

Physicochemical Properties, GC-MS Analysis and Impact of Different Material Size on Yield of Himalayan *C. deodara* Essential Oil

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ABSTRACT

Aromatic oils are called Essential Oils (EO) because of their ease of solubility in alcohol to form essence. These oils are found in the uncombined form in various parts of plants and employed for flavours, perfumes, cosmetics, beverages, disinfectants, medicines, and stabilizers. EO possess various constituents mainly sesquiterpenoids, benzenoids, phenylpropanoids etc. The demand for *C. deodara* plant material and its essential oil has been increasing in pharmaceutical, chemical, food and perfumery industries because of its favourable physicochemical characteristics and therapeutic efficacy. *C. deodara* has been used as antiseptic, insecticides, anti-inflammatory, molluscicidal and anti-fungal. Therefore, the main objective of this experimental work is to investigate the effect of different particle sizes of plant material on the yield of essential oil; to investigate the effect of processing time on *C. deodara* EO yield and their physicochemical characteristics and analysis of essential oil constituents using GC-MS. Different size of *C. deodara* material namely, grade A (large size), grade B (medium size) and grade C (small size) material has been extracted using Clevenger's apparatus to measure its physicochemical properties. The processed grade B material was extracted with n-hexane by the maceration process to analyse variation in physicochemical properties. From the results, it has been observed that medium-size material of *C. deodara* has a higher percentage of yield because it has an optimum surface area to release its constituents. Temperature variations have a direct impact on physical as well as chemical properties. Higher temperatures and pressures result in a 'harsh' aroma that changes the aromatic properties and decrease the oil's therapeutic values. Distillation time has also an impact on the yield as well as on the physical and chemical properties of the material. Thin-layer chromatography showed 10 spots of different colours in essential oil. The major constituent's percentage obtained after GC-MS analysis as α -himachlene (13.83%), γ -himachlene (12.00%), β -himachlene (37.34%), Deodaron (0.43%), α -atlantone (4.53%), Z, γ -atlantone (2.77%), E, γ -atlantone (3.34%) and E, α -atlantone (10.63%).

Key words: *Cedrus deodara*, Physicochemical properties, GC-MS analysis, Percentage yield, Optical rotation, Thin-layer Chromatography (TLC).

INTRODUCTION

Aromatic oils found in uncombined form in various parts of plants and employed for flavors, perfumes, disinfectants, medicines, and stabilizers. The essential oils are of four general classes: The *pinenes* or *terpenes* of coniferous plants, containing carbon and hydrogen of the empirical formula $C_{10}H_{16}$.^[1-3]

Essential oils (EOs) possess various constituents mainly sesquiterpenoids, benzenoids, phenylpropanoids,^[4] etc. which exert strong therapeutic effect on humans, animals, and other plants.^[5] Essential oil's (EOs) and aroma chemicals are high-value products used in perfumery, cosmetics, feed, food, beverages and pharmaceutical industries. Aromatherapy with EOs covers the other side of the "classical" medical uses. Encapsulation is a technique widely utilized in pharmaceutical, chemical, food, and feed industries to render EOs more manageable in formulations. EOs are

not only appealing to humans but also to animals. EOs are used as feed additives and for treating diseases in pets and farm animals. EOs are produced and utilized in developing countries worldwide. Storage and transport of EO's are crucial issues since they are highly sensitive to heat, moisture, and oxygen. Therefore, special precautions and strict regulations apply for their handling in storage and transport.^[6]

In water or hydro distillation, the chopped plant material is submerged and remained in direct contact with boiling water. In steam distillation, the steam is produced in a boiler separately and blown through a pipe into the bottom of the still. The high-pressure steam-type distillation is often applied for peppermint, spearmint, lavender etc. The condensed distillate, consisting of a mixture of water and oil,

