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Chemical composition, herbicidal, antifeedant and cytotoxic activity of *Hedychium spicatum* Sm.: A Zingiberaceous herb

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ABSTRACT

Essential oils have been used traditionally as herbal medicine in various ailments and can be used as a strong alternative to chemical pesticides. The yield of essential oil from *Hedychium spicatum* Sm. was 0.9 mg kg^{-1} regarding its fresh weight. The essential oil was then subjected to column chromatography for separation into polar ethyl acetate and non-polar hexane fractions. The chemical composition of hexane and ethyl acetate column fraction were determined by gas chromatography-mass spectrometry. In the present investigation, the marked effect of anticancer activity from hexane and ethyl acetate fraction of oil of *H. spicatum* Sm. rhizomes on A431 and MCF cell lines was assessed through micro-culture tetrazolium assay (MTT). The test samples were screened for cytotoxicity against the cell lines at different concentrations of 50, 100, 150 and 200 µL to determine the IC_{50} value. The hexane and ethyl acetate fractions were screened for their herbicidal activities against *Raphanus raphanistrum* and also assessed for the feeding performance of *Spilosoma obliqua* in the laboratory. For antifeedant activity, the antifeedant index calculated over 36 h for neonate larvae increased significantly with concentration in the treated diet. The sprout inhibition activity on *Raphanus raphanistrum* was studied using petri dish bioassay. The root and shoot length were measured for each concentration after evaluating the activity for 5 days. Based on all the observations, the results indicate that the different fractions of essential oil contain phytotoxic compounds that could be used to develop botanical pesticide and also in the field of cancer drug development.

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1. Introduction

Medicinal plants are presumed to be useful in the indigeneous system of traditional medicine for decades. Herbal medicines have been used since ancient times by people in India and other parts of the world. The therapeutic and effective properties of medicinal plants are remarkably pleasant. Due to noxious side effects of various conventional synthetic drugs (Mohammadhosseini et al., 2017; Mohammadhosseini et al., 2019), the search of new aromatic and medicinal drugs is gaining its focus in the present scenerio. Aromatic plants are natural source of essential oils and secondary metabolites. The essential oils account for the known phytochemical and biological properties of the plants. They are considered to be the secondary metabolites, obtained by hydrodistillation procedures and consist of a variety of volatile molecules such as terpenes and terpenoids, phenol-derived aromatic and aliphatic components, esters, saturated and unsaturated hydrocarbons, alcohol, terpenes, aldehydes, ketones and oxides which produce distinctive perfume (Mohammadhosseini, 2017). Various reports in literature describe the chemical compositions and biological properties of medicinal plants in treating a variety of diseases and also their uses in various insecticidal and pesticidal formulations. Owing to their strong fragrance and medicinal properties, the essential oils are used

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as antiseptic, antimicrobial, analgesic, sedative, anti-inflammatory and splasmolytic remedies (Rawat et al., 2018; Sarker and Nahar, 2018; Wansi et al., 2018; Wansi et al., 2019; Venditti et al., 2018). Work is still being carried on to validate Ayurveda as a well-defined alternate and complementary system for pharmacovigilance of conventional herbal medicines (Rawat et al., 2018).

The genus *Hedychium* (Zingiberaceae) is distributed in Asia and Madagascar and grows as herb of perennial tuberous rootstocks. Various species of this genus are used in traditional medicines for the treatment of asthma, bronchitis, blood purification, gastric diseases, as anti-emetics, as well as eye diseases especially among the hill tribes of Uttarakhand (Joshi et al., 2008). *H. spicatum* Sm. rhizome is described as shati in Ayurvedic texts (Ghildiyal et al., 2012). The plant has been used in various treatments of CNS depressant, analgesic, stomachache, carminative, bronchodilator stimulant and toxic, cough, wound ulcer, fever, respiratory problems, hiccough, cancer, AIDS etc. (Rawat et al., 2018). The antioxidant, antimicrobial, antidiabetic, anthelmintic, antiasthmatic, hepatoprotective, antiemetic, antidiarrheal, analgesic, expectorant, anti-inflammatory, emmenagogue, antifungal, hypoglycaemic, insect repellent, hypotensive, pediculicidal and cytotoxic activities have also been reported in *H. spicatum* Sm. (Asolkar and Chopra, 1992; Gopanraj et al., 2005; Bhatt et al., 2008). The essential oil from rhizomes of *H. spicatum* Sm. is reported to show insect repelling property (Jadhav et al., 2007). Various traditional and ethanobotanical uses of *H. spicatum* Sm. rhizomes in India are listed in Table 1.

Synthetic insecticides have been commonly used for controlling the pest growth and thus keeping pest populations below the economic approach. These synthetic pesticides tremendously increase the crop production. However, continuous use of these conventional insecticides has become the root cause of developing the more resistant individuals; therefore increasing serious environmental problems such as hormonal imbalance, polluted ground water and ozone depletion due to their use (Montenegro et al., 2018). The interest in medicinal and aromatic plants and their products is growing rapidly in recent years in the developing countries as well as in the developed countries, mainly due to their better acceptability, compatibility, minimal negative effects and inexpensivity (Pal and Shukla, 2003). *Spodoptera littoralis* (Lepidoptera: Noctuidae), a cotton leafworm, is one of the most destructive pests in many countries. A wide range of vegetables and crops including soybean, cotton, alfalfa, peanut, potato, pepper and tomato are injured by this insect (Magd and Gengaihi, 2000; Kandil et al., 2003; Adham et al., 2009). *H. spicatum* Sm., a ginger lily is tremendously present near front line of Himalayan region. This plant is a good source of local medicines for villagers since time immemorial. Hence, antifeedant properties of this plant have shown very good protection against most notorious insect pests of pulse crop, *S. obliqua*, in future formulation of this plant product which may play an important role in integrated pest management of many crops. *Spilosoma obliqua* Walker, commonly known as Bihar hairy caterpillar (Lepidoptera: Arctiidae) is a polyphagous, destructive pest responsible for damaging a wide range of field crops, vegetables, medicinal and ornamental plants (Tandon et al., 2009). This is a key plant pest in the northern plains of India that attacks more than 20 cash crops (Butani and Juneja, 1984), 2nd and 3rd in stars of the insect feed gregariously on the plants often leading to its death (Sadek, 2003). Weeds are considered as serious enemy of crop production, because of their interference with crop functions and suppressing their growth and development. Reduction in the yield by weeds is usually more than the losses caused by disease, pests and insects (Jabran et al., 2015). In recent decades, synthetic herbicides have been used to eliminate weeds; however, their use has begun to be questioned due to their toxicity in animals, plants, human health and the environment (Ben El Hadj et al., 2015; Jalaei et al., 2015; Kleinowski et al., 2016). New herbicides with various modes of action are need of today's scenario for crop production because of resistance developed by weed against synthetic herbicides. Natural compounds can be a novel source for the same as they form structural diversity and evolved biological activities (Dayan and Duke, 2014). The use of biochemical herbicides is a promising, economically and environmentally sustainable alternative. The search for new potential bioactive compounds in medicinal plants is a realistic and promising strategy for prevention and cure of many deadly diseases. The excessive use of essential oils in pharmaceutical industries including antiseptics, soaps, deodorants, flavours, colouring and dentistry products increases the possibility of necessitating

Table 1

Traditional and ethanobotanical uses of *Hedychium spicatum* Sm. in India (Prakash et al., 2016; Rawat et al., 2018).

Plant part	Traditional use
Rhizome powder	inflammation, pain, asthma, foul breath, vomiting, diarrhoea, bronchitis, hiccough and blood diseases Asthma, bronchitis, eye diseases, gastric diseases, tonic and blood purifier
Whole herb	Spices and medicines
Rhizome powder	Poultice for various acnes and pains
Decoction of rhizome	Carminative, digestive and emmenagogue disorders
Rhizome powder	Hiccough, vomiting, tridosha and blood diseases
Rhizome paste	Hair loss

Fig. 1. *Hedychium spicatum* Sm. herb cultivated in Pantnagar (Photograph) (A-B) aerial part and rhizomes.

research on their cytotoxicity conditions (Prashar et al., 2004). The present report reveals the composition of essential oil from *H. spicatum* Sm. rhizomes, its herbicidal activity on Radish (*Raphanus raphanistrum*) seeds and the antifeedant activity against the Bihar hairy caterpillar, *Spilosoma obliqua* Walker. The results from this study can be used to support the application of *H. spicatum* Sm. essential oil as a potential product for further discovery as a botanical pesticide. These compounds have the potential for further development as a botanical pesticide. However, therapeutic usage of the oil may require higher dosages. So, we evaluated the oils in order to study the cytotoxicity in A431 and MCF cell lines at concentrations showing significant activities.

