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EPOXIDE-RICH CHEMOTYPE OF *Artemisia indica* FLOWERS: PHYTOCHEMICAL PROFILE, PHYSICOCHEMICAL PROPERTIES, AND ANTIOXIDANT POTENTIAL

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ABSTRACT

Epoxide-rich Artemisia indica essential oil has potential applications in pharmaceuticals, cosmetics, agriculture, and food preservation. This study aligns with Sustainable Development Goal (SDG) 3: Good Health and Well-being by investigating the phytochemical composition, physicochemical properties and antioxidant efficacy of essential oil obtained from the flowers of *Artemisia indica* Willd. collected from Pithoragarh, Kumaon Himalaya, India. The essential oil, extracted by steam distillation, was analysed by GC and GC–MS, revealing 60 constituents, of which 36 compounds (92.82% of the oil) were identified. Oxygenated monoterpenes (77.97%) were dominant, with trans-carane-4,5-epoxide (32.72%), camphor (16.25%), and 1,8-cineole (14.07%) as the major constituents. Physicochemical parameters such as specific gravity (0.924), specific viscosity (0.110), refractive index (1.553), acid value (13.253), and saponification number (34.435) were established as quality indicators. Antioxidant activity, assessed by DPPH and H_2O_2 scavenging assays, showed strong radical scavenging with maximum inhibition of 90.51% and 84.58% at 10 mg/mL, respectively. The IC_{50} values for DPPH and H_2O_2 assays were 2.41 ± 0.042 mg/mL and 3.63 ± 0.032 mg/mL, comparable to standard antioxidants. This is the first comprehensive report on *A. indica* flower oil from the Kumaon Himalaya, highlighting its distinctive epoxide-rich chemotype and potent antioxidant potential. The findings suggest promising applications in food preservation, cosmetics, and natural health products.

Keywords: *Artemisia indica*, 2,2-diphenyl1-picrylhydrazyl, Sesquiterpenes, Antioxidant, Physicochemical
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INTRODUCTION

Medicinal herbs are a foundational pillar of conventional healthcare systems in the high-altitude region and continue to hold high value in codified systems such as Ayurveda, Traditional Chinese Medicine, and local folk practices. Their popularity stems from their natural origin, affordability, and generally favourable safety profile, which has led to growing interest in exploring their biological activities. Among these, the genus Artemisia (family Asteraceae) is particularly significant. Named after the Greek goddess Artemis, it is commonly referred to as “Sage Brush” or “Wormwood.”[1] With more than 500 species reported worldwide, of which about 47 occur in India, Artemisia represents a diverse and economically important group of plants valued for their therapeutic and nutritional potential.[2] Indigenous to the Indian Subcontinent, Artemisia indica Willd, popularly known as "Titepati"-is a resilient perennial herb that grows in the Western and Central Himalayas, especially in the Kumaun hills of Uttarakhand. Despite its widespread use in traditional medicine, this species has received relatively little scientific attention compared to other Artemisia members.[1] Its ethnomedicinal uses are varied: local communities employ it to treat chronic fever, dyspepsia, and hepatobiliary disorders, while its leaves and stems are valued for antihelminthic, antiseptic, and antispasmodic properties. In Nepal, leaf juice is used for diarrhoea, dysentery, abdominal pain, and skin ailments, while the tender shoots are consumed as a leafy vegetable or flavouring agent.[3,4] Similarly, the Garo tribes of Meghalaya fry the young shoots as a food source, and reports also highlight its insecticidal and culinary applications.[5,6] Beyond these traditional uses, experimental studies suggest diverse pharmacological activities, including antioxidant, cytotoxic, antimicrobial, antimalarial, antipromastigote, and antidiabetic effects.[7-11] Although the essential oil

composition of *A. indica* has been examined in aerial parts (leaves and stems) across different regions[12-18], the phytochemistry and antioxidant potential of its flowers remain largely unexplored. To address this gap, the present study investigates the yield, physicochemical properties, the chemical nature and antioxidant effects of *A. indica* flower essential oil sourced from Pithoragarh in the Kumaon Himalaya, Uttarakhand. By focusing on floral tissues, our work provides novel insights into a lesser-studied plant part and contributes to a deeper understanding of this species' therapeutic potential.

EXPERIMENTAL

Plant Material and Authentication

Fresh flowers of *Artemisia indica* Willd. (2kg) were collected in September 2022 from Pithoragarh, Uttarakhand, India (altitude: 1645 m; 29.58°N, 80.22°E). The plant species was authenticated at the Systematic Botany Division of the FRI, Dehradun (Accession No. 1324).

Essential Oil Extraction

The flowers were steam distilled for 6 hours using a Clevenger-type device. The oil that was taken out was dried over anhydrous sodium sulphate and kept in amber vials in a BOD incubator at 4 °C until it could be tested again.

