cymen-8-ol	1184	0.1	-
26	α -terpineol	1192	1.6	-
27	linalyl acetate	1248	0.6	-
28	bornyl acetate	1281	1.9	-
29	α -terpenyl acetate	1343	0.4	-
30	α -cubebene	1351	-	t
31	<i>cis</i> -carvone oxide	1357	0.1	-
32	α -copaene	1372	0.1	-
33	geranyl acetate	1376	0.1	-
34	(<i>E</i>)- β -elemene	1386	0.1	-
35	(<i>E</i>)- β -caryophyllene	1415	3.8	-
36	α -guaiene	1441	0.1	-
37	α -humulene	1451	5.3	1.5
38	β -selinene	1485	13.9	3.2
39	valencene	1488	9.6	-
40	α -selinene	1491	1.1	1.7
41	(<i>E,E</i>)- α -farnesene	1502	0.3	-
42	7-epi- α -selinene	1514	5.2	-
43	(6 <i>E</i>)-nerolidol	1558	3.5	-
44	caryophyllene oxide	1576	2.5	-

S.N.	Compounds	LTPRI ^{Exp}	Oleoresin(%)	Essential oil(%) †
45	<i>n</i> -hexadecane	1600	0.1	-
46	humulene oxide	1603	2.7	-
47	10- <i>epi</i> - γ -eudesmol	1616	0.1	-
48	1- <i>epi</i> -cubenol+ allohedycariol	1625	0.4	-
49	3-iso-thujopsanone	1629	0.1	-
50	δ -cadinol	1639	0.2	-
51	β -eudesmol	1648	0.4	-
52	α -cadinol	1650	0.4	-
53	selin-11-en-4 α -ol	1652	-	t
54	α -eudesmol	1658	21.3	-
55	7- <i>epi</i> - α -eudesmol	1662	-	4.1
56	aromadendrene oxide	1667	0.7	-
57	6-hydroxycaryophyllene	1669	0.4	-
58	(<i>E,Z</i>)-farnesol	1742	0.2	-
59	(<i>Z</i>)-lanceol	1758	0.1	-
60	(<i>E,E</i>)-farnesol	1790	3.2	-
61	farnesyl acetate	1845	0.1	-
62	occidol acetate	1971	0.1	-
63	coranorin-E	2112	1.8	-
Total			96.6%	83.7%

t=trace>0.01%, †Prakash *et al.* (2007)

Table II. Anti-inflammatory activity of oleoresin of *Alpinia allughas*: carrageenan-induced paw edema (Mean $\pm$ SE, N=6)

Treatment	Dose (mg/kg BW)	Change in paw thickness		% Inhibition
		0 hr	4 hr	
Control	0	2.18 $\pm$ 0.02	2.17 $\pm$ 0.04	00.48
Ibuprofen	40	2.97 $\pm$ 0.05	1.78 $\pm$ 0.04 ^a	40.06
Oleoresin	50	2.60 $\pm$ 0.08	1.84 $\pm$ 0.08 ^b	29.23
Oleoresin	100	2.58 $\pm$ 0.08	1.55 $\pm$ 0.17 ^b	39.92

^a = significant (p<0.05) compared to control; ^b = significant (p<0.05) compared to ibuprofen

the anti-inflammatory activity of the drug is due to its interference with the production of prostaglandins (Smith and Willis, 1971; Seibert *et al.*, 1994). In the present report the oleoresins of *A. allughas* showed a dose dependent effect on paw volume thickness at the dose level of 50mg/kg BW and 100mg/kg BW respectively compared to ibuprofen (Table II).

For studying sub-acute anti-inflammatory activity, arthritis was produced by using formaldehyde on day one of the experiment and the test samples were given orally every day for 10 days. The significant anti-arthritis effect was observed during present investigation.