2. Experimental

2.1. Collection of plant material

The cultivated plant material (rhizomes) were collected from Tarai region of Pantnagar Uttarakhand in the month of July and August, 2018 (Fig. 1). The plant was taxonomically identified with a herbarium number of GBPUH - 986 / 20-5-2019 by Dr. D.S. Rawat, Assistant Professor (Plant Taxonomist), Department of Biological Sciences, College of Basic Sciences and Humanities, Pantnagar. The sampling area, Pantnagar lies in the longitudinal and latitudinal coordinates of 29º1'15.74" N and 79º29'23.06" E respectively lying at an elevation of 344 m above mean sea level.

2.2. Isolation of essential oil

Essential oils from rhizomes of the *H. spicatum* Sm. were isolated using hydrodistillation method in a Clevenger apparatus (Clevenger, 1928). The rhizomes were cut into small size pieces, crushed and hydrodistilled for 7-8 h. The oil was extracted with hexane and desiccated over anhydrous sodium sulphate. The solvent was evaporated under vacuum and the obtained essential oil was stored at a low temperature for further use.

2.3. Extraction of polar column fraction of oil

The oil was subjected to column chromatography for separation of its major constituents. Accordingly, 15 g of the essential oil was loaded on the column (60 cm × 2.5 cm) packed with silica gel (60-120 mesh) in hexane. Firstly, the column was eluted with 100% hexane and then 100% ethyl acetate in order to separate the polar molecules. The polar fraction of ethyl acetate was used for the biological activity. The separation of polar compounds from non-polar compounds were monitored by TLC.

2.4. Analysis of the oil components

The composition of essential oil and polar column fraction from *H. spicatum* Sm. rhizomes were analyzed by gas chromatography-mass spectrometry (GC-MS) using a GC MS-QP 2010 Plus equipment gas chromatograph. EI (70 eV) was used for this analysis. The carrier gas was He, with a flow rate of 1.21 mL/min and a linear velocity of 39.9 cm/s. A 1 µL sample was injected with a split ratio of 22.0. The injection and detector were maintained at temperatures of 260 ºC and 280 ºC, respectively. The oven temperature was programmed as 50 ºC to 210 ºC at 3 ºC/min, isothermal for 2 min, and then increased to 280 ºC at 6 ºC/min, with final isothermal status for 11 min. The compounds of essential oil and column fraction were identified by comparing the Kovats index of peaks on DB-5 column with literature values and comparing data with NIST-MS library (Adams, 2007).

2.5. Herbicidal activity of essential oil and polar column fraction

Herbicidal activities of *Hedychium spicatum* Sm. rhizome essential oil (HSREO) and *Hedychium spicatum* Sm. rhizome column fraction (HSRCF) on seed germination and seedling growth were determined by petri dishes bioassay. Activated and fungus treated seeds of Radish (*Raphanus raphanistrum*) were collected from Vegetable Research Centre, Pantnagar. In order to insure the germination capability of the seeds, the seeds were kept dipped in water for around 24 h. The essential oil and the column fraction were used in pure form with some trace amount of DMSO to solubilize in water for herbicidal applications. To test the herbicidal activity of HSREO and HSRCF, 2 mL of different formulation concentrations (50, 100,150, and 200 µL) were added to petri dishes lined with two sheets of germination paper (JProlab®), and 10 seeds of *R. raphanistrum* were placed in each dish. A solution

of DMSO in distilled water served as the control. Three petri dishes for each treatment were arranged as replicates in a completely randomized design. The experiment was conducted at room temperature conditions of 25-28 ºC. The germination rate and the lengths of both root and shoot were measured at five days after treatment. Seed inhibition and germination was measured at the intervals of 24, 48, 72, 96 and 120 h after exposure for 10 seeds per treatment according to a previously described method of Turk and Tawaha (2003). Percent seed inhibition was calculated as per the following formula (Eqn. 1):

Seed inhibition (%)=[(Seeds grown in control-Seeds grown in treatment)/Seeds grown in control] * 100 (Eqn. 1)

2.6. Antifeedant activity

Larvae of *Spilosoma obliqua* Walker were reared in the laboratory after collecting them from the soybean fields. First-generation progeny (Fj) were used for all the bioassays at the normal conditions. HSREO and HSRCF were also prepared of 50 ppm, 100 ppm, 150 ppm, 200 ppm and 250 ppm in water. Tween-20 was used as the stabilizing agent in all the prepared samples. Fresh soybean (*Glycine max*) leaves were dipped in oil samples of different concentrations and then shade dried for 10 min. One 2nd instar larvae was kept in different concentration samples after keeping them starved for 24 hours. Three replicates for each sample concentration were showcased. Data on leaf area consumed were plotted on graph sheets after 12 h, 24 h and 36 h and subjected to analysis for antifeeding activity. The antifeedant activity was calculated by the formula given by Sadek (2003) (Eqn. 2).

Antifeedant activity (%)=[(Leaf area consumed in control-Leaf area consumed in treatment)/(Leaf area consumed in control+Leaf area consumed in treatment)]*100 (Eqn. 2)

2.7. Cytotoxicity study of oils

2.7.1. Subculturing of the cell lines

The cytotoxicity assay of HSREO and HSRCF was carried out using A431 cells (epidermal) and MCF cell lines (lung). The human cell lines were obtained from the National Centre for Cell Sciences (NCCS, Pune, India). The A431 and MCF cell lines were grown in DMEM media enhanced with foetal bovine serum (10%), pencillin (100 µ/mL), streptomycin (100 g/mL) and amphotericin B (5 g/mL) as recommended. Cells were then transferred aseptically to the tissue culture flask containing 7 mL of complete growth medium aseptically and incubated in CO_2 incubator (Heraeus, Germany) at 5% CO_2 atmosphere and 90% relative humidity, 37 ºC until reaching a uniform distribution. The cell lines were detached from the growth surface of the culture flask and then reinoculated into a fresh medium in a new culture flask whose growth medium had been changed a week in advance. A minimum amount of trypsin-EDTA was added to make a thin layer and incubated at 37 ºC for 5 min. After detachment, cell suspension was made with complete growth medium. An aliquot was taken out to count the cells with the help of haemocytometer. Trypan blue was used to check the cell viability. More than 98% of cell viability of the cell stock was to be accepted for determination of *in vitro* cytotoxicity.

2.7.2. Preparation of test solutions

The solutions of HSREO and HSRCF were prepared by dissolving in dimethyl sulphoxide (DMSO). The DMSO has been found to have no effect on the cell lines below 1% v/w. Different concentrations (50, 100, and 200 µL) were dissolved in minimum amount of DMSO and then completed growth medium containing 50 µg/mL of gentamycin to obtain working test solutions, as per the requirement. To obtain required concentration, further thinned with DMEM enriched with inactivated FBS (2%) to attain clean solution. A sequential 2-fold dilutions were primed from the stock for cytotoxicity studies. The experiments were conducted in triplicates.

2.7.3. Cell viability determination by MTT assay

This study checks for the ability of the cells to persist a poisonous dose. Reduction of 3-(4,5-dimethylthiasol2-yl)-2,5-diphenyltetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase into a blue colored product called as formazan is measured spectrophotometrically (Pham et al., 2018). The quantity of viable cells has been reported to be proportional to the extent of formazan produced (Hussain et al., 1993). The A431 and MCF cell lines were cultured monolayerlike on DMEM with 10% FBS solution. The cells were trypsinized and the cell count was adjusted to 1.0×10^5 cells/mL. 100 µL of cell suspension was added to 96-well microtiter plate and incubated for 24 h resulting in the formation of partial monolayer. The supernatants were removed and the cell layer was washed with the medium. To the wells, different concentrations of oil and polar fraction were added and incubated for 3 days at 37 ºC with 5% CO_2 (growth condition). After 3 days, the supernatant (extracts solution) from the wells were flipped off, to this 50 µL of MTT prepared in PBS was added. The plates were shaken and maintained in same growth condition for 3 h. Thereafter, supernatant was segregated from the wells. The blue colored formazan crystals formed in the cells were dissolved with DMSO (100 µL/mL) and shaked gently. The results were obtained by measuring the optical density using a microplate reader at 540 nm. The percentage of growth inhibition was calculated using the following formula (Eqn. 3), and the concentration of a test drug needed

to inhibit cell growth by 50% (CTC50) was determined from a dose-response curve for each cell line.

Growth inhibition (%)=100-[OD in test sample/OD in control]*100 (Eqn. 3)

2.8. Statistical analyses

Each experiment was arranged in a Completely Randomized Design with three replicates for four to five concentrations in both of the samples. Raw data were analyzed using double factor CRD (ANOVA); the mean values and standard deviation (SD) were calculated by statistical analysis. Percentage data were subjected to angular transformation (Snedecor et al., 1968).

