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CHEMICAL SCIENCE

Comparative study on eucalyptol and camphor rich essential oils from rhizomes of *Hedychium spicatum* Sm. and their pharmacological, antioxidant and antifungal activities

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Abstract: The aim of present study was to evaluate chemical composition and different biological activities viz., pharmacological and antioxidant activities of essential oils. The chemical composition of essential oils was determined by gas chromatography/mass spectrometry while biological activities were evaluated by standard protocols. Essential oils of *Hedychium spicatum* Sm. from two different ecological niches viz; Nainital (Site-I) and Himachal Pradesh (Site-II) of India revealed the qualitative and quantitative chemo-diversity. Both the oils were dominated by oxygenated terpenoids. Major marker compounds identified were eucalyptol, camphor, linalool, α-eudesmol, 10-epi-γ-eudesmol, and iso-borneol. Both the oils exhibited anti-inflammatory activity suppressing 17.60 % to 33.57 % inflammation at 100mg/kg b. wt. dose levels compared to ibuprofen-treated group (40.06 %). The sub-acute inflammation in oils-treated mice groups (50 and 100 mg/kg b. wt.) increased on day 2 but showed a gradual decrease from day 3 onwards and then recovered to normal by day 10. The antinociception percentage for doses (50 and 100 mg/kg b. wt.) ranged from 33.70-40.46 % in Site-I and 30.34-42.39 % in Site-II compared to standard drug, ibuprofen (43.08 %). The oils also showed a good antipyretic effect by suppressing Brewer's yeast (*Saccharomyces cerevisiae*) induced pyrexia after oil dose injection. The oils also exhibited good antioxidant activity.

Key words: Antioxidant activity, camphor, eucalyptol, *Hedychium spicatum.*

INTRODUCTION

Hedychium spicatum Sm., a perennial rhizomatous herb belonging to the family Zingiberaceae is commercially known as Kapoor Kachri, Ginger lily or Ban-Haldi in Indian traditional medicine (Prakash et al. 2016). In Indian subcontinent, it is distributed in the subtropical regions including Andhra Pradesh, Arunachal Pradesh, Assam, Himachal Pradesh, Karnataka, Manipur, Meghalaya, Mizoram, Nagaland, Orissa, Sikkim and Uttarakhand ascending to an altitude of 100 to 3000 m (Rao et al. 2011, Chhetri et al. 2005, Rawat et al. 2018). Besides India, this plant is cosmopolitantly spread in Bhutan, Nepal, Japan, Pakistan, China, Myanmar, Thailand, Mauritius, Seychelles and Madagascar (Mukherjee 1970, Sirirugsa 1999, Rawat et al. 2018). This herb has been used in various traditional therapies like bronchitis, indigestion, eye disease, inflammations and diarrhoea. The decoctions and infusions of the leaves, flowers and rhizomes of this herb are recommended for its blood purifying, stomachic and carminative properties in Ayurvedic and traditional Chinese medicine (Rawat et al. 2019). The modern pharmacological studies

indicated that this herb exhibited diverse biological activities such as anti-inflammatory, anti-asthmatic, anti-allergic, analgesic, ulcer protection, blood pressure lowering properties, hepatoprotective properties, antihyperglycemic properties, nootropic effects and memory restorative properties, anticancer and cytotoxic properties, hair growth promoting properties, tranquillizing properties, antioxidant and radical scavenging properties, antimicrobial properties and anthelmintic and pediculicidal properties (Prakash et al. 2016, Rao et al. 2011, Rawat et al. 2018, 2019, 2021, Chaturvedi & Sharma 1975, Ghildiyal et al. 2012, Joshi et al. 2008, Reddy et al. 2009).

The production of secondary metabolites like terpenoids, in plant systems rely on the environmental and edaphic conditions. The same plant species from different ecological niches may differ in its chemical composition both qualitatively and quantitatively hence also differ in biological activities. Thus, it becomes essential to check the chemical variation of a plant growing in different ecological regions/ stress conditions. In continuation to our previous research on family Zingiberaceae, the aim of present investigation was to analyse the chemical variation in essential oils from two different ecological niches viz., Nainital (Uttarakhand) and Renuka, Dadahu (Himachal Pradesh), India and to evaluate several biological activities such as antioxidant activity, anti-fungal activity, *in-vivo* anti-inflammatory activity, antinociceptive activity and antipyretic activity of traditionally and ethnobotanically important Zingiberaceous herb *Hedychium spicatum*.

MATERIALS AND METHODS

Plant material and isolation of essential oils

The fresh rhizomes were collected from Nainital (Uttarakhand) (1950 m, 29° 39' 31''North Latitude, 79° 44'84'' East Longitude) and Renuka, Dadahu (Himachal Pradesh), (1100 m, 30°61'50''North Latitude, 77°44'85''East Longitude) India in the month of September, 2012. The plants were identified and authenticated by Plant Taxonomist, Dr. D.S. Rawat, G. B. Pant University of Agriculture and Technology, Pantnagar, India. The crushed fresh rhizomes (1 kg) subjected to Clevenger apparatus for 3-4 h for hydro distillation. The process was repeated for 3 times to get sufficient amount of essential oils. The neat essential oils were collected and stored in refrigerator at 4 °C for further analysis. The essential oil isolated from two different regions were cited as *Hedychium spicatum* essential oil (HSEO) Site-I and Site-II.

Identification of components

The components of the oils were identified using gas chromatography and mass spectrometry (GC/MS). The analysis was performed under the following experimental conditions:System: Nucon, GC 5765; ionization voltage: 70 eV; ion sources temperature: 210 °C; carrier gas: helium (linear velocity of 32 cm/s, measured at 100 °C); flow rate: 50 kg/cm^2; Detector: FID and MS; column: fused silica capillary column DB-5, 30 m × 0.32 mm; injection volume: 0.1 µL; film thickness: 0.25 µm, temperature program: 50-300 °C, increasing by 3 °C/min, injector temperature of 210 °C, detector temperature 210 °C. The chemical constituents of the oils were then identified by comparison of their mass spectral fragmentation pattern and Kovatt indices with Wiley Registry of Mass Spectral Data 8th edition, NIST Mass Spectral Library, and literature and quantification were simply done by measuring the peak areas of gas chromatograms (Adams 2007).