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is usually separated in a so-called Florentine flask, a glass jar, or more recently in a receptacle made of stainless steel with one outlet near the base and another near the top. Generally, the process of steam distillation is the most widely accepted method for the production of essential oils on a large scale.^[7] Expression or cold pressing is a process in which the oil glands within the peels of citrus fruits are mechanically crushed to release their content. EOs (Essential oils) are the complex mixtures of volatile compounds, separated as secondary metabolites from plant parts by steam/ Hydro distillation, solvent-less extraction and other physical means such as cold pressing. The demands of EO is increasing drastically in food and beverage; fragrances; cosmetics; flavoring agents in air fresheners and deodorizers; household and aromatherapy. *Cedrus deodara* (Pinaceae) EO is one of the important oil, which has high demand in the market because of its varied pharmacological activities.

C. deodara and its Pharmacological activities

Cedrus (true Cedar) is one of 11 commonly accepted genera in Pinaceae, first described by Trew in 1757. It comprises four species *C. deodara*, *C. libani*, *C. brevifolia* and *C. atlantica*. Himalayan deodara grows in the slope of the Himalayas and at the elevation of 1650m to 2400m above the sea level. In Himachal Pradesh, the total 68,872 hectare area is covered under *C. deodara* forest. The plant is found in Chamba, Manali, Kinnaur, Sirmour, Shimla, Kangra and Mandi region of Himachal Pradesh. The height of deodars is approximately 65–85m tall and 4m diameter at breast height (DBH). The leaves of deodara are stiff, sharp pointed having length of 23–37 mm long and the bark is greyish brown, dark in colour.^[8,9] The extracted oil from deodara has been used as antiseptic, insecticides, anti-fungal, anti-inflammatory and molluscicidal. The sizable stumps and roots of the plant left after cutting of trees are utilized for the Hydro/ Steam distillation of Essential oil (EO). The description of *C. deodara* is illustrated in Table 1.

In the traditional Ayurveda therapeutic system, all plant parts of the Deodar Cedar are used against various diseases. Among others, they are used to treat inflammation, dyspepsia, insomnia, cough and cold. They are also given against fever, urinary discharges, bronchitis, leucoderma, elephantiasis, tuberculosis glands, mind disorder, and skin and blood diseases.^[10,11] In the Persian – Arabic traditional Unani therapeutic system, both Cedar wood and the bark with the essential oil are utilized. Wood is used as a diuretic, expectorant, for relieving rheumatism, as well as for treating epilepsy and urinary tract diseases. The bark is used in formulations that are administered as astringents, antipyretics and anti-diarrhoeal. Finally, Cedar oleoresin is applied for the healing of wounds and the treatment of skin rashes.^[12]

Anti-inflammatory activity

Steam distillate of the wood of *C. deodara* was examined for its oral anti-inflammatory and analgesic activity at the doses of 50 and 100 mg/kg body weight. It produced significant inhibition of carrageenan-induced rat paw edema and of both exudative–proliferative and chronic phases of inflammation in adjuvant arthritic rats at doses of 50 and 100 mg/kg body weight. The oil at both tested doses was found to possess analgesic activity against acetic acid-induced writhing and hot plate reaction in mice.^[13]

Antibacterial activity

Volatile oil and constituents obtained from leaf and cone part of plant were evaluate *in vitro* antimicrobial activity against Gram-positive and Gram-negative bacteria. Volatile oil, α -pinene and β -pinene showed good antibacterial activity. Ethanolic extract from the wood part of plant was evaluated against three-gram positive (*Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus cereus*) and three-gram negative (*Klebsiella*

Table 1: Characteristics of *C. deodara*.