3. Results and Discussion

3.1. Identification of the chemical composition of HSREO and HSRCF

Hydrodistillation of fresh *H. spicatum* Sm. rhizomes gave a dark purple essential oil (HSREO) with a yield of 0.9 mg.kg^{-1} with respect to its fresh weight. The polar ethyl acetate column fraction (HSRCF) had an orange colour. Both of the oils of HSREO and HSRCF obtained from *H. spicatum* Sm. contained a complex mixture of terpenoids with higher amounts of monoterpenes.

Sixty-three constituents constituting 96.6% of the total oil were identified in HSREO. Camphor (27.3%) was found to be major component of the oil followed by 1,8-cineole (12.2%), curdione (9.3%), isoborneol (7.1%), (+)-linalool (4.5%), (-)-borneol (3.9%), khusimone (4.6%), camphene (4.8%) and (*E,E*)-germacrone (4.3%). The other major compounds identified were (+)-α-terpineol (2.1%), curzerene (2.1%), camphene hydrate (2.0%), α-pinene (1.3%) and myrcene (1.2%). The column fraction of oil eluted in 100% ethyl acetate after pre-elution by 100% hexane (HSRCF) revealed the presence of forty-six constituents contributing to 95.8% of the total fraction. Camphor (30.0%) was found to be major component of the oil followed by curdione (14.5%), 1,8-cineole (9.5%), isoborneol (8.3%), (-)-borneol (4.4%), khusimone (3.0%), (+)-linalool (5.7%), (-)-terpinen-4-ol (2.4%), camphene hydrate (2.3%) and γ-elemene (2.0%).

In terms of class composition of terpenoids,

Table 2
Comparative analysis of composition of HSREO and HSRCF.

S. No.	Compound Name	K.I.	% Composition	
			HSREO	**HSRCF**
1	Heptan-2-ol	896	t	t
2	Tricyclene	926	0.2	-
3	α-Thujene	930	t	-
4	α-Pinene	938	1.3	-
5	**Camphene**	**963**	**4.8**	-
6	(+)-Sabinene	976	0.1	-
7	Myrcene	989	1.2	-
8	δ-2-Carene	1001	t	-
9	α-Phellandrene	1005	t	-
10	α-Terpinene	1018	t	-
11	**1,8-Cineole**	**1035**	**12.2**	**9.5**
12	β-Ocimene	1040	t	-
13	(-)-Terpinen-4-ol	1076	0.7	2.4
14	*trans*-Linalool oxide	1078	-	t
15	*m*-Cymene	1091	t	-
16	**(+)-Linalool**	**1098**	**4.5**	**5.7**
17	*n*-Undecane	1100	-	0.2
18	**Camphor**	**1141**	**27.3**	**30.0**
19	Camphene hydrate	1149	2.0	2.3
20	β-Pinene oxide	1159	0.6	-
21	**Isoborneol**	**1160**	**7.1**	**8.3**
22	**(-)-Borneol**	**1169**	**3.9**	**4.4**
23	*trans*-Furfuryl acetone	1183	-	t
24	**(+)-α-Terpineol**	**1189**	**2.1**	**-**
25	Verbenone	1205	-	t
26	(-)-*trans*-Isopiperitol	1208	t	-
27	*trans*-Carveol	1216	t	t
28	*cis*-Carveol	1229	-	t
29	(-)-Carvone	1243	0.1	0.2
30	δ-Elemene	1338	0.3	-
31	(-)-β-Elemene	1390	1.5	-
32	(+)-Sativene	1391	0.1	-
33	β-*cis*-Caryophyllene	1419	0.2	-
34	γ-Elemene	1436	-	2.0

Table 2 (Continued)

35	Aromadendrene	1454	t	-
36	α-Humulene	1454	0.3	-
37	*allo*-Aromadendrene	1460	t	-
38	Epiglobulol	1468	0.3	-
39	γ-Muurolene	1479	0.1	-
40	**Germacrene *D***	**1485**	**0.7**	**4.5**
41	α-Vetispirene	1490	t	-
42	α-Selinene	1498	-	0.1
43	Curzerene	1499	2.1	0.7
44	α-Muurolene	1500	t	-
45	*cis-Z*-α-Bisabolene epoxide	1507	-	0.1
46	Germacrene *A*	1509	0.4	-
47	δ-Cadinene	1523	0.3	-
48	*trans-Z*-α-Bisabolene epoxide	1529	-	t
49	10-*epi*-Cubebol	1535	t	0.2
50	Elemol	1549	t	-
51	Germacrene *B*	1561	0.8	-
52	Longipinanol	1569	0.1	-
53	Spathulenol	1578	0.5	-
54	Caryophyllene oxide	1583	0.1	0.3
55	β-Elemenone	1589	0.4	-
56	Globulol	1590	-	0.4
57	Hexadecane	1600	-	0.2
58	β-Elemenone	1602	-	0.7
59	**Khusimone**	**1604**	**4.6**	**3.0**
60	Dodecyl acetate	1607	-	t
61	Humulene epoxide II	1608	-	0.3
62	*trans*-Isolongifolanone	1626	0.1	0.2
63	(-)-Spathulenol	1630	t	1.0
64	τ-Cadinol	1640	t	0.3
65	β-Eudesmol	1649	-	t
66	α-Cadinol	1654	0.6	0.6
67	Neocurdione	1657	0.2	0.2
68	Atractylone	1658	-	t
69	Selina-4,11-diene	1659	t	-
70	Neo-intermedeol	1660	-	2.0
71	**(*E,E*)-Germacrone**	**1693**	**4.3**	**-**
72	Juniper camphor	1694	-	t
73	Heptadecane	1700	-	0.1
74	Curcumenol	1734	-	1.2
75	(−)-Curcumol	1754	1.2	-
76	β-Costol	1767	t	-
77	(+)-Lepidozene	1779	-	0.2
78	**Curdione**	**1792**	**9.3**	**14.5**
79	Cryptomeridiol	1813	t	-
80	Coronarin *E*	1836	-	t
81	1-Hexadecanol	1875	-	t
	TOTAL		**96.6**	**95.8**

HSREO = *H. spicatum* rhizomes essential oil; **HSRCF** = *H. spicatum* rhizomes polar column fraction of essential oil; **K.I.** - Kovats Indices, **GC**- Gas chromatography, **MS**- mass spectrometry, **t**- trace amount (contribution less than 0.1%).

HSREO was potentially rich in oxygenated monoterpenoids (60.5%). The oil was found to contain oxygenated sesquiterpenoids (19.2%), hydrogenated monoterpenoids (7.6%) and hydrogenated sesquiterpenoids (4.7%), whereas HSRCF was potentially rich in oxygenated monoterpenoids (63.5%). The oil was found to contain oxygenated sesquiterpenoids (27.2%) and hydrogenated sesquiterpenoids (2.5%). No trace amounts of hydrogenated monoterpenoids were found. The quantitative and qualitative analytical results of identified compounds by GC and GC-MS with their class composition classification are presented in Table 2 and gas chromatogram in Fig. 2(A) for HSREO and Fig. 2(B) for HSRCF.

Raina and Negi (2015) have also reported the essential oils from *H. spicatum* Sm. to be potentially rich in oxygenated monoterpenes (18.3-75.7%) followed by oxygenated sesquiterpenes (8.1-43.8%), sesquiterpene hydrocarbons (1.6-25.3%) and monoterpene hydrocarbons (0.9-10.0%). 1,8-Cineole, the marker compound of family Zingiberaceae, has been reported as the most abundant oxygenated monoterpenoid besides other monoterpenoid compounds like α-pinene, β-pinene, linalool, terpinen-4-ol and α-terpineol. Verma and Padalia (2010) have reported the chemical compositions of *H. spicatum* Sm. from two different