Antioxidant activity

DPPH radical scavenging method

According to protocol developed by Burits & Bucar 2000, different concentrations of HSEO (Site- I and II) (50-250 µg/mL) were added to 5.0 mL of a 0.004 % methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). Finally the absorbance was taken on Thermo Scientific EVOLUTION 201 series against a blank at 517 nm after 30 min of incubation at room temperature. All the observations were taken as triplicate. BHT, catechin and gallic acid were used as the standard antioxidant. Inhibition of free radical by DPPH was calculated by % DPPH radical scavenging capacity= (Ao – At / Ao) × 100.

Where, Ao and At are the absorbance values of control and test essential oils respectively. Percent inhibition was plotted against concentrations and the equation for the line was used to obtain the IC_{50} values.

Hydroxyl radical scavenging method

60 µL $FeSO_4.H_2O$ (1.0 mM) was added to 90 µL of aqueous 1, 10 phenanthroline (1.0 mM), 2.4 mL of 0.2 M phosphate buffer (pH 7.8) and 150 µL of hydrogen peroxide (0.17 mM) and 1.5 mL to different concentrations of HSEO (Site-I and II) (50-250 µg/mL). The absorbance was then observed at 560 nm against blank (Dinis et al. 1994). Ascorbic acid was used as the standard. % IC was calculated as % hydroxyl radical scavenging capacity = (Ao – At / Ao) × 100. Where, Ao and At are the absorbance values of control and test essential oils respectively. Percent inhibition was plotted against concentrations and the equation for the line was used to obtain the IC_{50} values.

Nitric oxide (NO) scavenging method

As per the method developed by Naskar et al. 2010, 2.0 mL sodium nitroprusside (10 mM) in phosphate buffer saline (pH 7.4) was mixed with different concentrations of HSEO (Site-I and II) (50-250 µg/mL) and incubated at 25 °C for 3 h. To the mixture add 1.0 mL of Griess reagent was added. The absorbance of the pink coloured solution obtained was measured at 546 nm. Ascorbic acid was used as the standard. % IC was calculated as % nitric oxide radical scavenging capacity = (Ao – At / Ao) × 100. Where, Ao and At are the absorbance values of control and test essential oils respectively. Percent inhibition was plotted against concentrations and the equation for the line was used to obtain the IC_{50} values.

Superoxide anion ($O_2^{\cdot-}$) scavenging method

In brief, 1.0 mL of nitrobluetetrazolium solution (100 µM of NBT in 100 mmol/L phosphate buffer, pH 7.4), 1.0 mL of NADH (468 µmol in 100 mM phosphate buffer, pH 7.4) solution and concentrations of HSEO (Site-I and II) (50-250 µg/mL) were mixed. The reaction was started by the addition of 100 µL of phenazinemethosulfate solution (100 mM phosphate buffer, pH 7.4). The reaction mixture was then incubated at 30 °C for 15 min. The absorbance was measured at 560 nm. Ascorbic acid was used as the standard (Naskar et al. 2010). % IC was calculated as % superoxide anion scavenging capacity = (Ao – At / Ao) × 100. Where, Ao and At are the absorbance values of control and test essential oils respectively. Percent inhibition was plotted against concentrations and the equation for the line was used to obtain the IC_{50} values.

Fe^{2+} metal chelating method

The chelation of Fe^{2+} by essential oils was determined by method developed by Pulido et al. 2000. In brief 0.1 mL of 2.0 mM $FeCl_2.4H_2O$, 0.2 mL of 5.0 mM ferrozine was added to different concentrations of HSEO (Site-I and II) (50-250 µg/mL). The solutions were mixed and allowed to

react for 10 min. The absorbance was measured at 562 nm. EDTA and Citric acid was used as the standard. % IC was calculated as % Fe^{2+} metal chelating capacity = (Ao – At / Ao) × 100. Where, Ao and At are the absorbance values of control and test essential oils respectively. Percent inhibition was plotted against concentrations and the equation for the line was used to obtain the IC_{50} values. A lower IC_{50} value indicates greater antioxidant activity.

Anti-fungal activity and determination of minimum inhibitory concentration (MIC) by broth microdilution

Disc diffusion technique was used to determine the antifungal activity against the growth of tested fungi viz., *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, *Sclerotium rolfsii* and *Colletotrichum falcatum* (Murray et al. 1995). The cultures were obtained from Department of Plant Pathology, College of Agriculture, G.B.P.U.A.&T, Pantnagar. Potato dextrose agar (PDA) was inoculated when cooled at 45 °C and added 20 mL PDA in each sterilized petri plates (6 mm diameter) in laminar flow and kept undisturbed till solidification. Each essential oil was diluted with DMSO to obtain the final concentrations of 1000, 750, 500, 250 µg/mL respectively. The pathogenic fungi were cultured on PDA media and incubated at 30 °C for 1 week. The essential oils were diluted in DMSO to dissolve the essential oils. The plates were then impregnated with various concentrations of all samples. The plates were incubated at 28 °C for 3-4 days in which the fungal growth was monitored. PDA plates containing only DMSO served as the negative control and carbendazim was used as positive control. The percentage ofinhibition of mycelia growth was calculated from the mean values of colony diameter of treated and control (DMSO). Percentage inhibition was calculated by using the following formula:

$$\% \text{ Inhibition} = C\text{-}T/C*100$$

C = mean of the diameter of growth in control, T = mean of the diameter of growth with the oil.

The minimum inhibitory concentrations (MICs) of Site-I and Site-II were determined by broth micro dilution method (Gulluce et al. 2004). MICs were described as the lowest concentration of HSEO in Site-I and Site-II resulting in the slight reduction of the inoculum.

Animals

The pharmacological activities of HSEO in Site-I and Site-II were carried out on 3-4 months aged male 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 consisting of six mice in each. The animals were acclimatized under laboratory-controlled conditions of 27±3 °C with food and water. Priot to conducting the experiment, the mice of all groups were weighed individually to quantify the dose of essential oils for each group. The animals were habituated to laboratory environment for two weeks. All the experiments were regulated between 9:00 to 17:00 h. The animals were kept under observation after the completion of experiments for one week, to note acute or sub-acute toxicity, if any. To conduct the experiments on mice the permission was taken from Institutional Ethical Committee, CPCSEA with reference number (Ref: IAEC/Chem/CBSH/118).