Species	Ripe color	Tree Length (m)	DIA. (m)
<i>Cedrus Deodara</i>	Reddish brown	5.5-6	1-3
Seed Size			
	Length (cm)	Width (cm)	
	2.5-3.7	2-2.5	
Leaf Size			
	Length (cm)	Thickness (mm)	
	2.5-2.7	0.9-1	

pneumonia, *Pseudomonas aeruginosa*, *Escherichia coli*) microorganism, found to have a good antibacterial action.^[14-16]

Antifungal activity

The fungicidal activity is persisted for longer period in essential oils of the plant. The antifungal activity of root oil and its compounds isolated from the oil were evaluated against *Candida albicans* and *Aspergillus fumigatus*. *C. deodara* oil at the concentration of 150 μ g/disc showed zone of inhibition against *A. fumigatus* but at the same concentration did not show any antifungal activity against *C. albicans*. Trans-atlantone and allo-himachalol, isolated from the oil did not possess any antifungal activity, while himachalol at the concentration of 150 μ g/disc showed zone of inhibition against *A. Fumigates*.^[17,18]

Insecticidal activity

Himalayan Cedar wood oil showed insecticidal property against adult Indian mosquitoes.^[19-22] Chromatographic fractions bio assayed against the Pulse beetle (*Callosobruchus*) and housefly (*Musca domestica*) showed insecticidal activity against both the test species.^[23] *C. deodara* also showed good pesticidal activity.^[24]

Molluscicidal activity

Fruit powder of *Embelia ribes* in combination with *Azadirachta indica* and *C. deodara* oil with synergists MGK- 264, piperonylbutoxide (PB) in binary and tertiary combinations were tested against *Lymnaea acuminata*. Combination of three plants in the ratio of 1:1:1 revealed maximum inhibition against *Lymnaea acuminata*.^[25]

Anti-tubercular activity

Chloroform and acetone extract obtained from the leaf and cone part of plant showed antitubercular activity against *Mycobacterium tuberculosis* in tuberculosis gland by broth dilution method. Crude extracts showed significant activity.^[26]

Anxiolytic and anticonvulsant activity

The heart wood extracts of *C. deodara* (ALCD) was studied for anxiolytic activity using three experimental models viz., Elevated plus maze test, Light dark model, locomotor activity by actophotometer. Anticonvulsant activity was studied by using Pentylene tetrazole induced convulsions and Maximal electro-shock induced convulsions. Pretreatment with ALCD followed by estimation of GABA in rat brain tissues was performed to study the effect of ALCD on GABA levels of brain. The ALCD at 50, 100 and 200 mg/kg doses significantly increased the time spent and number of entries in to the open arm indicating the test drugs could reduce the fear and anxiety in the mice. In Light dark model, ALCD (50, 100,

200 mg/kg) increased the time spent and number of entries in to the light compartment.

In pentylenetetrazole induced convulsions model the ALCD (100 or 200 mg/kg) significantly increased the onset of clonus, onset of tonus and percentage protection when compared to control group. In MES induced convulsions model, ALCD (100 or 200 mg/kg) significantly decreased the duration of tonic extensor and increased the percentage protection when compared to the control group.^[27] Seven days treatment with ALCD (30 mg/kg, 100mg/kg p.o.) and further GABA estimation in brain showed significant enhancement of GABA levels in cerebellum and whole brain other than cerebellum compared to control group.

Anti-diabetic activity

Formulation of *C. deodara* wood carried (per capsule: crude extract 190 mg, micro-crystalline cellulose 159 mg, di-calcium phosphate 71.4 mg, methyl paraben sodium 6.6 mg, propyl paraben sodium 5 mg, magnesium stearate 11 mg, talc 7 mg) showed significant anti diabetic activity, thus, revealed that the study had a vital role in the management of diabetes.^[28]

Antioxidant activity

The chloroform and purified fraction of *C. deodara* exhibited significant antioxidant property in DPPH assay. Isolated compounds viz. (-)-matairesinol, (-)-nortrachelogenin, and adibenzylbutyrolactollignan (4, 4', 9-trihydroxy-3, 3'- dimethoxy-9, 9'-epoxy) were responsible for activity.^[15,16]

Diuretic and Antiurolithiatic activity

Petroleum ether extract (PECD) of the heart wood of *C. deodara* was tested for its diuretic and antiurolithiatic activity. Sodium oxalate (70 mg/kg, i.p) for 10 days was experimentally used to induce urolithiasis. In sodium oxalate treated rats, crystal was observed in urine under light microscope and elevation of serum parameters indicated the development of nephrolithiasis in the control.^[28] The rats treated with PECD showed increase in urine output and significant antiurolithiatic activity.

