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Cytotoxic activity and GC-MS analysis of the constituents of essential oil of *Nepeta govaniana* (Wall.ex Benth) from Jammu and Kashmir, India

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ABSTRACT
The chemical composition of the essential oil of *Nepeta govaniana* was determined by GC-FID with relative abundance and relative retention indices (RRI). The GC-FID analysis led to identification of ten compounds accounting for 86.02% of total oil composition. The dominant compounds present were 1, 2, 3, 6-tetramethylbicyclo [2, 2, 2] octa-2, 5-diene (32.35%) followed by geijerene (20.65%) and pregeijerene (6.18%). The oil was screened for cytotoxic activity against a panel of five cancer cell lines viz lung (A-549), breast (MCF-7), pancreas (MiaPaCa-2) and Colon (HCT-116 and Colo-205) using sulforhodamine B assay (SRB). The *Nepeta govaniana* essential oil exhibited a concentration-dependent as well as cell line-dependent growth inhibition of cancer cells and was found to be most active against the lung (A-549; IC_{50} of 19.1 ± 0.9 µg/ml) followed by colon (colo-205; IC_{50} of 21.3±1.1 µg/ml) cancer cell lines.

Keywords: Geijerene, Pregeijerene, relative abundance, relative retention indices, cytotoxic activity.

1. Introduction
The genus *Nepeta* commonly called as catmint belongs to family Lamiaceae and is distributed in central and southern parts of Europe, Asia and Middle East. It consists of 250 species among them 30 species are distributed in temperate Himalayas and plains of India [1]. Several species of *Nepeta* are used as laxative and for treatment of tooth trouble [2]. They are also used as antispasmodic, antiseptic, antiasthmatic and febrifuge [3]. Some species are used to reduce serum lipids and have anti-inflammatory effects [4]. The diverse biological activity like feline attractant, canine attractant and insect repellant are attributed to the presence of active irridoids in several species of *Nepeta* [5-10]. *Nepeta govaniana* (yellow catmint) is a perennial herb with 2-4 feet erect sturdy stem having pale yellow tubular flowers throughout the summer. The 4-angled aromatic, fragrant leaves have hairy velvety appearance. It is found in Himalayas at the altitudes of 2400-3300 m from Pakistan to Uttarakhand. Literature survey reveals that *Nepeta govaniana* has been in the focus for essential oil from its aerial parts since long back [11-14]. However, there are no reports of the cytotoxic activity and essential oil composition of *Nepeta govaniana* from high Himalayas of Jammu and Kashmir. In lieu with the institute's programme to screen the rich flora of the region for new aromatic chemicals, the objective behind the current study was to check the cytotoxic activity of the characterized essential oil of *Nepeta govaniana* growing in Jammu and Kashmir (India) against various cancer cell lines and determine its chemical composition using GC-MS analytical techniques.

2. Materials and Methods
2.1 Plant material
Fresh plant material of *Nepeta govaniana* was collected from high Himalayas of Jammu and Kashmir, India in October, 2011. The plant was taxonomically identified and authenticated at the Department of Botany, University of Kashmir against the Herbarium number Kash-1123. The plant was air-dried for three days, pulverized, and subjected to hydrodistillation in a Clevenger-type apparatus for 4 h, to give 0.25% calculated on fresh weight basis of the plant material. The oil was dried over anhydrous sodium sulfate and stored in a sealed vial under refrigeration prior to analysis.

2.2 GC-FID analysis
GC/FID was carried out on Perkin Elmer auto system XL Gas Chromatograph 8500 series with flame ionization detector (FID) and head space analyzer using a fused silica capillary

RTX-5 column (30m x 0.32 mm, film thickness 0.25 µm) coated with dimethyl polysiloxane. Oven temperature was programmed from 60 to 280 ^{0}C at 3^{0}C/min, with injector and detector temperature 230 ^{0}C and 250 ^{0}C respectively. The injection volume was 0.5 µl and nitrogen was used as a carrier gas (1.3 ml/min).