Four different groups with six mice in each group were used for conducting various pharmacological activities. The experimental dose of 50 mg/kg and 100 mg/kg b.wt. concentrations were selected as per % inhibition calculations. Small amount of Tween-20 and saline water was added to pulverize 50 and 100 µL

of oils separately to make final volume of 10 mL. Paracetamol, ibuprofen and indomethacin were used as positive and saline water as negative control respectively. The essential oils, standard drugs and saline water were orally administered at the dose level of 0.1 mL/10 g b.wt.

Anti-inflammatory assay

Acute Anti-inflammatory assay

Carrageenan induced hind paw edema method was performed to investigate anti-inflammatory activity by intradermally injecting 0.1 mL of 1.0 % suspension of carrageenan into the subplantar region of the right hind paw of mice (Kasahara et al. 1985). The post carrageenan injection paw volume was measured by plethysmometer (UGO Basile, Italy) at an interval of 1 h and 4 h. HSEO of Site-I and Site-II (50 mg/kg and 100 mg/kg b.wt.) and standard anti-inflammatory drug, ibuprofen suspension (40 mg/kg b.wt.) was also administered orally to the mice. The reduction in hind foot paw volume compared to control was calibrated as the anti-inflammatory activity.

Sub-acute anti-inflammatory assay

Formaldehyde induced arthritis method was used to determine the subacute activity of the rhizome essential oils by injecting 0.1 mL formaldehyde (1.0 %) solution intradermally in the right hind paw of the mice on day one of the experiment (Kasahara et al. 1985). HSEO of Site-I and Site-II with doses of 50 and 100 mg/kg b.wt. were administered orally, daily in the morning for 10 days. Ibuprofen (dose of 10 mg/ kg b.wt.) was used as standard anti-inflammatory drug while control group received saline water only. Paw volume of all the mice were measured plethysmometrically in the evening hours for 10 days.

Antinociceptive assay

Writhing effect

Glacial acetic acid (0.1 mL/10 g body weight) was injected 40 min after 0.2 mL HSEO (Site-I and Site-II) oils and the positive control drug (40 mg/kg b.wt. ibuprofen) were orally administered intraperitoneally to four different groups of six experimental mice each to induce pain sensation (Collier et al. 1968, Antonisamy et al. 2017). The number of writhing for every mouse was counted per min for 15 min after acetic acid injection. The inhibition of writhing and percentage of pain protection in mice was calculated.

Hot plate method

Analgesic response latencies were evaluated using standard hot-plate test (Langerman et al. 1995). The mice were placed into Perspex cylinder on the heated surface of the hot-plate (55±2 °C). Response latency (time in seconds) was recorded to induce discomfort reaction *viz*., licking paws or jumping. The measurements were recorded at 0, 30, 60, 120 and 150 min of oral administration of the essential oils at 50 and 100 mg/kg b.wt. concentrations. Standard drug indomethacin (5 mg/kg b.wt.) and saline water (0.2 mL) were orally administered as positive and negative control respectively. A latency period of 20 sec was marked as complete analgesia and measurements were stopped if it exceeded the latency period to avoid injury.

Antipyretic assay

Brewer's yeast (*Saccharomyces cerevisiae*) induced pyrexia model was used to perform antipyretic activity in mice weighing 18-23 g, four groups of six mice each (Rao et al. 1997). To record basal line body temperature, a thermometer was inserted into the rectum after restraining the mice. All the experimental mice were induced

by a subcutaneous injection of 20 % suspension of Brewer's yeast (*Saccharomyces cerevisiae*) (10 mL/kg b.wt.) except the control group. The rectal temperature was recorded again at nineteenth hour. 0.1 mL/10 g b.wt. of HSEO (Site-I and Site-II) in selected dose levels of 50 mg/kg and 100 mg/kg b.wt. Paracetamol (33 mg/kg b.wt.), the antipyretic drug was orally administered immediately to standard group. Control group received 0.2 mL normal saline water only. The rectal temperature was again recorded at hourly intervals upto 3 h. The percentage reduction in rectal temperature was calculated by considering the total fall in temperature to normal level as 100%.

Acute toxicity assessment

Acute toxicity study was performed in accordance with OECD guidelines (Toxicity-Up 2001) prior to the pharmacological activities. The lethality of HSEO (Site-I and Site-II) were examined on mice by oral administration of essential oils at 400, 600 and 800 mg/kg b.wt. After injection, mice were observed continuously for 24 h for any toxic manifestations or behavioural changes. Casualties were monitored up to 48 h.

Statistical analysis

Data were expressed as Mean±Standard error of the mean. Statistical comparison between the groups was analysed using programme 43 (software) one way ANOVA. $p<0.05$ was considered to be statistically significant.

RESULTS

Chemical composition

The essential oil extraction yield (w/v) was of 0.04 % and 0.05 % for Site-I and Site-II respectively. The GC/MS analysis of HSEO (Site-I and Site-II) revealed the presence of over 18 and 34 constituents contributing 91.5 % and 92.6 %of the total oil respectively. The oils were dominated by oxygenated terpenoids (83.8 % in Site-I and 82.7 % in Site-II). The major constituents in Site-I were eucalyptol (44.0 %) and linalool (6.6 %) while camphor (35.6 %), isoborneol (13.2 %), eucalyptol (8.0 %), borneol (8.2 %), linalool (5.8 %),α-terpinene (2.0 %) were identified as major constituents in Site-II respectively. The oxygenated sesquiterpenoids contributed 32.8 % in Site-I with α-eudesmol (26.8 %), 10-*epi*-γ-eudesmol (5.4 %), 1-*epi*-cubenol (0.3 %), *E*-nerolidol (0.2 %) and caryophyllene oxide (0.1 %) while 8.3 % in Site-II being represented by germacrene-D-4-ol (7.4 %), α-cadinol (0.4 %), caryophyllene oxide (0.3 %), spathulenol (0.1 %) and cubinol (0.1 %). The hydrocarbons contributed 7.2 % and 9.9 % of the total oil in Site-I and Site-II respectively. The major monoterpene hydrocarbons (4.6 % in Site-I and 3.6 % in Site-II) were β-pinene (2.5 %), α-pinene (1.0 %) in Site-I and α-terpinene (2.0 %) in Site-II. The sesquiterpenes (2.6 % in Site-I, 6.3 % in Site-II) were represented by *E*-β-farnesene (1.4 %) and α-selinene (1.6 %), germacrene B (1.8 %) respectively as the major constituents besides other minor constituents. Compounds that were present in Site-I and absent in Site-II were (*E*)-β-farnesene (1.4 %), 10-*epi*-γ-eudesmol (5.4 %) and α-eudesmol (26.8 %) whereas compounds that were present in Site-II and absent in Site-I were camphor (35.6 %), isoborneol (13.2 %), α-selinene (1.6 %), germacrene B (1.8%) germacrene-D-4-ol (7.4 %), borneol (8.2 %), and α-terpinene (2.0 %). It was observed that altitude of plant habitat had an explicit effect on both composition of essential oils (EOs) and content of its ingredients. Composition of EOs as well as the concentration of each constituent show remarkable variations in response to changing environment. For that reason, potential synergistic and antagonistic effects among essential oil constituents and the environmental variables that affect EOs compositions are crucial for EOs quality and

bioactivity. The detailed comparative composition of HSEO (Site-I and Site-II) with earlier reports in terms of constituents and class composition has been recorded in Table I and Table II. The results thus demonstrate the presence of different chemotypes of *H. spicatum*.