The demand of *C. deodara* plant material and its essential oil has been increasing in pharmaceutical, chemical, food and perfumery industries because of its favorable physico-chemical characteristics and therapeutic efficacy. This has created pressure on various industries to produce quality products of *C. deodara* material. It has been considered that *C. deodara* of Himalayan region represents good quality after lot of work done on *C. deodara* of different regions. Thus, Himalayan *C. deodara* is selected for the present investigations.

The main objective of this experimental work is to investigate the effect of different particle size of plant material on the yield of essential oil; to investigate the effect of processing time on *C. deodara* EO yield and their physicochemical characteristics and analysis of essential oil of *C. deodara* using GC-MS.

Methodologies and Experimental Setup

The plant material *C. deodara* wood was collected from Shimla division of Theog forest region. The collected material was divided into three different sizes i.e. coarse, medium and fine size. The identity of plant material was confirmed by comparing its microscopic characteristics with available official literature. The authentication of plant was done in Pharmacognosy laboratory of Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala.

Essential oil of *C. deodara* was extracted using Clevenger's apparatus. The apparatus is made up of one round bottom flask (RBF) of various sizes

that hold the raw material. Through the connector, the RBF is connected to the condenser. The experimental set up is shown in Figure 1.

Different sizes of *C. deodara*, ground material, i.e. grade A (Large sized material), grade B (medium sized material) and grade C (small size material) were used in present study. The selected material for this experimental study is shown in Figure 2.

In this present experimentation, the different grade of material selected with their size ranges is shown in Table 2.

Experimentation

The powdered wood was collected and divided into 3 grades according to particle size. Grade A (large size; 15-30 mm and above), Grade B (medium size; 8-12 mm) and C (small size; 0.5-1 mm). The extraction of essential oil from different grades of plant was carried out separately using hydro distillation process. The plant drug (500 g) was boiled with 2.5 L of distilled water in a Clevenger apparatus separately for 6 hr, 8 hr, 10 hr, 12 hr, 14 hr, 16 hr, 20 hr, 22 hr and 24 hr. The plant material was boiled with water initially at 100°C then the constant temperature was

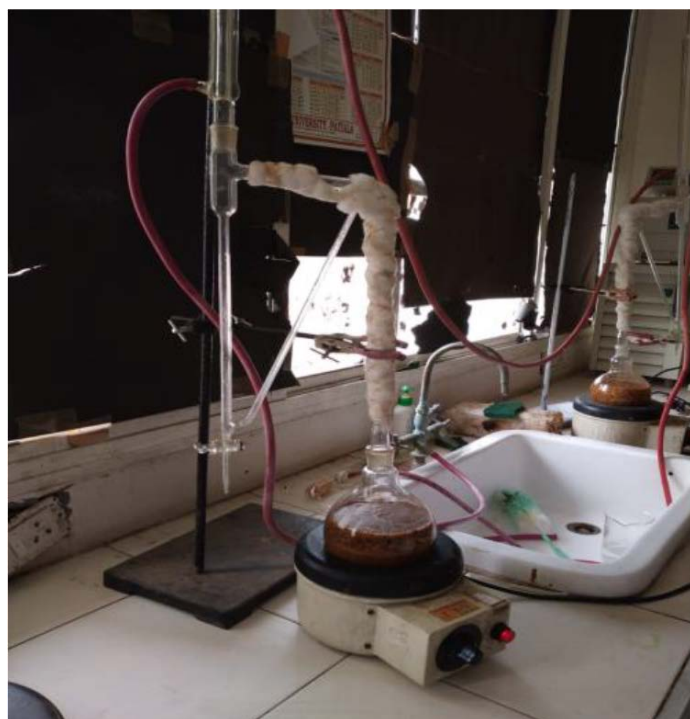


Figure 1: Experimental Set up.