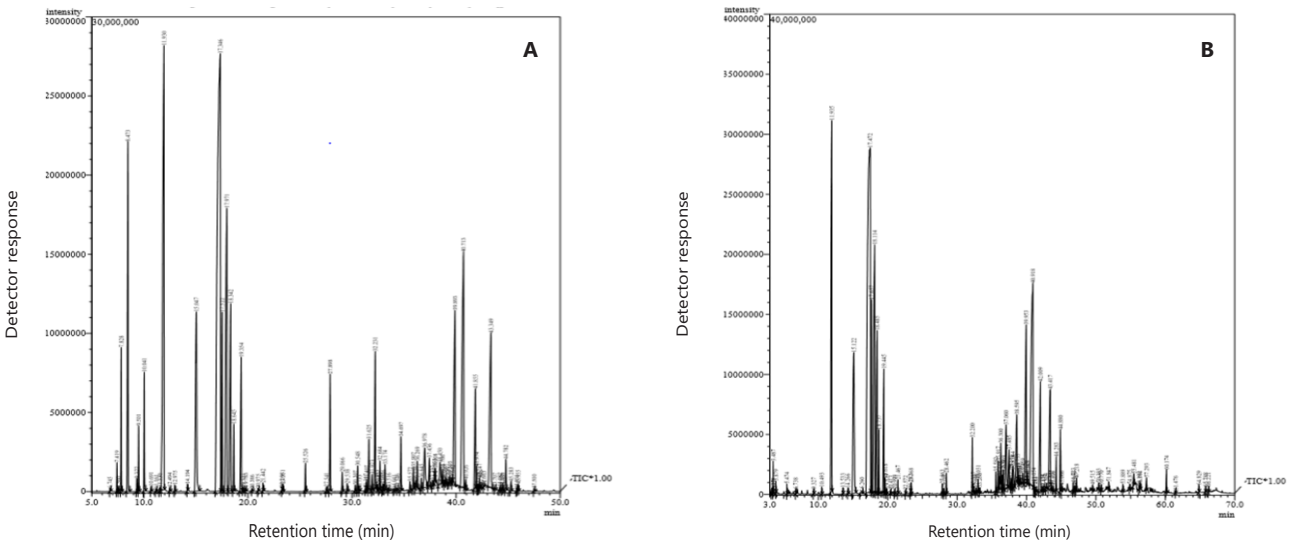

Fig. 2. (A) Gas chromatogram of HSREO and (B) Gas chromatogram of HSRCF.

sites, Song and Bhowali, Uttarakhand. The characterized oils accounted for the presence of monoterpenoids and oxygenated monoterpenoids involving 1,8-cineole and α-terpineol as major compounds in the roots essential oil. β-Pinene, α-pinene and camphene were the representing monoterpene hydrocarbons constituting the total oil content in leaf essential oil. Rhizomes essential oil has also been reported to be dominated by monoterpenoids and oxygenated monoterpenoids with maximum abundance of 1,8-cineole. Significant amount of sesquiterpene alcohol has been reported in rhizome oil as compared to that of root oil. Gopichand et al. (2017) have analyzed the chemical components of the volatile oil of cultivated *H. spicatum* Sm. and reported the dominant presence of oxygenated monoterpenes viz; 1,8-cineole, linalool (12.8%), β-pinene (10.1%), γ-terpinene (8.2%), terpinolene (5.0%), α-terpinene (3.8%) and α-terpineol (3.4%) as major compounds besides terpinolene, α-terpinyl acetate, γ-eudesmol, and γ-muurolene. 1,8-Cineole (17.6%), α-eudesmol (17%), 10-*epi*-γ-eudismol (9.7%), δ-cadinene (7.5%), cubenol (6.9%), germacrene D (6.8%) and γ-cadinene (5.4%) as major compounds from *H. spicatum* rhizomes collected wildly from Kumaun region of Central Himalaya have also been reported previously from our laboratory with comparative study as reported by various workers (Joshi et al., 2008; Prakash et al., 2010).

It has been reported that the oxygenated terpenoids like camphor, 1,8-cineole, curdione, isoborneol, (+)-linalool and (-)-borneol exhibit potent biological activities (Pavela, 2008; Goren et al., 2011). In order to study the biological activity of oxygenated terpenoids, the essential oil was fractioned into hydrocarbons and oxygenated parts by using column chromatography. To the best of our knowledge, no work has been reported in the literature dealing with the screening of chemical composition of the column fraction of the *Hedychium spicatum* essential oil.

A simple comparison of the present results with those reported from our laboratory and various workers revealed that the characterized essential oil composition in the present study has different qualitative and quantitative make up. The marker compound, 1,8-cineole, as reported by many researchers only constituted about 12.2% of the oil profile. However, the major compound identified in present oil was camphor (27.3%) which was totally missing in previous reports; hence it is a different chemotype of *H. spicatum* which may either be due to genetic variation or change in climatic conditions.

3.2. Seed germination and seedling growth bioassay

Several studies revealed that essential oils extracted from aromatic plants exhibit phytotoxicity (Muller et al., 1964; Kohli and Singh, 1991; Ahmad and Misra, 1994; Dudai et al., 1999; Scrivanti et al., 2003). Various researches confirm the strong inhibitory effects of oxygenated monoterpenes on weed germination. Inhibition of seedling germination by essential oils obtained from rosemary (*Rosmarinus officinalis* L.), thyme (*Thymus vulgaris* L.), and savory (*Satureja montana* L.) has been reported due to the presence of major constituents like 1,8-cineole, α-pinene and borneol (Angelini et al., 2003). The allelopathic influence on the seedling growth of fifty-two plant species and twenty-seven soil algal populations has been reported due to significant delay in germination and causing a reduction of radicle and shoot length, leaf area and leaf number (Schenk, 2009). The herbicidal properties of camphor, 1,8-cineole, borneol, α-terpineol and terpinen-4-ol found in the essential oils of *H. raphanistrum* are in total agreement with the studies reported by several authors (Vokou et al., 2003; Zunino and Zygadlo, 2004; Salamci et al., 2007; Kordali et al., 2008; Marei et al., 2012). Herbicidal activity of the oil can also be due to

synergistic effect of other major or minor constituents.

The effects of HSREO and HSRCF on the seed germination and seedling growth of *R. raphanistrum* seeds were tested by dose-response bioassay. The HSREO and HSRCF strongly inhibited both seed germination and seedling growth in a dose-dependent manner, with significantly higher effect on seedling growth (root and shoot length) than on seed germination. Seedling germination was completely inhibited at 200 ppm concentration of HSREO. HSRCF also showed excellent herbicidal activity at 150 and 200 ppm concentrations by completely inhibiting the germination. HSRCF inhibited the growth to 62.77% and 77.42% at 50 and 100 ppm concentration, respectively. HSREO at 150 ppm inhibited the growth by 70.59%. Seedling growth, with partial reduction at lower concentrations (50 and 100 ppm) and complete inhibition at the highest concentration of 150 and 200 ppm in the HSREO was observed, whereas HSRCF showed complete inhibition of seedling growth even at lower concentrations. HSREO rich in oxygenated monoterpenes, especially camphor and 1,8-cineole, exhibited strong inhibitory effects on the seed germination and seedling growth of *R. raphanistrum*. These results agree with literature on the inhibitory effect by essential oils against weed germination and seedling growth. It generally may be attributed to the allelopathic potential of some monoterpenes (De Martino et al., 2010; Amri et al., 2017). The herbicidal activity of HSREO and HSRCF has been presented in Table 3(a) and 3(b) and Fig. 3.

3.3. Root and shoot length growth inhibition bioassay

At fifth day of germination, the saplings were observed to record root and shoot length. Effects of the HSREO and HSRCF were very high in inhibiting the seedling germination, thus the effect on root and shoot growth inhibition activity was examined. Although both seedling root and shoot growth were inhibited, the effect was more marked in the case of HSRCF. HSRCF inhibited the root growth and shoot growth by 96% and 94% respectively at 50 ppm concentration but the maximum root and shoot growth inhibition (100%) was seen for all the higher concentrations. HSREO also inhibited the root and shoot length growth to 100% at 150 and 200 ppm (Table 4 and Fig. 4).