Antioxidant activity

HSEO (Site-I and Site-II) exhibited good to strong DPPH radical scavenging activity. The radical scavenging potential in the form of their IC_{50} values revealed the order Site-I (IC_{50} = 10.00±0.08 µg/mL) > Site-II (IC_{50} = 25.40±0.16 µg /mL) compared to the standards antioxidants, gallic acid (IC_{50} = 1.26±0.01 µg/mL) > catechin (IC_{50} = 1.61±0.01 µg/mL) > BHT (IC_{50} =7.99±0.01 µg/mL). The antioxidant power of HSEO (Site-I and Site-II) may be attributed to their hydrogen donating ability to DPPH free radicals. HSEO (Site-I and Site-II) scavenge hydroxy free radicals as a function of selected dose levels compared to ascorbic acid. The results for radical scavenging activity obtained in terms of IC_{50} values were 11.7±0.12 µg/mL and 12.6±0.09 µg/mL for Site-I and Site-II respectively as compared to ascorbic acid (IC_{50}=2.65±0.10 µg/mL). Nitric oxide (NO) scavenging ability of Site-I and Site-II exhibited different degree of antioxidant potential as indicated by IC_{50} values in the order of Site-I (IC_{50}= 5.82±0.02 µg/mL) >Site-II (IC_{50}= 7.40±0.15 µg/ mL) compared to ascorbic acid (IC_{50} = 4.22 ±0.01 µg/mL). Site-I and Site-II were able to scavenge the superoxide anion with an IC_{50} of 1.45±0.16 µg/mL and 18.62±0.24 µg/mL respectively in comparison to ascorbic acid (IC_{50} = 6.08±0.01 µg/ mL). The IC_{50} values of HSEO for Site-I and Site-II towards their antioxidant potentiality in terms of chelating ability was observed as Site-I (IC_{50}= 5.79±0.01 µg/ mL) > Site-II (IC_{50}= 3.10±0.23 µg/ mL) compared to Na_2EDTA (IC_{50}= 2.13±0.07 µg/mL) and citric acid (IC_{50}= 1.93±0.09 µg/mL) respectively (Table III).

Anti-fungal activity and determination of minimum inhibitory concentration (MIC) by broth microdilution

HSEO (Site-I and Site-II) exhibited dose dependent inhibitory effect against the growth of all tested fungi. The oils suppressed the mycelial growth of *Sclerotinia sclerotiorum* at all the tested dose levels followed by *Rhizoctonia solani, Sclerotium rolfsii* and *Colletotrichum falcatum*. HSEO of Site-I was found to exhibit strong inhibitory effect against *Sclerotinia sclerotiorum* (32.5 %), *Rhizoctonia solani* (39.17 %), *Colletotrichum falcatum* (30.83 %) and *Sclerotium rolfsii* (19.17 %) even at a lower dose level of 250 µg/mL. HSEO Site-II was observed to be moderately active against the mycelial growth of all the fungi at a dose level of 1000 µg/mL. The MIC values of HSEO (Site-I and Site-II) ranged from 31.25 to 125 and 62.5 to 250 µg/ mL respectively. The study indicated that both the essential oils were found to have potent fungicidal activity as per their inhibition action against all the tested pathogenic fungi (Table IV).

Acute toxicity assessment

After the oral administration of essential oils at 400, 600 and 800 mg/kg b.wt. within 24 h no acute toxicity was observed.

Anti-inflammatory Assay

Acute anti-inflammatory assay

The perusal of Table V indicates significant anti-inflammatory effect of HSEO (Site-I and Site-II). HSEO (Site-I and Site-II) suppressed 17.60 % and 33.57 % inflammation respectively at 100 mg/kg b. wt. dose level. Maximum inhibition (40.06 %) was recorded in ibuprofen treated group, almost parallel to essential oils at 100 mg/kg b. wt. dose level.

Table I. Class composition of essential oil of collections Site-I and Site-II and earlier reports.