Several essential oil components are reported to exhibit 5-lipoxygenase inhibitors which include β -caryophyllene (Kamatou *et al.*, 2006), α -bisabolol (Kamatou and Viljoen, 2010). These compounds have been reported to possess anti-inflammatory activity due to the inhibition of leukotriene synthesis. The rhizome of *Alpinia murchisii* Ridl., *Alpinia scabra* (Blume) naves and leaves of *Alpinia pabangensis* Ridl. have been reported to exhibit 5-lipoxygenase inhibitor activity (Syamsir, 2009). The sesquiterpenes santoline, spathulenol, caryophyllene oxide, α -humelene, kaurene, elemol, γ -eudesmolandsabineneetc. Have been reported to possess anti-inflammatory activity

Table III. Effect of oleoresins of *Alpinia allughas* on formaldehyde induced arthritis (Mean±SE, N=6)

Treatment	Dose (mg/kg)	Volume of inflammation										
		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Control	0	2.14	2.20	2.16	2.25	2.15	2.18	2.16±	2.29	2.16	2.19	2.23
		±	±	±	±	±	±	0.15	±	±	±	±
		0.06	0.10	0.13	0.12	0.07	0.08		0.11	0.12	0.11	0.13
Ibuprofen	10	2.18	2.35	2.28	2.34	2.31	2.31	2.29	2.28	2.26	2.17	2.16
		±	±	±	±	±	±	±	±	±	±	±
		0.08	0.09 ^a	0.12	0.03	0.08 ^a	0.11 ^a	0.10	0.10	0.09	0.05	0.06
Oleoresin	50	2.12	2.30	2.28	2.26	2.26	2.24	2.23	2.21	2.18	2.10	2.16
		±	±	±	±	±	±	±	±	±	±	±
		0.05	0.03	0.02	0.02	0.04 ^a	0.04	0.02	0.02	0.08	0.07	0.07
Oleoresin	100	2.09	2.20	2.24	2.24	2.22	2.22	2.21	2.21	2.20	2.10	2.18
		±	±	±	±	±	±	±	±	±	±	±
		0.06	0.11	0.11	0.11	0.11	0.10	0.09	0.08	0.07	0.06	0.06

^a = significant (p<0.05) compared to control ^b = significant (p<0.05) compared to ibuprofen

Table IV. Peripheral analgesic activity of oleoresins of *Alpinia allughas* (Writhing Effect) (Mean±SE, n=6)

Treatment	Dose (mg/kg BW)	No of Writhings	% Writhing	% Inhibition
Control	0	217.00±1.26	100.00	0
Ibuprofen	40	123.50±3.44	56.91	43.08
Oleoresin	50	141.50±1.37	65.20	34.79 ^a
Oleoresin	100	123.16±0.40	56.75	43.24

^a= significant (p<0.05) compared to ibuprofen

(Medeiros *et al.*; Fernandes *et al.*, 1994; Chao *et al.*, 2005; Yoon *et al.*, 2009).

Antinociceptive assay Writhing effect

Oleoresin showed significant peripheral analgesic activity in writhing effect with 34.79% reduction at a dose level of 50mg/kg BW and 43.24% at a dose level of 100mg/kg BW respectively compared to ibuprofen (Table IV).

Hot plate method

A perusal of table V reveals that the discomfort reaction time in indomethacin treated mice was maximum at 60 min while oleoresins exhibited a dose dependent manner increase in the paw licking and jumping time. In the present study the oleoresins significantly attenuated the response in a dose dependent manner.

1, 8-cineole a major constituent of the essential oils of most *Eucalyptus* species and also present in our plant exhibit antinociceptive effect by reducing the acetic acid-induced pain sensation in experimental animal (Santos and Rao, 2000). It has been reported that 1,8-cineole inhibited the paw licking response in both the phases of formalin test, with a significant inhibition only at the second phase (de Lima *et al.*, 2009).

(-)-Linalool is a monoterpene present in aromatic plants viz; *Oscimum basilicum*, *Origanum vulgare*, *Aeolant hussuaveolens* and also marker compounds of zingiberaceous herbs. The antinociceptive activity of linalool in inflammation and neuropathic pains has been reported. It has been reported that the monoterpene significantly reduced the acid-induced writhing and in the hot plate test (Peana *et al.*, 2004). Batista *et al.*, 2010 also reported the significant

Table V. Central analgesic activity of oleoresins of *Alpinia allughas* (Hot plate method) (Mean±SE, n=6)

Treatment	Dose (mg/kg BW)	Hot plate reaction time (min)				
		0	30	60	20	150
Control	0	3.01±0.00	3.11±0.01	2.95±0.01	2.84±0.02	3.03±0.02
Indomethacin	50	3.27±0.03	3.83±0.03 ^a	4.93±0.04 ^a	4.07±0.09 ^a	3.84±0.03 ^a
Oleoresin	50	2.71±0.09	2.24±0.02 ^{a,b}	2.91±0.05 ^b	2.78±0.07 ^b	2.60±0.08 ^{a,b}
Oleoresin	100	2.79±0.09	3.23±0.05 ^b	2.75±0.12 ^{a,b}	3.23±0.08 ^{a,b}	3.04±0.05 ^b

a = significant (p<0.05) compared to control; b = significant (p<0.05) compared to indomethacin