Figure 2: Selection of material of different sizes- (a) Large Size; (b) Medium Size and (c) Smaller Size.

Table 2: Different grade of material with their size ranges.

Grade	Material Size	Size Range (mm)
A	Large	15-30
B	Medium	8-12
C	Small	0.01-1

**Figure 3:** *C. deodara* essential oil obtained by hydro-distillation process.

maintained between 40-50°C so that water vapor with essential oil were condensed when passed through the condenser at the rate of 2 drops per second. Due to less density of essential oil in comparison to water, the essential oil was collected on the top of water layer. The oil of *C. deodara* extracted by hydro-distillation is shown in Figure 3.

Re-processing of extracted material with non-polar solvent

The processed grade B plant material (100 g) was extracted with n-hexane by maceration process. The processed material was placed in some quantity of hexane so that this material completely immersed in a stoppered container. Then it was allowed to stand for 4-5 days with occasional stirring so that soluble constituents get dissolved into the hexane.

After completion of 4-5 days of this process, the extract was filtered and marc was pressed. Solubilize components with menstrual (solvent used for extraction) were collected. For extracting the oil from the dissolved hexane, distillation process was carried out. The solvent (n-hexane) was evaporated first and collected in a suitable container and remaining liquid residue of oil in the RBF was collected. The residue of liquid was kept in china dish followed by slightly heating on water bath to remove remaining n-hexane, and the oil was collected.

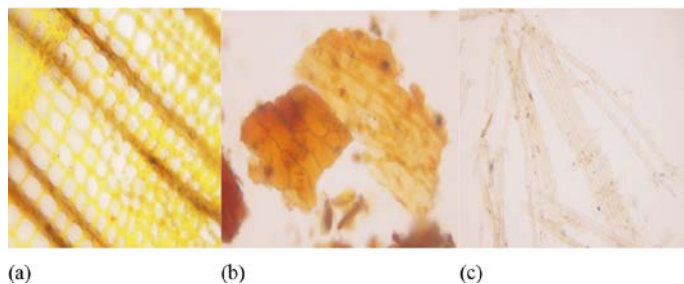
RESULTS AND DISCUSSION

Characteristics of *C. deodara*

Powder microscopy showed the presence of Xylem parenchyma, Patches and fibres. The results obtained through microscopic examination are represented in Figure 4.

Percentage yield of *C. deodara*

The yield of essential oil of *C. deodara* was calculated by using following Eq. 1 and shown in Table 3.

**Figure 4:** Photomicrographs (X 400) of *C. deodara* powdered drug showing microscopic characteristics. (a) Medullary rays with secondary xylem; (b) Patches of parenchyma; (c) xylem fibres.**Table 3: Percentage yield of essential oil of different grade raw material.**

Grade	Material Size	Size Range (mm)	% Yield (W/w)
A	Large	15-30	2.5-3.5%
B	Medium	8-12	4.5-6%
C	Small	0.01-1	2-3%

$$\text{oil \%} = \frac{\text{Weight of the oil}}{\text{Weight of the material taken}} \times 100 \quad (\text{Eq. 1})$$

From results, it was observed that small-size materials had the lowest yield. This occurred as a result of oil evaporation in the environment, which resulted in an increase in surface area due to smaller particle sizes. It has also been observed that oil recovered from smaller materials is not pure and contains smaller particles suspended in the oil. Medium-size plant material produced a higher yield because it has an optimum surface area that allows essential oil to be released in a best efficient manner. Moreover, it has been noticed that, due to uneven surface of the materials, water vapors could not penetrate properly resulted into less yield of essential oil. Finally it is suggested that, medium-size material is appropriate for better yields.