3.4. Antifeedant effects

Antifeedant activity was found to be dose-dependent. HSREO showed maximum antifeedant activity of 68.69% at 200 ppm and 92.89% at 250 ppm. HSRCF also showed excellent antifeeding activity of 63.01% and 92.79% at 200 ppm and 250 ppm, respectively. Antifeedant activity of HSREO and HSRCF has been presented in Table 5(a), 5(b) and Fig. 5. No antifeedal properties of *H. spicatum* Sm. oil has been reported against *S. obliqua* but major components like camphor, 1,8-cineole, curdione, isoborneol, (+)-linalool, germacrene D, (-)-borneol, khusimone and camphene are known to exhibit insecticidal property. Numerous

Table 3

(a) Herbicidal activity of HSRCF on *R. raphanistrum* in the laboratory conditions. (b) Herbicidal activity of HSREO on *R. raphanistrum* in the laboratory conditions.

a

CONC.	Percent Seed Germinated					Mean	Percent Inhibition
	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours		
50 ppm	00	2.71	7.33	12.13	12.13	6.86±5.47	62.77
100 ppm	00	00	00	10.40	10.40	4.16±5.69	77.42
150 ppm	00	00	00	00	00	00	100
200 ppm	00	00	00	00	00	00	100
Control	18.43	18.43	18.43	18.43	18.43	18.43±0	0
CD1(5%)	1.12						
CV	25.99						
CD2(5%)	1.12						
CV	25.99						
CD3(5%)	2.51						
CV	25.99						

Here, CD1=variation with respect to concentration, CD2=variation with respect to time, CD3=variation with respect to interaction between both, CV=coefficient of variance.

b

CONC.	Percent Seed Germinated					Mean	Percent Inhibition
	24 Hours	48 Hours	72 Hours	96 Hours	120 Hours		
50 ppm	7.33	9.26	11.74	12.13	13.16	10.72±2.37	41.83
100 ppm	1.91	4.62	9.54	11.28	11.28	7.72±4.24	58.11
150 ppm	1.91	1.91	7.15	7.67	8.46	5.42±3.23	70.59
200 ppm	00	00	00	00	00	00	100
Control	18.43	18.43	18.43	18.43	18.43	18.43±0	0
CD1(5%)	1.765						
CV	28.44						
CD2(5%)	1.765						
CV	28.44						
CD3(5%)	3.948						
CV	28.44						

Here, CD1=variation with respect to concentration, CD2=variation with respect to time, CD3=variation with respect to interaction between both, CV=coefficient of variance.

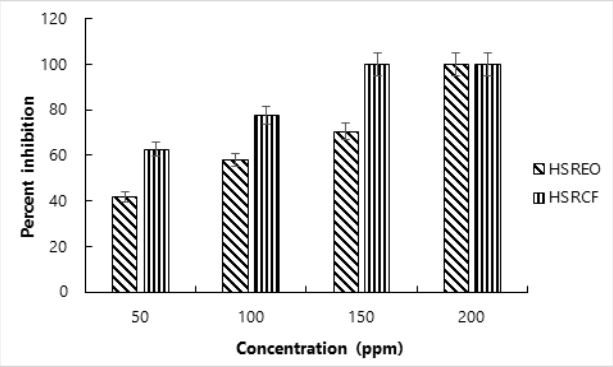

Fig. 3. Herbicidal activity of HSREO and HSRCF on *R.raphanistrumin* the laboratory conditions.HSREO=*Hedychium spicatum* rhizomes essential oil. HSRCF=*Hedychium spicatum* rhizomes column fraction.

studies have indicated that camphor has insect repellent activity against stored-product pests. Impregnation of camphor on the surface of grains and inside the grain kernels have been reported to the development of eggs in immature stages as well as the progeny emergence of *Sitophilus granarius*, *S. zeamais*, *Prostephanus truncates* and *Tribolium castaneum* (Obeng-Ofori et al., 1998). A report published by Rozman et al. (2006) confirmed that camphor, a pure compound, exhibited contact and fumigant activity against *S. oryzae* and *Rhyzopertha dominica*, and did not affect *Tribolium castaneum* at a dose of 0.1 µL/720 mL. Liska et al. (2010) have published that camphor along with 1,8-cineole and eugenol exhibited the highest mortality (78.5%) against *Tribolium castaneum*.

3.5. *In-vitro* anticancer activity

The search for new potential bioactive compounds in medicinal plants is a realistic and promising strategy for prevention and cure of many deadly diseases. In the present study, the marked effect of anticancer activity from HSREO and HSRCF on A431 and MCF cell lines was assessed through micro-culture tetrazolium assay (MTT). The test samples were screened for cytotoxicity against the cell lines at different concentrations of 50, 100, 150 and 200 µL to determine the IC_{50} value, i.e. the concentration (µg/mL) causing 50% cell death. HSREO showed moderate cytotoxicity against both the cell lines i.e., 76.51% for A431 cell lines and 78.08% for MFC cell lines. HSRCF was found to show potent cytotoxicity against both the cell lines i.e., 81.25% for A431 cell lines and 88.45% for MFC cell lines. The results indicated that the percentage of growth inhibition increased steadily with increasing concentrations up to 200 µg/mL (Table 6 and Fig. 6). Thus, the IC_{50} value of the assay was 22.345 µg/mL for essential oil against A431 cell lines and 20.445 µg/mL against MFC cell lines. The IC_{50} value for polar fraction was 20.49 µg/mL against A431 cell lines and 22.7 µg/mL against MCF cell lines (Fig. 7). This finding proposes that the decline examined in the viable cells after treatment with botanicals is due to cell death.

No cytotoxicity activity has been reported for HSREO and HSRCF against A431 and MCF cell lines in the literature search. In this study, we have focused to elucidate anti-cancer properties of *Hedychium spicatum* Sm. rhizomes and found that both HSREO and HSRCF showed very good cytotoxicity against the tested cancerous cell lines.

Each component present in the oil was not evaluated for anticancer activity against the tumor cell lines, therefore it is difficult to comment on which of these compounds would have been responsible for the observed effects. The anti-cancer activity can be the result of presence of major components present in the oil and the polar column fraction such as camphor followed by 1,8-cineole, curdione, isoborneol, (+)-linalool, germacrene D, (-)-borneol, khusimone, camphene and (*E,E*)-germacrone and also with the synergistic effect of minor components like (+)-α-terpineol, curzerene, camphene hydrate, α-pinene and myrcene. Camphor have been potentially demonstrated to enhance enzymatic breakdown of carcinogens and to increase radiation susceptibility of cancer cells (Chen et al., 2013). Yeh et al. (2009) reported the anticancer activity of the essential oil extracted from the leaves of camphor tree, *Cinnamomum kanehirae* which was abundantly rich in the composition of camphor. Moteki et al. (2002) observed specific induction of apoptosis by 1,8-cineole in human leukemia Molt 4B and HL-60 cells. Cha et al. (2010) utilized the essential oil of *Artemisia lavandulaefolia* to extract 1,8-cineole to investigate its property to induce apoptosis in KB cells. Murata et al. (2013) reported the anti-proliferative effect of 1,8-cineole HCT116 and RKO (human colon) cancer cell lines by WST-8 and Brdu methods. The proliferation was suppressed by the induction of apoptosis. Germacrene was found to induce and exhibit anti-cancer activity (Aggarwal et al., 2013). Curdione provided some molecular performance for inducing anti-cancer property and anti-tumour property against MCF-7 cells by inducing apoptosis (Li et al., 2014). Other major and minor components present in HSREO and HSRCF have also been reported to induce apoptosis and cytotoxic activity (Yan et al., 2005; Li et al., 2008; Hassan et al., 2010; Lakshmi et al., 2011; Aydin et al., 2013; Kong et al., 2013).

4. Concluding remarks

The present investigation showed that HSREO is characterized by the noticeable amount of oxygenated monoterpenoids, primarily camphor, 1,8-cineole, curdione, isoborneol, (+)-linalool, germacrene D, (-)-borneol, khusimone, camphene, (*E,E*)-germacrone, (+)-α-terpineol, curzerene, camphene hydrate, α-pinene and myrcene. HSRCF also revealed the presence of oxygenated monoterpenoids viz., camphor, curdione, 1,8-cineole, isoborneol, camphene, khusimone,

Table 4

Root and shoot length growth inhibition of *R. raphanistrum* seeds grown in HSREO and HSRCF calculated on fifth day.

CONC.	HSREO				HSRCF			
	Mean Root length(cm)	Mean Shoot length(cm)	Root growth inhibition	Shoot growth inhibition	Mean Root length(cm)	Mean Shoot length(cm)	Root growth inhibition	Shoot growth inhibition
50 ppm	7.15	7.55	70	47	0.78	0.78	96	94
100 ppm	2.70	2.70	89	81	00	00	100	100
150 ppm	00	00	100	100	00	00	100	100
200 ppm	00	00	100	100	00	00	100	100
Control	24.59	14.30	0	0	24.60	14.30	0	0
CD (5%)	2.76	2.36			1.13	1.21		
CV	22.07	26.50			12.25	22.21		

Results obtained using One-way ANOVA ($p < 0.05$), HSREO = *Hedychium spicatum* rhizomes essential oil, HSRCF = *Hedychium spicatum* rhizomes column fraction.