Compounds	KI	Sabulal et al. 2007	Our Work						
			Previous					Present	
			Joshi et al. 2008			Prakash et al. 2010	Rawat et al. 2019		
			I	II	III			Site-I	Site-II
tricyclene	926	-	-	-	-	-	0.2	-	-
α-pinene	938	-	1.8	2.8	3.7	11.0	1.3	1.0	-
camphene	963	0.3	0.4	0.3	0.1	-	4.8	t	0.5
sabinene	976	0.1	0.1	t	13.0	t	0.1	0.3	-
β-pinene	980	1.3	4.0	5.3	5.9	1.4	-	**2.5**	0.1
β-myrcene	989	0.1	0.3	0.4	8.7	t	1.2	-	0.1
δ-2-carene	1001	0.1	-	-	-	-	t	-	-
α-phallendrene	1005	0.1	-	-	-	-	t	-	-
Δ3-carene	1011	-	-	-	-	-	-	-	0.1
α-terpinene	1018	0.1	-	-	-	-	t	0.8	**2.0**
p-cymene	1031	0.1	t	t	0.7	t	-	t	0.1
eucalyptol	1035	44.3	42.8	45.7	4.3	17.6	12.2	44.0	8.0
γ-terpinene	1058	0.1	-	-	-	-	**-**	-	-
(-)-terpinen-4-ol	1076	**-**	**-**	**-**	**-**	**-**	0.7	**-**	**-**
trans-linalool oxide	1078	0.2	-	-	-	-	**-**	-	0.2
cis-linalool oxide	1087	0.2	-	-	-	-	-	-	0.2
limonene	1089	0.5	t	t	0.2	t	-	-	0.7
linalool	1098	25.6	6.4	0.6	2.1	2.6	4.5	**6.6**	**5.8**
camphor	1141	0.4	-	-	-	-	27.3	-	**35.6**
camphene hydrate	1149	-	-	-	-	-	2.0	-	**-**
β-pinene oxide	1159	-	-	-	-	-	0.6	-	**-**
***iso*-borneol**	1156	-	-	-	-	-	7.1	-	**13.2**
borneol	1165	-	t	0.1	0.6	0.1	3.9	0.1	**8.2**
terpineol-4-ol	1176	1.3	0.9	1.1	16.5	0.2	-	-	0.6
α-terpineol	1189	1.9	1.6	1.7	0.1	-	2.1	0.8	**2.0**
δ-terpineol	1171	0.3	-	-	-	-	-	-	-
iso-bornyl formate	1239	-	-	-	-	-	-	-	0.3
δ –carvone	1243	-	-	-	-	-	0.1	-	0.1
iso-bornyl acetate	1285	-	-	-	-	-	-	-	0.2
δ –elemene	1338	-	-	-	-	-	0.3	-	0.2
β-elemene	1339	-	-	-	-	0.2	1.5	-	0.3
α –cubebene	1351	-	-	-	-	0.1	-	-	-
furfuryl hexanoate	1367	-	-	-	-	0.1	-	-	-
α-copaene	1378	-	-	-	-	0.5	-	-	0.3
(+)-sativene	1391	-	-	-	-	-	0.1	-	-
α-(*Z*)-bergamotene	1415	0.7	-	-	-	0.1	-	-	-
(*E*)-caryophyllene	1418	-	-	-	-	1.5	-	0.3	-
(*Z*)-caryophyllene	1419	0.2	-	-	-	0.1	0.2	-	-
aromadendrene	1441	-	-	-	-	-	-	-	0.2
(*Z*)-β-farnesene	1445	-	-	-	-	0.1	-	-	-

Table I. Continuation.

α –himachalene	1447	-	-	-	-	0.1	-	-	-
aromadendrene	1454	0.1	-	-	-	-	t	-	-
α-humulene	1454	0.3	-	-	-	2.0	0.3	-	0.2
(*E*)-β-farnesene	1458	-	2.0	1.3	-	t	-	1.4	-
9-*epi*-β-caryophyllene	1458	0.1	-	-	-	-	-	-	-
dehydroaromadendrene	1459	-	-	-	-	1.0	-	-	-
epi-globulol	1468	-	-	-	-	-	0.3	-	-
γ –muurolene	1477	-	-	-	-	1.5	0.1	-	-
germacrene-*D*	1489	-	-	-	-	-	0.7	-	0.3
β-selinene	1490	-	-	-	-	-	-	-	0.7
epi-cubibol	1492	0.2	-	-	-	-	-	-	-
α-selinene	1493	-	0.1	0.3	1.4	3.2	-	0.1	1.6
curzerene	1499	-	-	-	-	-	2.1	-	-
α-murrolene	1500	0.2	-	-	-	-	t	-	0.4
germacrene-*A*	1509	-	-	-	-	-	0.4	-	-
γ-cadinene	1513	0.3	-	-	-	5.4	-	0.8	0.3
cubebol	1514	-	0.8	0.8	-	t	-	-	-
β-himachalene	1516	0.1	-	-	-	-	-	-	-
δ-cadinene	1524	0.9	-	-	-	7.5	0.3	-	-
hedycaryol	1555	2.6	-	-	-	-	-	-	-
germacrene-*B*	1561	-	-	-	-	-	0.8	-	1.8
cis-sesquisabinene hydrate	1562	0.7	2.0	1.1	-	-	-	-	-
(*E*)-nerolidol	1564	0.3	-	-	-	0.6	-	0.2	-
longipinanol	1569	-	-	-	-	-	0.1	-	-
germacrene-*D*-4-ol	1574	-	-	-	-	6.8	-	-	**7.4**
spathuenol	1578	0.6	12.4	12.0	1.2	-	0.5	-	0.1
caryophyllene oxide	1582	0.5	-	-	-	-	0.1	0.1	0.3
β-elemenone	1589	-	-	-	-	-	0.4	-	-
khusimone	1604	-	-	-	-	-	4.6	-	-
10-*epi*-γ-eudesmol	1619	-	-	-	-	9.7	-	5.4	-
trans-isolongifolanone	1626	-	-	-	-	-	0.1	-	-
1-*epi*-cubenol	1639	0.5	-	-	-	6.9	-	0.3	-
cubenol	1646	-	-	-	-	-	-	-	0.1
β-eudesmol	1649	2.2	-	-	-	t	-	-	-
α-eudesmol	1652	2.3	-	-	-	17.0	-	**26.8**	-
(-)-α-cadinol	1653	2.3	-	-	-	-	0.6	-	0.4
neocurdione	1657	-	-	-	-	-	0.2	-	-
γ-muurolol	1671	4.4	-	-	-	-	-	-	-
agarospirol	1682	0.9	-	-	-	-	-	-	-
8-*epi*-β-bisabolol	1690	0.5	-	-	-	-	-	-	-
(*E, E*)-germacrone	1693	-	-	-	-	-	4.3	-	
oplopanone	1733	-	-	-	-	0.1	-	-	-
(-)-curcumol	1754	-	-	-	-	-	1.2	-	-
curdione	1792	-	-	-	-	-	9.3	-	-
Total		97.9%	75.6%	73.5%	58.3%	97.4%	96.6%	91.5%	92.6%

Table II. Class composition of essential oil of collections Site-I and Site-II and earlier reports.

Class composition		Sabulal et al. 2007	Joshi et al. 2008			Prakash et al. 2010	Rawat et al. 2019	Present Study	
			I	II	III			Site-I	Site-II
Mono-terpenoids	Hydrocarbons	2.8	6.6	8.8	32.3	12.4	7.6	4.6	3.6
	Oxygenated	74.2	51.7	49.2	23.6	21.2	60.5	51.5	74.4
Sesqui-terpenoids	Hydrocarbons	2.9	2.1	1.6	1.4	23.3	4.7	2.6	6.3
	Oxygenated	18.0	15.2	13.9	1.0	40.5	19.2	32.8	8.3
Total (%)		97.9	75.6	73.5	58.3	97.4	92	91.5	92.6

Site-I= Nainital, U.K. collection, Site-II= Renuka, Dadahu, H.P. collection

Table III. Antioxidant potential in term of IC_{50} values of HSEO of Site-I and Site-II.