Physicochemical parameter of *C. deodara*

The physical properties of essential oil of *C. deodara* were observed and presented in Table 4.

Specific density

The calculated density of Himalayan *C. deodara* is obtained at 20°C is 0.939 Kg/m³ as shown in Table 5.

Table 4: Physical properties of *C. deodara* oil.

Characteristics	Observation
Color	Pale yellow Liquid
State	Liquid-oil
Odour	Characteristics
Taste	Pungent and cooling
Flash Point	175.5°F
Density of oil	0.93
Solubility	1. Water insoluble 2. Alcohol miscible having high concentration or in anhydrous alcohol 3. Miscible in oil; fats; paraffin; ether; chloroform and glacial acetic acid.

Optical Rotation

A Polarimeter was used to measure optical rotation of essential oil obtained from different grades of *C. deodara* material. The oil extracted within the first 12 hr of extraction showed higher optical activity than oil extracted after 13 hr. The optical rotation values obtained from different grade materials are given below in Table 6.

It has been further observed that, the oil produced from medium size materials possessed excellent optical rotation values than large or fine sized material. Optical rotation of volatile oil obtained after re-extraction of medium sized plant material was found to be 47.

Refractive Index

The refractive index of essential oil produced from different grades of *C. deodara* material was measured. It's evident from Table 7 that not much difference exists in refractive index (RI) of different samples of essential oil obtained from different grade material of *C. deodara*.

Viscosity

Brookfield viscometer was used to determine viscosity of essential oil obtained from different *C. deodara* grade material in the sample fluid. Viscosity results for *C. deodara* are shown in Table 8.

TLC fingerprint profile of volatile oil

TLC fingerprint profile of essential oil of different *C. deodara* grade material showed similar separation pattern of components in the solvent system Toluene: Ethylacetate (9:1) after spraying with 1% vanillin

Table 5: Specific density of essential oil of *C. deodara*.

Sl. No.	Measurement	Values
1.	Specific density at 20°C	0.9399

Table 6: Optical rotation of essential oil obtained from different grade material of *C. deodara*.

Grade	Material Size (mm)	Time (Hr)	OR Value (degrees)
A	Large (15-30)	6	65
		8	63
		10	61
		12	59
		15	57
Average Value		After 15 Hrs.	61
B	Medium (8-12)	6	69
		8	68
		10	67
		12	65
		15	59
Average Value		After 15 Hrs.	67
C	Small (0.01-1)	6	68
		8	68
		10	66
		12	64
		15	59
Average Value		After 15 Hrs.	63

sulphuric acid reagent followed by following by heating. The results of the TLC are shown in Figure 5 and Table 9.

GC-MS analysis of volatile oil

The volatile oil obtained from *C. deodara* (Grade B material) was analysed by using GC-MS. The Chromatogram as shown in Figure 6 showed "141" peaks, out of these 8 compounds were found to be major constituents of volatile oil. The main constituents were observed to be β - himachlene with 37.34% followed by α - himachlene (13.83), γ - himachlene (12%), etc.

List of major constituents found after GC-MS analysis are as given below in Table 10;

Table 7: Refractive index of essential oil obtained from different grade material.

Refractive index (RI) of sample of different grades				
Time/ duration of extracted oil	Error in refractometer	RI (A)	RI (B)	RI (C)
6 Hrs	0.022	1.503	1.532	1.527
8 Hrs	0.022	1.531	1.533	1.531
10 Hrs	0.022	1.531	1.533	1.530
12 Hrs	0.022	1.532	1.531	1.529

Table 8: Viscosity of essential oil of *C. deodara*.

Rotation (rpm)	Spindle number	Viscosity
100 rpm	64 spindle	163 cps
50 rpm	64 spindle	12 cps

Table 9: Results of TLC fingerprint profile of *C. deodara* essential oil.