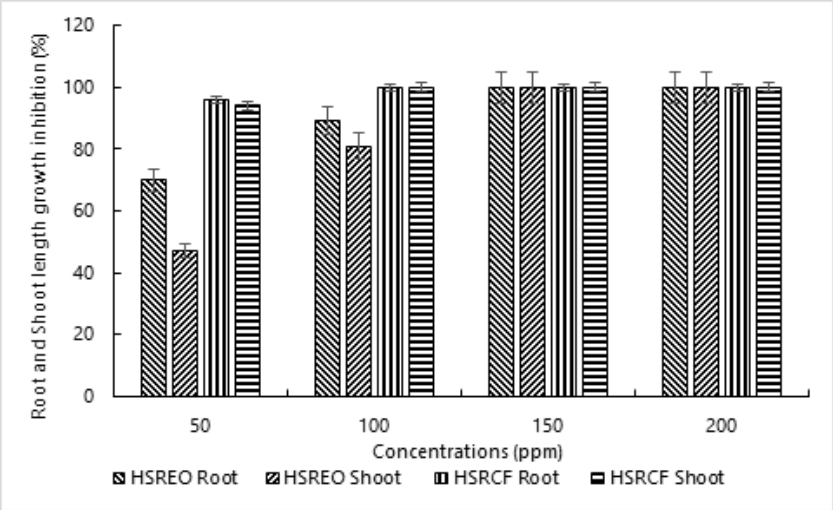

Fig. 4. Root and shoot length growth inhibition of *R. raphanistrum* seeds grown in HSREO and HSRCF calculated on fifth day. HSREO=*Hedychium spicatum* rhizomes essential oil, HSRCF=*Hedychium spicatum* rhizomes column fraction.

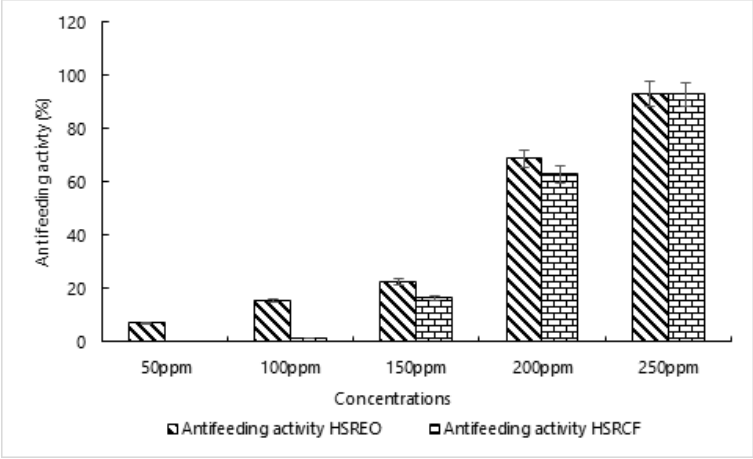

Fig. 5. Antifeedant activity of HSREO and HSRCF against *Spilosoma obliqua* in the laboratory conditions: HSREO=*Hedychium spicatum* Sm. rhizomes essential oil, HSRCF=*Hedychium spicatum* Sm. rhizomes column fraction.

(+)-linalool, (-)-terpinen-4-ol, camphene hydrate and γ-elemene.

Both the botanicals showed moderate anti-feedant against *S. obliqua* when feeded with soybean leaves. Herbicidal activity at 200 ppm also showed complete inhibition of the *R. raphanistrum* seeds. This could be related to its chemical account. We suggest that a combination of these natural products with

Table 5(a)

Antifeedant activity of HSREO against *Spilosoma obliqua* in the laboratory conditions.

CONC.	Percent Leaf Area Consumed			Mean	Feeding Percent	Antifeedant activity	Feeding inhibition	Preference Index
	12 Hours	24 Hours	36 Hours					
50 ppm	11.64	19.85	28.94	20.1±8.65	80.56	7.01	3.63	0.963
100 ppm	10.67	18.38	25.85	18.30±7.59	73.2	15.51	8.40	0.915
150 ppm	9.33	17.99	22.94	16.75±6.88	67	22.66	12.78	0.872
200 ppm	4.20	7.34	8.81	6.78±2.35	27.12	68.69	52.32	0.476
250 ppm	00	0.73	3.89	1.54±2.06	6.16	92.89	86.72	0.132
Control	14.10	21.40	29.49	21.66±7.69	86.64	0	0	1
CD1 (5%)	3.97							
CV	41.38							
CD2 (5%)	5.61							
CV	41.38							
CD3 (5%)	9.73							
CV	41.38							

CD1=variation with respect to concentration, CD2=variation with respect to time, CD3=variation with respect to interaction between both, CV=coefficient of variance.

Table 5(b)

Antifeedant activity of HSRCF against *Spilosoma obliqua* in the laboratory conditions.

CONC.	Percent Leaf Area Consumed			Mean	Feeding Percent	Antifeedant activity	Feeding inhibition	Preference Index
	12 Hours	24 Hours	36 Hours					
50 ppm	16.17	21.34	27.45	21.66±5.64	86.64	0	0	1
100 ppm	15.70	21.23	27.05	21.32±5.67	85.25	1.56	0.79	0.992
150 ppm	13.29	19.42	21.47	18.06±4.25	72.24	16.62	9.06	0.909
200 ppm	3.28	10.38	10.38	8.01±4.09	32.04	63.01	46.00	0.539
250 ppm	00	1.93	2.75	1.56±1.41	6.24	92.79	86.56	0.134
Control	14.10	21.40	29.49	21.66±7.69	86.64	0	0	1
CD1 (5%)	2.94							
CV	28.29							
CD2 (5%)	4.16							
CV	28.29							
CD3 (5%)	7.20							
CV	28.29							

Here, CD1=variation with respect to concentration, CD2=variation with respect to time, CD3=variation with respect to interaction between both, CV=coefficient of variance.

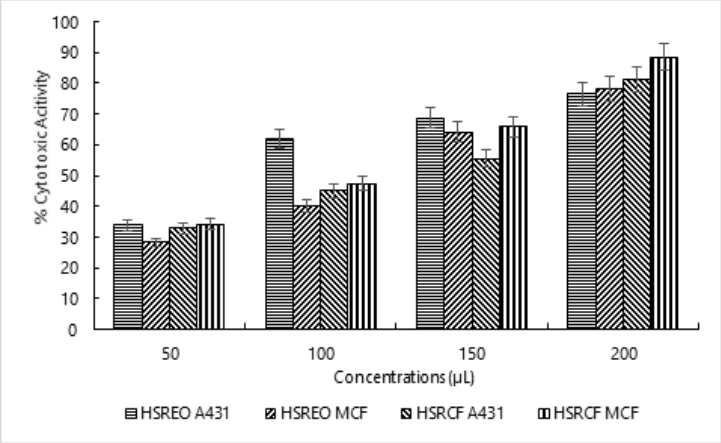

Fig. 6. Cytotoxicity activity of HSREO and HSRCF against A431 (epidermal) cell lines and MCF (lungs) cell lines in the laboratory conditions: HSREO=*Hedychium spicatum* Sm. rhizomes essential oil, HSRCF=*Hedychium spicatum* Sm. rhizomes column fraction.

conventional drugs might be a viable option for future oversights in pesticides and herbicides preparations. This phytotoxicity has generally been attributed to the allelopathic potential of some monoterpenes. In addition to these effects, botanicals also showed moderate cytotoxic activity against A421 and MCF cell lines. Their pleasant odour may have the added advantage of being more acceptable to patients.

Conflict of interest

The authors declare that there is no conflict of interest.

Table 6
Cytotoxicity activity of HSREO and HSRCF against A431 (epidermal) cell lines and MCF (lungs) cell lines in the laboratory conditions.

CONC.(µL)	Cytotoxicity activity (HSREO)		Cytotoxicity activity (HSRCF)	
	A431	MCF	A431	MCF
50	33.80	28.20	32.83	34.22
100	62.06	40.11	45.02	47.47
150	68.78	64.20	55.58	65.97
200	76.51	78.08	81.25	88.45
Control	0	0	0	0

Results obtained using One-way ANOVA ($p < 0.05$), HSREO=*Hedychium spicatum* rhizomes essential oil, HSRCF=*Hedychium spicatum* rhizomes column fraction.

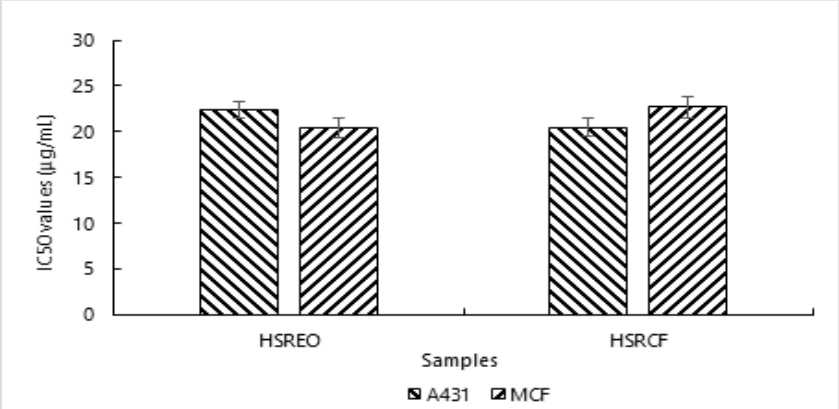

Fig. 7. IC_{50} values of HSREO and HSRCF: HSREO=*Hedychium spicatum* Sm. rhizomes essential oil, HSRCF=*Hedychium spicatum* Sm. rhizomes column fraction.