Table VI. Effect of oleoresins of *Alpinia allughas* on Brewer's yeast induced pyrexia in mice (Mean±SE, N=6)

Treatment	Dose (mg/k)	Body temperature (°C)		Temperature after administration of drug (°C)		
		Before injection of Brewer's yeast	After injection of Brewer's yeast	1 hr	2 hr	3 hr
Control	0	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)
Paracetamol	33	37.53±0.06	38.82±0.04	37.63±0.04 ^a (92.86±3.28)	37.54±0.02 ^a (99.47±5.49)	37.49±0.01 ^a (103.60±5.20)
Oleoresin	50	37.54±0.05	38.79±0.07	38.10±0.04 ^a (56.66±6.31)	38.02±0.05 ^{a,b} (62.50±2.76)	37.84±0.07 ^{a,b} (77.57±5.88)
Oleoresin	100	37.56±0.05	38.86±0.04	37.81±0.08 ^{a,b} (80.49±7.66)	37.68±0.06 ^{a,b} (90.75±6.57)	37.57±0.06 ^{a,b} (98.95±3.90)

Percentage reduction in temperature is given within parenthesis

^a = significant (p<0.05) compared to control; ^b = significant (p<0.05) compared to paracetamol

antinociceptive effects of linalool in mice, specifically in mechanical hypersensitivity induced by the neuropathic pain and in the mechanical and cold hypersensitivity induced by a chronic inflammatory model. Similarly, borneol also present in our sample reported to posses antinociceptive activity (Almeida *et al.*, 2013).

The antinociceptive effect of farnesol conducted by acetic acid-induced writhings and formalin assays has also been reported by Oliveira *et al.*, 2013. Similarly Paula-Freire *et al.*, 2014 has reported the antinociceptive properties of (E)-caryophyllene.

It has been reported that the pharmacological activities of *Copaiba* oleoresin were because of the presence of sesquiterpenes like β-caryophyllene, caryophyllene oxide, α-humulene, δ-cadinene, α-cadinol, α- and β-selinene, β-elemene, α-copaene and α-eudesmol (Leandro *et al.*, 2012). These compounds are

also identified in present analysis of *A. allughas* oleoresin. However, the other constituents including minor once in the oleoresin may interact synergistically in the promotion of the observed pharmacological activities.

Brewer's yeast induced pyrexia

The oleoresin of *A. allughas* showed a reduction in body temperature in Brewer's yeast induced pyrexia in mice. Oleoresin produced a significant antipyretic effect in a dose dependent manner with 50mg/kg BW and 100mg/kg BW dose level. Negative control mice did not show any reduction in the body temperature on oral administration of saline. The maximum inhibition was observed at 3h (Table VI).

An appreciable antipyretic effect was noticed in oleoresins in dose dependent manner which was comparable to paracetamol. Previous studies reveal that lipid peroxidation

process is responsible to increase body temperature. We have also reported antioxidant activity of *Alpinia allughas* which may be responsible for the activity observed in the experiments (Sethi *et al.*, 2015). The antioxidant supplementation decreases the lipid peroxidation processes hence reduce pyrexia (Brzezinska-Slebodziniska, 2001).

Oleoresins of *Alpinia allughas* possess significant anti-inflammatory, anti-nociceptive and antipyretic properties, which can be used for the preparation of various formulations for the treatment of inflammation, pain and pyrexia.

Toxicity

On giving oral doses of 400, 600, 800mg/kg BW of *Alpinia allughas* oleoresin, no change in behaviour or physiology was observed for two days and on a subsequent day. No lethality was observed during the experiment. All reflexes (pedal and corneal) and rectal temperature as well heart, respiration rates were within normal physiological limits in the extract treated mice.

CONCLUSION

The results suggest that *Alpinia allughas*, the member of family Zingiberaceae can be utilized for its chemical constituents and pharmaceutical activities. Being a wild plant species of this region, it can be taken for commercial cultivation and exploitation for use as herbal drug and source of perfumery chemicals.

ACKNOWLEDGMENT

Thanks are due to U.G.C., New Delhi, India for a research fellowship to Ravendra Kumar and Summer Chandra F.R.I. Dehradun, India for plant identification.

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