Spot No.	Color Observed	Rf Value
1	Navy Blue	0.10
2	Purplish Pink	0.21
3	Light purple	0.25
4	Purplish Blue	0.29
5	Purple	0.39
6	Pink	0.45
7	Blue	0.51
8	Dark Green	0.64
9	Greenish blue	0.71
10	Pink	0.85



Figure 5: TLC fingerprint profile of *C. deodara* essential oil.

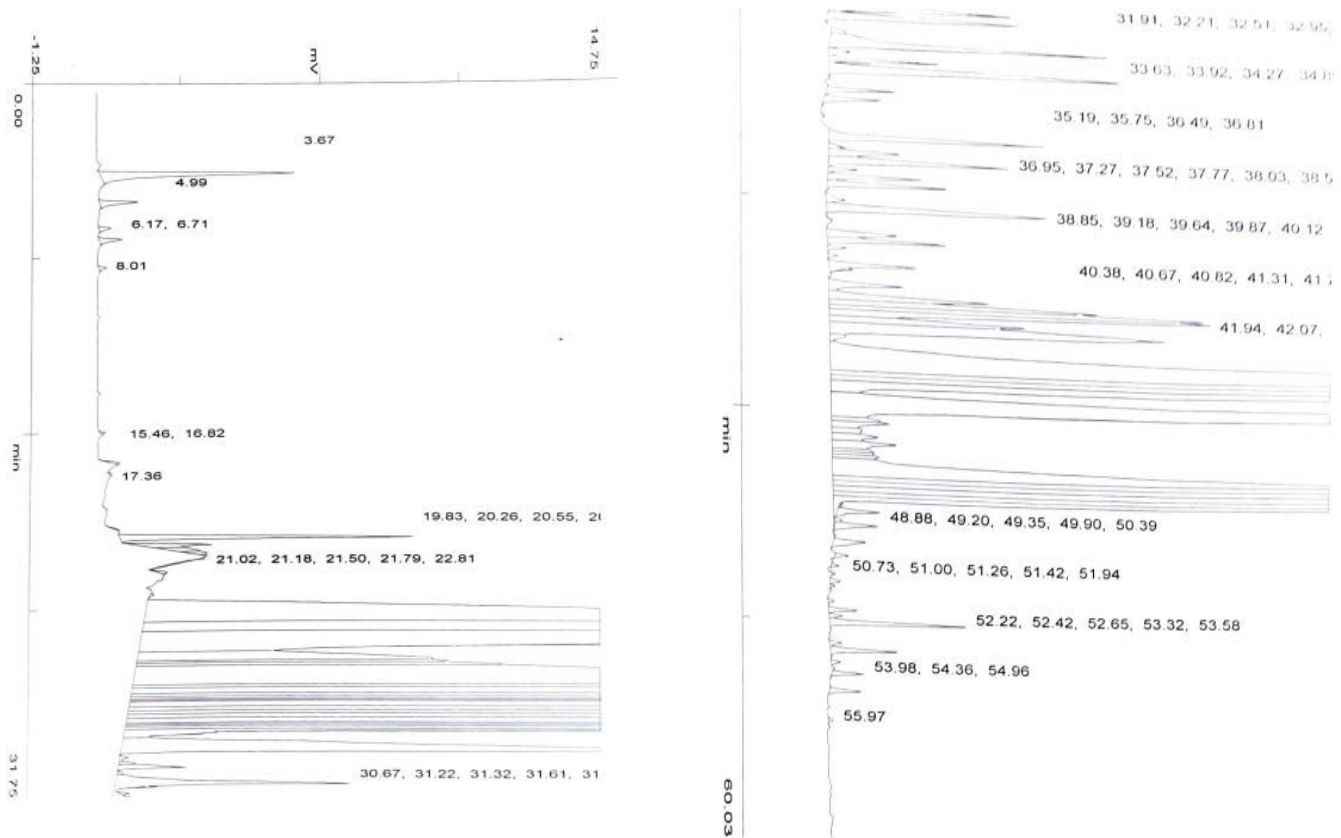


Figure 6: GC-MS profile of *C. deodara* essential oil.