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References

Adams, R.P., 2007. Identification of essential oil components by gas chromatography mass spectroscopy. Allured Publishing Corporation, USA.

Adham, F.K., Rashad, E.M., Shoukry, I.F., Nasr, E.E., 2009. Host plants shifting affects the biology and biochemistry of *Spodoptera littoralis* (boisd.) (Lepidoptera: noctuidae). Egy. Acad. J. Biol. Sci. 2, 63-71.

Aggarwal, B.B., Yuan, W., Li, S., Gupta, S.C., 2013. Curcumin-free turmeric exhibits anti-inflammatory and anticancer activities: Identification of novel components of turmeric. Mol. Nutr. Food Res. 57(9), 1529-1542.

Ahmad, A., Misra, L.N., 1994. Terpenoids from *Artemisia annua* and constituents of its essential oil. Phytochemistry 37, 183-186.

Amri, I., Hanana, M., Jamoussi, B., Hamrouni, L., 2017. Essential oils of *Pinus nigra* J.F. Arnold subsp. *laricio* Maire: chemical composition and study of their herbicidal potential. Arabian J. Chem. 10, 3877-3882.

Angelini, L.G., Carpanese, G., Cioni, P.L., Morelli, I., Macchia, M., Flamini, G., 2003. Essential oils from Mediterranean Lamiaceae as weed germination inhibitors. J. Agric. Food Chem. 51(21), 6158-6164.

Asolkar, L.V., Chopra, R.N., 1992. Second Supplement to Glossary of Indian Medicinal Plants with Active Principles. Publications & Information Directorate.

Aydin, E., Türkez, H., Geyikoğlu, F., 2013. Antioxidative, anticancer and genotoxic properties of α-pinene on N2a neuroblastoma cells. Biologia 68(5), 1004-1009.

Ben El Hadj, A.I., Chaouachi, M., Bahri, R., Chaieb, I., Boussaid, M., Harzallah-Skhiri, F., 2015. Chemical composition and antioxidant, antibacterial, allelopathic and insecticidal activities of essential oil of *Thymus algeriensis*. Boiss. et Reut. Ind. Crops Prod. 77, 631-639.

Bhatt, I.D., Prasad, K., Rawat, S., Rawal, R.S., 2008. Evaluation of antioxidant phytochemical diversity in *Hedychium spicatum*: a high value medicinal plant of Himalaya. Pharmacogn Mag. 4, 202-205.

Butani, D., Juneja, S.S., 1984. Pests of radish in India and their control. Pesticides 18(5), 10-12.

Cha, J.D., Kim, Y.H., Kim, J.Y., 2010. Essential oil and 1,8-cineole from *Artemisia lavandulaefolia* induce apoptosis in KB cells via mitochondrial stress and caspase activation. Food Sci. Biotechnol. 19(1), 185-191.

Chen, W., Vermaak, I., Viljoen, A., 2013. Camphor—a fumigant during the black death and a coveted fragrant wood in ancient Egypt and Babylon—a review. Molecules 18(5),

5434-5454.

Clevenger, J.F., 1928. Apparatus for the determination of volatile oil. J. Pharm. Sci. 17(4), 345-349.

Dayan, F.E., Duke, S.O., 2014. Natural compounds as next generation herbicides. Plant Physiol. 166, 1090-1105.

De Martino, L., Mancini, E., Almeida, L.F.R., De Feo, V., 2010. The antigerminative activity of twenty-seven monoteroines. Molecules 15, 6630-6637.

Dudai, N., Poljakoff-Mayber, A., Mayer, A.M., Putievsky, E., Lerner, H.R., 1999. Essential oils as allelochemicals and their potential use as bioherbicides. J. Chem. Ecol. 25, 1079-1089.

Ghildiyal, S., Gautam, M.K., Joshi, V.K., Goel, R.K., 2012. Pharmacological evaluation of extracts of *Hedychium spicatum* (Ham-ex-Smith) rhizome. Anc. Sci. Life 31(3), 117.

Gopanraj, G., Dan, M., Shiburaj, S., Sethuraman, M.G., George, V., 2005. Chemical composition and antibacterial activity of the rhizome oil of *Hedychium larsenii*. Acta Pharm. 55, 315-320.

Gopichand, R.L., Maurya, A.K., Agnihotri, V.K., Singh, R.D., 2017. Effect of growth hormones on seed germination and plant growth: with chemical components of Hedychium spicatum Ham. ex. Smith. Ornam. Med. Plants. 1: 1-14.

Goren, A.C., Piozzi, F., Akcicek, E., Kılıç, T., Çarıkçı, S., Mozioğlu, E., Setzer, W.N., 2011. Essential oil composition of twenty-two *Stachys* species (mountain tea) and their biological activities. Phytochem. Lett. 4(4), 448-453.

Hassan, S.B., Gali-Muhtasib, H., Göransson, H., Larsson, R., 2010. Alpha terpineol: a potential anticancer agent which acts through suppressing NF-κB signalling. Anticancer Res. 30(6), 1911-1919.

Hussain, R.F., Nouri, A.M.E., Oliver, R.T.D., 1993. A new approach for measurement of cytotoxicity using colorimetric assay. J. Immunol. Methods 160(1), 89-96.

Jabran, K., Mahajan, G., Sardana, V., Chauhan, B.S., 2015. Allelopathy for weed control in agricultural systems. Crop Prot. 72, 57-65.

Jadhav, V., Kore, A., Kadam, V.J., 2007. *In-vitro* pediculicidal activity of *Hedychium spicatum* essential oil. Fitoterapia 78(7-8), 470-473.

Jalaei, Z., Fattahi, M., Aramideh, S., 2015. Allelopathic and insecticidal activities of essential oil of *Dracocephalum kotschyi* Boiss. from Iran: a new chemotype with highest limonene-10-al and limonene. Ind. Crops Prod. 73, 109-117.

Joshi, S., Chanotiya, C.S., Agarwal, G., Prakash, O., Pant, A.K., Mathela, C.S., 2008. Terpenoid compositions, and antioxidant and antimicrobial properties of the rhizome essential oils of different *Hedychium* species. Chem. Biodivers. 5(2), 299-309.

Kandil, M.A., Abdel-Aziz, N.F., Sammour, E.A., 2003. Comparative toxicity of chlorfluazuran and leufenron against cotton leafworm *Spodoptera littoralis* (Boisd). Egypt J. Agric. Res. 2, 645-661.

Kleinowski, A.M., Ribeiro, G.A., Milech, C., Braga, E.J.B., 2016. Potential allelopathic and antibacterial activity from *Alternanthera philoxeroides*. Hoehnea 43, 533-540.

Kohli, R.K., Singh, D., 1991. Allelopathic impact of volatile components from *Eucalyptus* on crop plants. Biol. Plant. 33, 475-483.

Kong, Q., Sun, F., Chen, X., 2013. Impact of fixed-dose combination of germacrone, curdione, and furanodiene on breast cancer cell proliferation. Cell J. (Yakhteh). 15(2), 160.

Kordali, S., Cakir, A., Ozer, H., Cakmakci, R., Kesdek, M., Mete, E., 2008. Antifungal, phytotoxic and insecticidal properties of essential oil isolated from *Turkish Origanum acutidens* and its three components, carvacrol, thymol and *p*-cymene. Bioresour. Technol. 99(18), 8788-8795.

Lakshmi, S., Padmaja, G., Remani, P., 2011. Antitumour effects of isocurcumenol isolated from *Curcuma zedoaria* rhizomes on human and murine cancer cells. Int. J. Med Chem. Article ID 253962.

Li, J., Bian, W.H., Wan, J., Zhou, J., Lin, Y., Wang, J.R., Wang, Z.X., Shen, Q., Wang, K.M., 2014. Curdione inhibits proliferation of MCF-7 cells by inducing apoptosis. Asian Pac. J. Cancer Prev. 15(22), 9997-10001.

Li, M., Zhang, N., Lin, Q.Y., 2008. Determination of germacrone and curdione in *Radix Curcumae* by HPLC. West China J. Pharm. Sci. 23(1), 105.

Liska, A., Rozman, V., Kalinovic, I., Ivecic, M., Balicevic, R., 2010. Contact and fumigant activity of 1,8-cineole, eugenol and camphor against *Tribolium castaneum* (Herbst). 10th International Working Conference on Stored Product Protection. 425, 716-720.