2.3 Identification of components

Identification of the essential oil constituents was done on the basis of Retention Index [RI, determined with respect to homologous series of *n*-alkanes (C_5-C_{28}, Polyscience Corp., Niles IL) under the same experimental conditions], co-injection with standards (Sigma Aldrich and standard isolates), MS Library search (NIST 05 and Wiley), by comparing with the MS literature data (Adams, 2007).

2.4 Cell lines, growth medium and treatment conditions

Human cancer cell lines; lung (A-549), breast (MCF-7), pancreas (MiaPaCa-2) and colon (HCT-116, Colo-205) were procured from European collection of cell culture (ECACC), UK. Cells were grown in Minimum Essential Medium (MEM) supplemented with 10% FCS and 1% penicillin. Penicillin was dissolved in PBS and sterilized by filtering through 0.2 µm filter in laminar air flow hood. Cells were cultured in CO_2 incubator (New Brunswick, Galaxy 170R, eppendroff) with an internal atmosphere of 95% air and 5% CO_2 gas and the cell lines were maintained at 37 ^{0}C. The media was stored at low temperature (2-8 ^{0}C) and the medium for cryopreservation contained 20% FCS and 10% DMSO in growth medium.

2.5 Cytotoxicity using SRB assay

RPMI-1640 medium, streptomycin, fetal bovine serum, sodium bicarbonate, phosphate buffer saline, sulphorhodamine, trypsin, 5-flurouracil and gentamycin sulphate were purchased from Sigma Chemicals Co. Glacial acetic acid from Fischer scientific and trichloroactetic acid (TCA) from Merck specialities private limited. All the cells used were grown in RPMI-1640 medium containing 10% FBS, 100 unit penicillin/100 µg streptomycin per ml medium. Cells were allowed to grow in carbon dioxide incubator (Thermo scientific USA) at 37 °C with 98% humidity and 5% CO_2 gas environment.

In the present study the cytotoxic effect of the essential oil was evaluated using sulphorhodamine B (SRB) assay. The SRB dye binds to the basic protein of cells that have been fixed to tissue-culture plates by trichloroacetic acid (TCA). As the binding of SRB is stoichiometric, the amount of dye extracted from stained cells is directly proportional to the cell number. In the present case, all cell lines seeded in flat-bottomed 96-well plates were allowed to adhere overnight and then media containing different oil samples (varying concentrations) were added. The plates were assayed for 48 h. The cells were fixed by adding 50 µl of ice-cold 50% TCA to each well for 60 minutes. The plates were washed five times in running tap water and stained with 100 µl per well SRB reagent (0.4% w/v SRB) in 1% acetic acid for thirty minutes. The plates were washed five times in 1 % acetic acid to remove unbound SRB and allowed to dry overnight. SRB was solubilised with 100 µl per well 10 mM tris-base, shaken for 5 minutes and the optical density was measured at 570 nm.

3. Results and discussion

The chemical constituents of the essential oil were analyzed by capillary GC-FID and the constituents are listed in table-1 with relative abundance and relative retention indices (RRI). The oil yield was 0.25% calculated on fresh weight basis of the plant material. The GC-FID analysis led to identification of ten compounds accounting for 86.02 % of total oil composition. The dominant compounds present were 1,2,3,6-tetra methylbicyclo [2,2,2] octa-2,5-diene (32.35%) followed by Geijerene (20.65%) and Pregeijerene (6.18%).