Essential oil / Standard	Antioxidant assay				
	DPPH scavenging activity/ IC_{50}(µg/mL)	OH scavenging activity/ IC_{50}(µg/mL)	Nitric oxide radical scavenging activity/ IC_{50}(µg/mL)	Superoxide anion scavenging activity/ IC_{50}(µg/mL)	Metal chelating ability/ IC_{50}(µg/mL)
Site-I	10.00±0.08^{c}	11.70±0.10^{e}	5.82±0.02^{bc}	1.45±0.16^{a}	5.79±0.01^{d}
Site-II	25.40±0.16^{e}	12.61±0.12^{d}	7.40±0.15^{d}	18.62±0.24^{e}	3.10±0.23^{c}
BHT	7.99±0.04^{b}	-	-	-	-
Catechin	1.61±0.01^{a}	-	-	-	-
Gallic acid	1.26±0.01^{a}	-	-	-	-
Ascorbic acid	-	2.65±0.13^{a}	4.22±0.01^{b}	6.08±0.01^{d}	-
Na_2EDTA	-	-	-	-	2.13±0.07^{b}
Citric acid	-	-	-	-	1.93±0.09^{a}

- = Not applicable, **Values are means of three replicates ± SD. Within a column, mean values followed by the same letter are not significantly different according to Tukey's test (p<0.05),HSEO:** *H. spicatum* **essential oil, Site-I= Nainital, U.K. collection, Site-II= Renuka, Dadahu, H.P. collection.**

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. The sub-acute inflammation in HSEO (Site-I and Site-II) treated mice groups (50 mg/kg b. wt. and 100 mg/kg b. wt.) increased in day 2 but showed gradual decrease from day 3 onwards and then reverted to normal by day 10 (Table VI).

Table IV. Screening of antifungal activity of HSEO of Site-I and Site-II.

Sample	Conc (µg/mL)	% of mycelial growth inhibition [mm] (MIC [µg/mL])			
		C. falcatum	*R.solani*	*S. sclerotium*	*S. rolfsii*
Site-I	250	30.83±2.2^{bc}	39.17±0.8^{cd}	32.5±1.4^{b}	19.17±0.8^{b}
	500	37.5±1.4^{cd}(62.5)	45.83±2.2^{def}(31.25)	44.17±3.0^{cde}(62.5)	37.5±1.4^{c}(125)
	750	41.67±2.2^{cdef}	50±1.4^{efg}	58.33±2.2^{fgh}	48.33±3.0^{cdef}
	1000	50.83±1.6^{efgh}	57.5±2.5^{g}	95.83±0.8^{m}	72.5±2.8^{jk}
Site-II	250	35.83±2.2^{cd}	33.33±0.8^{bc}	34.17±0.8^{bc}	3.33±0.8^{a}
	500	39.17±0.8^{cd}(62.5)	40.83±1.6^{cde}(62.5)	41.67±1.6^{bcde}(62.5)	55.83±3.6^{efgh}(250)
	750	52.50±1.4^{fgh}	59.17±3.3^{g}	59.17±3.0^{gh}	65.83±2.2^{hijk}
	1000	60±2.8^{h}	80.83±2.2^{h}	80.83±2.2^{jkl}	69.17±1.6^{ijk}

Values are means of three replicates± SE. Within a column, mean values followed by same letter are not significantly different according to Tukey's test (p<0.05), HSEO: *H. spicatum* **essential oil, Site-I= Nainital, U.K. collection, Site-II= Renuka, Dadahu, H.P. collection**

Table V. Acute anti-inflammatory activity of HSEO of Site-I and Site-II.

Group	Treatments	Dose (mg/kg)	Change in paw thickness		% Inhibition
			1 hr	4 hr	
I	Control	0.2 mL	2.18±0.02	2.17±0.04	00.48±0.01^{a}
II	Ibuprofen	40	2.97±0.05	1.78±0.04^{a}	40.06±0.12^{a}
III	Site-I	50	2.62±0.19	2.35±0.15^{b}	10.30±0.11^{a}
IV	Site-I	100	2.67±0.18	2.20±0.15^{b}	17.60±0.11^{b}
V	Site-II	50	2.65±0.23	2.23±0.15^{b}	17.23±0.10^{a}
VI	Site-II	100	2.85±0.11	1.91±0.41^{a}	33.57±0.11^{b}

a = significant (*p*<0.05) as compared to control, b= significant (*p*<0.05) as compared to Ibuprofen, HSEO: *H. spicatum* essential oil, Site-I= Nainital, U.K. collection, Site-II= Renuka, Dadahu, H.P. collection.

Antinociceptive assay

In present communication the potential antinociceptive properties of the essential oils of *Hedychium spicatum* were studied in chemical (acetic acid writhing test) and thermal (hot plate test) methods of nociception. HSEO (Site-I and Site-II) showed significant (p<0.05) antinociceptive activity in a dose dependent manner using acetic acid induced writhing test. The antinociception percentage for the doses ranges as 33.70 % at 50 mg/kg and 40.46 % at 100 mg/kg b. wt. of HSEO Site-I and 30.34 % at 50 mg/kg and 42.39 % at 100 mg/kg b. wt. of HSEO Site-II compared to standard drug ibuprofen (43.08 % inhibition) (Table VII), which revealed significant antinociceptive activity in HSEO of Site-II. Using hot plate test method, HSEO (Site-I and Site-II) also exhibited a significant (p<0.05) antinociceptive activity (Table VIII). Discomfort reaction time in indomethacin treated mice was maximum at 60 min in both the essential oils.

Antipyretic assay

The antipyretic property of HSEO (Site-I and Site-II) has been presented in Table IX. A noticeable increase in rectal temperature was produced 18 h after the injection of brewer's yeast to mice. Both the oils at different dose level reduced the rectal

Table VI. Effect of HSEO of Site-I and Site-II on formaldehyde induced subacute inflammation.