Gas Chromatography (GC) and GC–MS Analysis

A Shimadzu GC QP 2010 was used for GC chemical profiling. The RTx-5 MS capillary column (1009701), measuring 30.0 m in length, the column featured a 0.25 mm internal diameter and a 0.25 mm film thickness. The temperature profile of the oven started at 50 °C, was maintained for two minutes, and then rose at a rate of 3 °C per minute to 200 °C. The temperature was raised by 10 °C/minute up to 280 °C and held constant for eight minutes. The detector and injector were operated at 260 °C and 250 °C, respectively, with nitrogen as the carrier gas flowing at 1.13 mL min^{-1}. GC-MS was performed using a Shimadzu GC QP 2010. This instrument was equipped with an RTx-5 MS capillary column, 1009701, which was 30.0 m long, 0.25 mm wide, and had a 0.25 mm film thickness. The oven was programmed to start at 50 °C, hold for 2 minutes, and then increase to 200 °C at a rate of 3 °C/min. It then rose to 280 °C at a rate of 10 °C/min, and finally, it was kept at 280 °C for 8 minutes. The injector temperature was 250 °C, whereas the detector temperature was 260 °C. Nitrogen was chosen as the carrier gas, having a flow rate of 1.13 mL/min. The analysis was carried out under the same chromatographic conditions as well as electron impact ionisation of 70 eV. The identification of constituents involved comparing mass spectra with the NIST14, WILEY08, and FFNSC2 libraries, along with calculating retention indices (RI) using C_6–C_{33} n-alkanes as a reference.[19] Only compounds having a spectral match of 85% or above were approved. The relative abundances were reported as the percentage of the peak areas.

Physicochemical Characterization

The essential oil's characteristics were assessed by its colour, yield, specific gravity (SG), specific viscosity (SV), refractive index (RI), optical rotation, acid number (AN), and saponification number (SN), adhering to established methodologies. Each parameter was measured three times, and the average values are shown.

Characterisation of the Physical Properties of Extracted Essential Oil from *Artemisia indica* Flowers Oil [20-22]

Characteristics give a baseline for oil aptness. The parameters of the oil NM-1 studied are colour, yield, (SG), (SV), (RI), (OR), (AN) and (SN).

Specific Gravity (SG)

Initially, a clean and dry pycnometer was weighed. It was subsequently filled with distilled water up to the calibration mark and reweighed. The mass of the distilled water was then determined. After emptying it and drying it in an oven, the pycnometer was filled to the brim with the essential oil under test. The following formula is used to calculate the weight of the pycnometer with oil:

Specific gravity (SG) = (Weight of the oil sample) / (Weight of an equivalent volume of water).

Specific Viscosity (SV)

The Ostwald viscometer was dried and cleaned. The viscometer's bulb was then filled with 10 ml of a 1% solution of the oil under investigation, dissolved in acetone. The viscometer was kept undisturbed while the solution was drawn to the upper mark. The duration of the solution's flow from the viscometer's upper to lower mark was noted. After that, the viscometer was filled with pure acetone, and the procedure was repeated. The relative viscosity of the essential oil was evaluated using the equation shown below:

$$\eta \text{ Rel} = \text{Flow time of 1\% solution of essential oil in solvent} / \text{Flow time of pure solvent}$$

Relative viscosity was calculated using the equation provided below:

$$\eta \text{ sp} = \eta \text{ rel}^{-1}$$

Where, η sp = SV, η rel $^{-1}$ = RV

Refractive Index (RI)

The refractive indices of the oils were obtained using an Abbe-type refractometer. The oil was put in between the two prisms of the device, which were cleaned using alcohol. First, the screw heads were properly tightened. After a few minutes, the refractometer was allowed to reach the same temperature as the oil. A wider coloured band was then obtained by moving the refractometer slightly back and forth. Tuning of the screw was done until the coloured bands were turned into a colourless line. The line was adjusted manually to match the cross-hairs intersection point. Refractive index was now read from the sector directly.

Acid Number (AN)

In a conical flask, the essential oil sample (1 ml) was first combined with 15 ml of 95% ethanol. This mixture was then titrated using a 0.1 N sodium hydroxide solution. Three drops of 1% phenolphthalein indicator were introduced to the flask during the titration process. The endpoint was identified when a light pink colour appeared and remained unchanged for at least 10 seconds. A blank experiment was also performed simultaneously under the same conditions but without adding the oil sample. The difference in the amount of sodium hydroxide used for the sample and the blank was recorded to determine the alkali required for neutralisation. Using this value, the acid number of the oil was calculated with the help of the given formula:

$$\text{Acid number (AN)} = (\text{Volume of 0.1 N alkali consumed} / \text{Weight of 1 ml of essential oil}) \times 5.6$$

Saponification Number (SN)

The screw adjustments on the compensator made the coloured band change into a colourless line.