Table 10: List of major constituents of *C. deodara* essential oil observed in GC-MS analysis.

Sl. No.	Constituent Name	Constituent Percentage
1.	Alpha-himachlene	13.83%
2.	Gamma-himachlene	12.00%
3.	Beta-himachlene	37.34%
4.	Deodaron	0.43%
5.	Alpha-atlantone	4.53%
6.	Z, gamma-atlantone	2.77%
7.	E, gamma-atlantone	3.34%
8.	E, alpha-atlantone	10.63%

CONCLUSION

This experimental work is carried out to establish physicochemical data and GC-MS analytical profile of essential oil of Himalayan *C. deodara*. In this experimental work, three different grade size materials, i.e. larger size, medium size and finer size material has been extracted through hydro distillation process. This work is carried out to measure the influence of material size, extraction temperature and hydro distillation time on % age of yield, physical and chemical properties of *C. deodara* essential oil. Higher % age of yield has been observed from medium size material because medium size plant material have optimum surface area to release its constituents, therefore, it releases the essential oil constituents in a gradual manner. Temperature variation has direct impact on physical

as well as chemical properties of the material. Distillation time has also impact on the yield as well as on the physical and chemical properties. The oil collected in first 12 Hr. showed the highest yield, better physical and chemical properties.

It has also been observed that small size material have the lowest yield value. This happened due to evaporation of oil in the environment. In larger size material it has been observed that due to uneven surface of material, water vapours could not penetrate properly into the plant material, therefore, proper quantity of oil is not obtained. It is finally concluded that the medium size material is most suitable to yield higher percentage of essential oil. Temperature variation has direct impact on physical as well as chemical properties of the material. Higher distillation temperature results in alteration of physicochemical properties and nature of components of essential oil. Higher temperatures and pressures result in a 'harsh' aroma and changes the aromatic properties and decrease the oil's therapeutic values. Distillation time has also impact on the yield as well as on the physical and chemical properties of material.

The essential oil obtained after re-processing of extracted material with n-hexane was found to be impure and showed undesirable physical properties such as optical rotation of 47 in contrast to optical rotation 59-69 of pure essential oil. This observation inferred that the oil obtained by solvent extraction method from already processed material by hydro distillation (Grade- B) material was impure moreover; it was not subjected further for physicochemical study. Thin layer chromatography showed 10 spots of different colours in essential oil. The major constituent's percentage obtained after GC-MS analysis as α -himachlene (13.83%), γ -himachlene (12.00%), β -himachlene (37.34%), Deodaron (0.43%), α -atlantone (4.53%), Z, γ -atlantone (2.77%), E, γ -atlantone (3.34%) and E, α -atlantone (10.63%).

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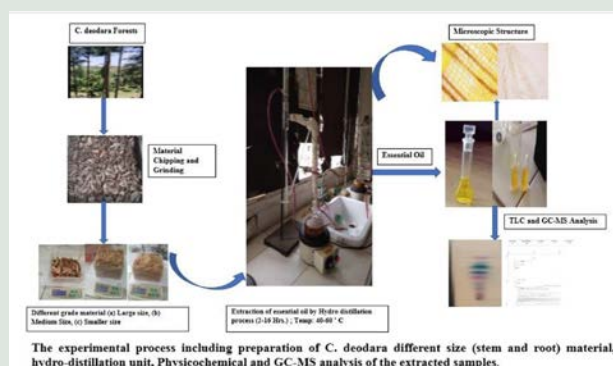
CONFLICT OF INTEREST

The authors declare that they have no conflicts of interests.

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GRAPHICAL ABSTRACT



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