Group	Dose (mg/kg)	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± 0.05	2.16± 0.06
Site-I (III)	50	2.08± 0.08	2.32± 0.10	2.37± 0.13 [a]	2.34± 0.04	2.35± 2.26 [a]	2.29± 0.07 [a]	2.29± 0.06	2.27± 0.04	2.25± 0.13	2.12± 0.11	2.15± 0.09
Site-I (IV)	100	2.09± 0.06	2.22± 0.06 [a]	2.38± 0.03 [a]	2.33± 0.05	2.25± 0.02 [a]	2.26± 0.03	2.17± 0.11 [b]	2.11± 0.05[a,b]	2.12± 0.06	2.11± 0.06	2.11± 0.06
Site-II (V)	50	2.06± 0.08	2.10± 0.21 [a]	2.41± 0.09[a,b]	2.39± 0.09	2.34± 0.08 [a]	2.33± 0.06 [a]	2.29± 0.06	2.27± 0.05	2.23± 0.05	2.17± 0.05	2.13± 0.08
Site-II (VI)	100	2.08± 0.04	2.14± 0.07	2.25± 0.09	2.23± 0.12[a,b]	2.19± 0.12 [b]	2.20± 0.09 [b]	2.15± 0.08 [b]	2.14± 0.07[a,b]	2.11± 0.06	2.10± 0.07	2.10± 0.06

a= significant (p<0.05) as compared to control, b = significant (p<0.05) as compared to Ibuprofen, HSEO: *H. spicatum* essential oil, Site-I= Nainital, U.K. collection, Site-II= Renuka, Dadahu, H.P. collection.

temperature right from 1 h onward. Maximum inhibition was observed at 3 h. HSEO (Site-I and Site-II) showed good to moderate antipyretic effect and pyrexia was recorded to reduce after the oil dose injections. Oral administration of paracetamol (33 mg/kg) significantly reduced the temperature. The reduction in temperature by the essential oils varied in a dose dependent manner. In negative control mice did not show any reduction in the body temperature on oral administration of saline.

DISCUSSIONS

The GC/MS analysis of *H. spicatum* essential oil has been compared with earlier reports by different workers and previous study by us. The result revealed that there was a qualitative and quantitative difference in HSEO composition. Previous studies indicated eucalyptol (1,8-cineole) as the marker compound of this family, while in present study eucalyptol (44.0 %) and camphor (35.6 %) has been identified as the major constituents in Site-I and Site-II respectively. Camphor was reported to be the dominating compound (27.3 %) in the previous report from our lab (Rawat et al. 2019) however it has not been reported in earlier reports except by Sabulal et al. 2007 in which the constituent has been reported to contribute only 0.4 % of the total oil. The compounds totally missing in earlier reports and identified in present study includes Δ3-carene, aromadendrene, isobornyl acetate, isobornyl formate, δ-carvone, β-selinene and cubenol. The variation in qualitative and quantitative makeup of the constituents in present investigation with earlier reports might be possibly due to the edaphic factors, climatic conditions or may be due to change in altitude.

Koundal et al. 2015 reported the antioxidant activity of the essential oils from rhizomes of *H. spicatum* collected from Himachal Pradesh in different seasons and reported the activity to be significantly high in December collection (IC_{50} = 39.02 µL/mL) and least in February collection (IC_{50} = 63.03 µL/mL). The antioxidant

Table VII. Antinociceptive activity of HSEO of Site-I and Site-II (Writhing effect).

Groups	Treatments	Dose (mg/kg)	No of Writhings	% Writhings	% Inhibition
I	Control (Saline Water)	0.2 ml	217.00±1.26	100	-
II	Ibuprofen	40	123.5±3.44	56.91±0.10	43.08±0.10
III	Site-I	50	144.82±0.74	66.22±0.10	33.70±0.01[a]
IV	Site-I	100	130.15±0.74	59.50±0.11	40.46±0.10[a]
V	Site-II	50	151.16±0.75	69.65±0.02	30.34±0.11[a]
VI	Site-II	100	125.00±1.89	57.60±0.01	42.39±0.02

a = significant ($p<0.05$) as compared to Ibuprofen, HSEO: *H. spicatum* essential oil, Site-I= Nainital, U.K. collection, Site-II= Renuka, Dadahu, H.P. collection.

activity in the essential oils of *H. spicatum* was also reported by many researchers but was not expressed in terms of their IC_{50} values (Joshi et al. 2008, Rawat et al. 2011, Rawat et al. 2014). Other species of *Hedychium* viz., *H. coronarium* and *H.aurantiacum* have also been reported to possess antioxidant potential (Joshi et al. 2008, Kumar et al. 2017). In present study all the essential oils showed antioxidant activity in a dose dependent manner compared to the standard antioxidant. The DPPH radical scavenging activities of HSEO (Site-I and Site-II) determined at selected dose level with respect to standard antioxidant showed good to moderate activity. The antioxidant activity in oils might be due to the presence of electron donors viz; various oxygenated terpenoids in essential oils and presence of double bonds in terpenoids. Antioxidant property of eucalyptol, linalool, camphor, borneol, spathulenol, α-terpinene and *E*-β-farnesene has also been reported (Horvathova et al. 2014, Shata et al. 2014). These compounds contribute major composition of the essential oils hence it may be concluded that the antioxidant activity in HSEO (Site-I and Site-II) might be possibly due to the presence of these compounds or by synergetic activity of other phytoconstituents present in the oils.

Camphor (35.6 %), the major constituent in HSEO of Site-II possibly being responsible for the potent antifungal activity of this important medicinal plant as substantiated by earlier reports on antifungal activity of camphor (Marei et al. 2012). It has been reported that the essential oils exhibit fungitoxicity due to the presence of various types of monoterpenoids and sesquiterpenoids. Eucalyptol (1,8-cineole), carvone, linalool, borneol, terpene-4-ol, *E*-nerolidol, caryophyllene oxide and α-pinene have also been reported to possess antifungal activity (Morcia et al. 2012, Dias et al. 2018). HSEO (Site-I and Site-II) in present study is also loaded with the above mentioned bioactive terpenoids. Based on the published data it can be inferred that the antifungal activity in HSEO (Site-I and Site-II) might be due to the presence of above-mentioned compounds or by synergetic activity of other phytoconstituents present in the complex mixture of essential oils.

The extracts of *Hedychium spicatum* rhizomes are reported to possess anti-inflammatory activity against carrageenan-induced hind paw oedema in rat and mice but however no reports exist on *H. spicatum* essential oil. Hexane soluble extract has been reported to show maximum activity of 42.16 %

Table VIII. Antinociceptive activity of HSEO of Site-I and Site-II (Hot Plate Method).