Magd El-Din, M., ElGengaihi, S.E., 2000. Join action of some botanical extracts against the Egyptian cotton leafworm, *Spodoptera* (Boised)) (Lepidoptera: Noctuidae). Egypt. J. Biol. P. Cont. 10, 51-56.

Marei, G.I.K., Rasoul, M.A.A., Abdelgaleil, S.A., 2012. Comparative antifungal activities and biochemical effects of monoterpenes on plant pathogenic fungi. Pestic. Biochem. Physiol. 103(1), 56-61.

Mohammadhosseini, M., 2017. The ethnobotanical, phytochemical and pharmacological properties and medicinal applications of essential oils and extracts of different *Ziziphora* species. Ind. Crops Prod. 105, 164-192.

Mohammadhosseini, M., Sarker, S.D., Akbarzadeh, A., 2017. Chemical composition of the essential oils and extracts of *Achillea* species and their biological activities: A review. J. Ethnopharmacol. 199, 257-315.

Mohammadhosseini, M., Venditti, A., Sarker, S.D., Nahar, L., Akbarzadeh, A., 2019. The genus *Ferula*: Ethnobotany, phytochemistry and bioactivities - A review. Ind. Crops Prod. 129, 350-394.

Montenegro, I.J., del Corral, S., Diaz Napal, G.N., Carpinella, M.C., Mellado, M., Madrid, A. M., Cuellar, M.A., 2018. Antifeedant effect of polygodial and drimenol derivatives against *Spodoptera frugiperda* and *Epilachna paenulata* and quantitative structure-activity analysis. Pest Manag. Sci. 74(7), 1623-1629.

Moteki, H., Hibasami, H., Yamada, Y., Katsuzaki, H., Imai, K., Komiya, T., 2002. Specific induction of apoptosis by 1,8-cineole in two human leukemia cell lines, but not a in human stomach cancer cell line. Oncol. Rep. 9(4), 757-760.

Muller, C.H., Muller, W.H., Haines, B.L., 1964. Volatile growth inhibitors produced by aromatic shrubs. Science 143, 471- 473.

Murata, S., Shiragami, R., Kosugi, C., Tezuka, T., Yamazaki, M., Hirano, A., Yoshimura, Y., Suzuki, M., Shuto, K., Ohkohchi,

N., Koda, K., 2013. Antitumor effect of 1,8-cineole against colon cancer. Oncol. Rep. 30(6), 2647-2652.

Obeng-Ofori, D., Reichmuth, C.H., Bekele, A.J., Hassanali, A., 1998. Toxicity and protectant potential of camphor, a major component of essential oil of *Ocimum kilimandscharicum*, against four stored product beetles. Int. J. Pest Manag. 44, 203-209.

Pal, S.K., Shukla, Y., 2003. Herbal medicine: Current status and the future. Asian Pac. J. Cancer Prev. 4, 281-288.

Pavela, R., 2008. Insecticidal properties of several essential oils on the house fly (*Musca domestica* L.). Phytother. Res. 22(2), 274-278.

Pham, H.N.T., Sakoff, J.A., Van Vuong, Q., Bowyer, M.C., Scarlett, C.J., 2018. Comparative cytotoxic activity between kaempferol and gallic acid against various cancer cell lines. Data in Brief 21, 1033-1036.

Prakash, O., Rajput, M., Kumar, M., Pant, A.K., 2010. Chemical composition and antibacterial activity of rhizome oils from *Hedychium coronarium* Koenig and *Hedychium spicatum* Buch-Ham. J. Essent. Oil Bear. Plants 13(2), 250-259.

Prashar, A., Locke, I.C., Evans, C.S., 2004. Cytotoxicity of lavender oil and its major components to human skin cells. Cell Prolif. 37(3), 221-229.

Raina, A.P., Negi, K.S., 2015. Essential oil composition of Hedychium spicatum Buch. -Ham. ex Smith. from Uttarakhand, India. J. Essent. Oil-Bear. Pl. 18(2): 382-388.

Rawat, S., Jugran, A.K., Bhatt, I.D., Rawal, R.S., 2018. *Hedychium spicatum*: a systematic review on traditional uses, phytochemistry, pharmacology and future prospectus. J. Pharm. Pharmacol. 70(6), 687-712.

Rozman, V., Kalinovic, I., Korunic, Z., 2006. Toxicity of naturally occurring compounds of Lamiaceae and Lauraceae to three stored-product insects. J. Stored Prod. Res. 43, 349-355.

Sadek, M.M., 2003. Antifeedant and toxic activity of *Adhatoda vasica* leaf extract against *Spodoptera littoralis* (Lep., Noctuidae). J. Appl. Entomol. 127(7), 396-404.

Salamci, E., Kordali, S., Kotan, R., Cakir, A., Kaya, Y., 2007. Chemical compositions, antimicrobial and herbicidal effects of essential oils isolated from Turkish *Tanacetum aucheranum* and *Tanacetum chiliophyllum* var. Biochem. Syst. Ecol. 35(9), 569-581.

Sarker, S.D., Nahar, L., 2018. Phytochemicals and phyto-extracts in cosmetics Trends Phytochem. Res. 2(4), 185-186.

Schenk, J.R., 2009. Phytochemistry, allelopathy and the capability attributes of camphor laurel (*Cinnamomum camphora* L.) Ness & Eberm.). Ph.D. Thesis, Southern Cross University, Lismore, Australia.

Scrivanti, L.R., Zunino, M.P., Zygadlo, J.A., 2003. *Tagetes minuta* and *Schinus areira* essential oils as allelopathic agents. Biochem. Syst. Ecol. 31, 563-572.

Snedecor, G.W., Cochran, W.G., 1968. Statistical Methods. Oxford/IBH Publishing, New Delhi. p. 593.

Tandon, S., Mittal, A.K., Pant, A.K., 2009. Growth-regulatory activity of *Trichilia connaroides* (syn. *Heynea trijuga*) leaf extracts against the Bihar hairy caterpillar *Spilosoma obliqua* (Lepidoptera: Arctiidae). Int. J. Trop. Insect Sci. 29(4), 180-184.

Tiwari, S.N., Bhattacharya, A.K., 1987. Artificial diet for *Spilosoma obliqua*. The Memoirs Entomological Society of India. No.12. IARI, New Delhi. p. 297.

Turk, M.A., Tawaha, A.M., 2003. Inhibitory effects of aqueous extracts of black mustard on germination and growth of Lentil. Agron. J. 1, 28-30.

Venditti, A., Frezza, C., Serafini, I., Pulone, S., Scardelletti, G., Sciubba, F., Bianco, A., Serafini, M., 2018. Chemical profiling of the fruits of *Styrax officinalis* L. from Monti Lucretili (Latium region, Central Italy): Chemotaxonomy and nutraceutical potential. Trends Phytochem. Res. 2(1), 1-12.

Verma, R.S., Padalia, R.C., 2010. Comparative essential oil composition of different vegetative parts of Hedychium spicatum Smith. from Uttarakhand, India. Int. J. Green Pharm. 4(4): 292-295.

Vokou, D., Douvli, P., Blionis, G. J., Halley, J. M., 2003. Effects of monoterpenoids, acting alone or in pairs, on seed germination and subsequent seedling growth. J. Chem. Ecol. 29(10), 2281-2301.

Wansi, J.D., Sewald, N., Nahar, L., Martin, C., Sarker, S.D., 2018. Bioactive essential oils from the Cameroonian rain forest: A review - Part I. Trends Phytochem. Res. 2(4), 187-234.

Wansi, J.D., Sewald, N., Nahar, L., Martin, C., Sarker, S.D., 2019. Bioactive essential oils from the Cameroonian rain forest: A review - Part II. Trends Phytochem. Res. 3(1), 3-52.

Yan, J., Gang C., Shengqiang T., Yeping F., Liuqing S., Jianzhong L., 2005. Preparative isolation and purification of germacrone and curdione from the essential oil of the rhizomes of *Curcuma wenyujin* by high-speed counter-current chromatography. J. Chromatogr. 1-2, 207-210.

Yeh, R.Y., Shiu, Y.L., Shei, S.C., Cheng, S.C., Huang, S.Y., Lin, J.C., Liu, C.H., 2009. Evaluation of the antibacterial activity of leaf and twig extracts of stout camphor tree, *Cinnamomum kanehirae*, and the effects on immunity and disease resistance of white shrimp, *Litopenaeus vannamei*. Fish Shellfish Immunol. 27(1), 26-32.

Zunino, M.P., Zygadlo, J.A., 2004. Effect of monoterpenes on lipid oxidation in maize. Planta 219(2), 303-309.