Table 1: GC-MS analysis of essential oil of *Nepeta govaniana*

S. No	Name of Compound	Percentage Abundance PA	Retention Time RT	Relative Retention Index (RRI)
1	Geijerene	20.65	25.219	1572
2	Pregeijerene	6.18	32.84	1628
4	β-Caryophylline	3.05	38.34	1418
5	4, 4-dimethyl adamantoin-2-ol.	15.87	38.69	1068
6	Germacrene-D	1.50	40.194	1576
7	α-Farnesene	1.35	41.54	1026
8	A-Copaen-11-ol	3.75	47.637	1128
9	B-Bourbonene	1.02	_	1383
10	Elemol	0.3	_	1552
11	1, 2, 3, 6-tetra methylbicyclo [2, 2, 2] octa-2, 5-diene (32.35%)	32.35	-	-

Nepeta govaniana essential oil was also studied in a calorimetric sulphorhodamine B assay against a panel of 5 human cancerous cell lines viz lung (A-549), breast (MCF-7), pancreas (Mia PaCa-2) and colon (HCT-116, Colo-205). Preliminary screening of the essential oil of various parts of *Nepeta govaniana* was first carried out at a fixed concentration of 50 µg/ml with mitomycin as standard. The oils that exhibited > 50% inhibition against any cell line were assayed further to determine IC_{50} value and a dose-dependent cytotoxicity relation was observed. *Nepeta govaniana* essential oil was found to be most potent against human lung (IC_{50} = 19.1 ± 0.9 µg/ml) followed by colon-205 (IC_{50} = 21.3±1.1 µg/ml) cancer cell lines. The results are shown in fig 1.

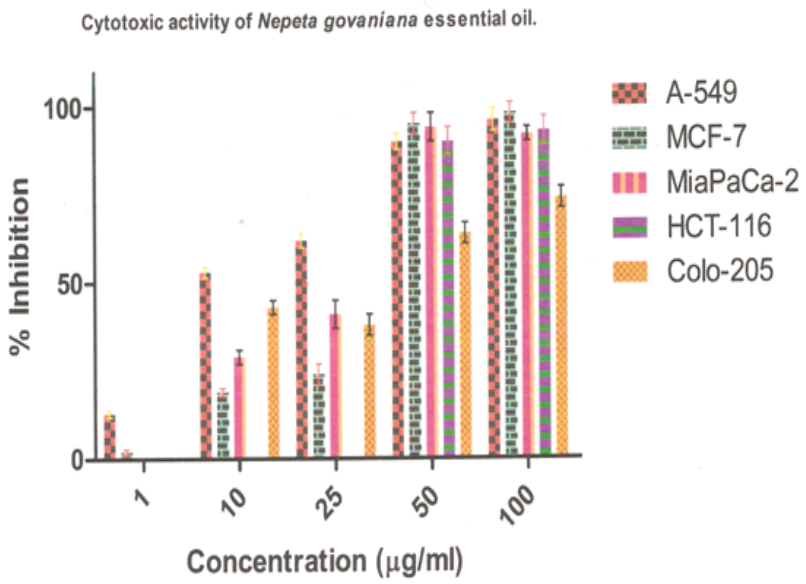

Fig 1: Cytotoxic activity of *Nepeta govaniana* essential oil.

The cytotoxic activity of *Nepeta govaniana* essential oil was evaluated against a panel of five cancer cell lines viz. lung (A-549), breast (MCF-7), pancreas (MiaPaCa-2) and colon (HCT-116 and colo-205) using SRB assay. The cell lines were subjected to increasing doses of the essential oil including 1, 10, 25, 50 and 100 µg/ml. The essential oil exhibited a concentration-dependent as well as cell line-dependent growth inhibition of cancer cells. It is evident from the figure that the oil exhibited no cytotoxic activity against the tested cancer cell lines at a concentration of 1µg/ml, exhibited cytoyoxicity at concentrations of 10 and 25 µg/ml against Lung (A-549), Breast (MCF-7), Pancreas (MiaPaCa-2) and Colon (colo-205) cell lines and was most active at a concentration of 50 µg/ml and 100 µg/ml against all the tested cancer cell lines.

4. Conclusion

The cytotoxic activity of *Nepeta govaniana* essential oil against a panel of five cancer cell lines have revealed it to be most active against the lung (A-549) and colon (Colo-205) cell lines.

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