Groups	Dose (mg/kg)	Hot plate reaction time (min)				
		0	30	60	120	150
Saline water (I)	**0.02 mL**	3.01±0.00	3.11±0.01	2.95±0.01	2.84±0.02	3.03±0.02
Indomethacin (II)	**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]
Site-I (III)	**50**	2.89±0.07	3.09±0.05[b]	3.24±0.08[a,b]	3.48±0.12[a,b]	3.03±0.10[b]
Site-I (IV)	**100**	2.93±0.04	3.03±0.03[b]	3.51±0.28[a,b]	3.84±0.03[a,b]	3.06±0.05b
Site-II (V)	**50**	2.92±0.04	3.34±0.41[a,b]	3.11±0.03[a,b]	2.60±0.30[a,b]	2.65±0.01[a,b]
Site-II (VI)	**100**	3.36±0.19	3.36±0.04[a,b]	3.40±0.03[a,b]	3.13±0.03[a,b]	2.86±0.12[a,b]

a= significant (*p*<0.05) as compared to control, b = significant (*p*<0.05) as compared to Indomethacin, HSEO: *H. spicatum* essential oil, Site-I= Nainital, U.K. collection, Site-II= Renuka, Dadahu, H.P. collection.

anti-inflammation in mice (200 mg/kg) and 27.2 % in rats (100 mg/kg) compared to 37 % in indomethacin (2.0 mg/kg) in mice and 27.2 % in phenylbutazone (30 mg/kg) in rats (Srimal et al. 1984). The hexane and benzene fractions of *Hedychium* have been reported to inhibit the granuloma formation by 8 and 5 %, respectively, at a dose level of 200 mg/kg compared to phenylbutazone (30 mg/kg) by 25 %. Similarly, the ethanolic extract (300 mg/kg) has been reported to significantly reduce the oedema volume (64.2 %) compared to 49.1 % in acetyl salicylic acid (300 mg/kg) (Tandan et al. 1997). Eucalyptol, camphor, linalool, borneol, *E*-nerolidol, caryophyllene oxide and α-pinene have also been reported to possess *in-vivo* anti-inflammatory activity (Martins et al. 2017, Silva-Filho et al. 2014). HSEO (Site-I and Site-II) are also comprised of these bioactive compounds hence the evidence-based literature substantiates that the anti-inflammatory activity in HSEO (Site-I and Site-II) might be due to the presence of above-mentioned compounds or by synergetic activity of other phytoconstituents present in the oils. The carrageenan induced anti-inflammatory activity of *Hedychium spicatum* Sm. can be probably due to its interference in production of prostaglandins and inhibition of cyclo-oxygenase pathways.

The acetic acid writhing test validates the peripheral analgesic characteristics of constituents/essential oil and indicates the binding of natural essential oil constituents to peripheral receptors in the peritoneum (Collier et al. 1968). Hot plate test confirms the involvement of components/essential oils showing antinociceptive activity by increasing the latency to discomfort in hot plate by activating the periaqueductal gray matter (PAG) to release endogenous peptides which then after inhibits the transmission of pain impulse at the synapse in the dorsal horn of spinal cord (Sulaiman et al. 2008). Various terpenes including eucalyptol, α-pinene, β-pinene, carvacrol, linalool, borneol, terpene-4-ol, *E*-nerolidol, caryophyllene and caryophyllene oxide have been reported to possess antinociceptive properties (Liapi et al. 2007, Damasceno et al. 2017). The present investigation on HSEO (Site-I and Site-II) are in total agreement with the bioactive constituents

Table IX. Effect of HSEO of Site-I and Site-II on yeast induced pyrexia in mice.

Groups	Treatment	Dose (mg/kg)	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	Saline	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	Site-I	50	37.65±0.03	38.86±38.91	38.27±0.06^{a,b} (50.66±10.04)	38.14±0.03^{a,b} (52.23±22.96)	37.85±0.07^{a,b} (83.40±8.09)
IV	Site-I	100	37.55±0.03	38.87±0.01	37.66±0.03^{a} (91.90±3.28)	37.45±0.04^{a,b} (108.08±3.78)	37.35±0.03^{a,b} (115.05±2.89)
V	Site-II	50	37.43±0.10	38.74±0.04	38.03±0.04^{a,b} (54.32±5.69)	37.98±0.04^{a,b} (68.74±13.88)	37.89±0.03^{a,b} (83.68±10.22)
VI	Site-II	100	37.65±0.04	38.91±0.09	37.83±0.39^{a,b} (86.39±3.80)	37.81±0.04^{a,b} (88.22±4.78)	37.72±0.08^{a,b} (94.02±6.78)

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 Paracetamol, HSEO: *H. spicatum* essential oil, Site-I= Nainital, U.K. collection, Site-II= Renuka, Dadahu, H.P. collection.

as reported above for their antinociceptive activity.

Eucalyptol (1,8-cineole), camphor and caryophyllene oxide have been reported to possess antipyretic property (Martins et al. 2017, Damasceno et al. 2017). In present study these compounds might be responsible for *Saccharomyces cerevisiae* induced anti pyretic activity or the synergic combinations of minor and trace terpenoids compositions of essential oils.

CONCLUSIONS

The present communication reveals the variation in essential oil composition of *Hedychium spicatum* collected from different altitudinal locations viz., Nainital, Uttarakhand and Renuka, Himachal Pradesh in India. Both the oils were found to be rich in oxygenated sesquiterpenes, hydrogenated sesquiterpenes and oxygenated diterpenes. The essential oils exhibited antifungal activity which might be possibly due to thepresence of several phytoconstituents. Thus, it can be concluded that oil of this important herb can be used for the development of natural fungicides, after proper screening and clinical studies. The ability of *Hedychium spicatum* to reduce the volume of paw edema validates the essential oil's ability to regulate the inflammatory mechanisms, and thus helping in the natural treatment of inflammatory swelling, ulcers, sores, glandular swelling, wounds and cuts. The essential oils of *H. spicatum* are also able to reduce the oxidative stress by scavenging the reactive oxygen species like free radicals, hydroxy free radicals, superoxide free radicals, alkoxy free radicals and non-radicals like hydrogen peroxides etc and can be helpful to cure various pathological conditions. Based on these findings, the herb *Hedychium spicatum* being a rich source of biologically active oxygenated terpenoids, can be utilised for the development of natural antioxidants, anti-inflammatory, anti-nociceptive and anti-pyretic drugs. Besides, the generation of

evidence-based database for future research and judicious *in-situ* exploitation of the herb.

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