To determine the refractive index, the compensator was adjusted until the coloured band transformed into a distinct colourless line. Subsequently, this colourless line was carefully aligned with the intersection point of the crosshairs, and the refractive index value was read directly from the instrument's sector scale. For the determination of the saponification value, 1 mL of essential oil and 10 mL of 0.5 N alcoholic sodium hydroxide were added to a 100 mL saponification flask. The flask was fitted with an air-cooled condenser (1 m × 1 cm). The reaction mixture was refluxed in a water bath for 1 h and subsequently cooled to room temperature. The unreacted alkali was then titrated with 0.5 N hydrochloric acid (HCl) solution using 1% phenolphthalein solution (three drops) as the indicator. A blank test was conducted in parallel using the same procedure. Based on the difference between the titration volumes of the blank and the sample, the saponification value of the essential oil was evaluated using the formula provided below:

$$\text{Saponification number (SN)} = (\text{Volume of 0.5 N acid consumed} / \text{Weight of 1 ml of essential oil}) \text{ X } 28.05.$$

Optical Rotation (OR)

A 100 mm polarimeter tube filled with the essential oil sample was inserted into the polarimeter between the polariser and analyser. The analyser was rotated until equal illumination was observed in both halves of the viewing field. Small adjustments were made to achieve the exact balance point where the intensity difference between the two fields was minimised. The direction of optical rotation was determined according to the analyser movement from the zero mark, with clockwise and anticlockwise rotations corresponding to dextrorotatory and laevorotatory characteristics, respectively. After proper alignment, the optical rotation angle was measured from the instrument scale, while the vernier scale was used to

obtain minute readings with improved precision. The measurement was repeated to verify reproducibility, and the difference between successive readings was maintained within 5'.

Antioxidant Assays

DPPH Radical Scavenging

With a few minor adjustments, the Brand-Williams *et al.* approach was used to calculate the scavenging activity.[23,24] A 0.5 mM DPPH solution was combined with different amounts of essential oil (made in methanol) and allowed to sit in the dark for half an hour. The absorbance at 517 nm was measured. The positive control used was ascorbic acid. This is how the % inhibition was computed:

$$\text{DPPH inhibition percentage} = (A_o\text{-}A_t)\ /Ao\ x100$$

Where A_o is the absorbance of the control, and A_t is the absorbance of the sample.

Hydrogen Peroxide (H_2O_2) Scavenging

The scavenging ability of the oil against H_2O_2 was evaluated by mixing the sample with 40 mM H_2O_2 prepared in phosphate buffer (pH 7.4). After 10 min incubation, absorbance was measured at 230 nm. BHT was used as the standard. The percentage inhibition was calculated using the same formula as above.[25,26]

Statistical Analysis

All experiments were conducted in triplicate, and the data are expressed as mean ± standard deviation (SD). Statistical analyses were performed using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test to determine significant differences among the groups. Differences were considered statistically significant at $p < 0.05$. All statistical analyses were carried out using SPSS software (version 16.0).

RESULTS AND DISCUSSION

Yield and Physicochemical Properties

Steam distillation of *A. indica* flowers yielded 0.7% (v/w) of a white-coloured essential oil. The oil exhibited a specific gravity of 0.924 and a refractive index of 1.553, while its specific viscosity was measured as 0.110. The acid value (13.253) and saponification number (34.435) further characterised the oil, providing baseline data for its physicochemical quality (Table-1).

Table-1: Characteristics Providing Baseline Data for its Physicochemical Quality

Parameter studied	Value NM-1
Yield	0.7%
Colour	White
SG	0.924
SV	0.110
RI	1.553
AN	13.253
SN	34.435

Phytochemical Composition of the Essential Oil

36 chemicals or 92.82% of the total oil content were found using GC and GC–MS analysis. Monoterpenoids were the dominant class with oxygenated monoterpenes (77.97%) far outweighing monoterpene hydrocarbons (7.37%). Sesquiterpenes and their oxygenated derivatives were present in lower amounts (2.64% and 4.84%, respectively). The major constituents were *trans*-carane-4,5-epoxide (32.72%), camphor (16.25%), and 1,8-cineole (14.07%), supported by notable amounts of chrysanthenone (4.02%), eudesmol (4.24%), lavandulyl acetate (2.72%), camphene (2.51%), and 4-terpineol (2.32%). Collectively, these results point to an epoxide-rich chemotype unique to the Kumaon flower oil. The GC chromatogram illustrating compound distribution is presented in Fig.-1 and detailed compositional data are summarized in Table-2.

Table-2: Chemical Composition of the Essential Oil of *A. indica* Flowers

S. No.	Chemical compounds	R.T.	GC%	R. $I_{cal.}$	R. $I_{lit.}$
1	Tricyclene	8.31	0.12	925	926

2.	α-Thujene	8.447	0.03	929	930
3.	α-Pinene	8.729	0.78	936	939
4.	Camphene	9.387	2.51	953	954
5.	Sabinene	10.313	0.62	975	975
6.	(-)-β-pinene	10.502	0.13	980	979
7.	Myrcene	11.037	0.66	992	990
8.	α-phellandrene	11.818	0.46	1009	1002
9.	α-Terpinene	12.012	0.91	1015	1017
10.	1,8-cineol	13.021	14.07	1039	1031
11.	β-Ocimene	13.575	0.18	1051	1050
12.	γ-Terpinene	14.111	1.10	1063	1059
13.	*trans*-Sabinene hydrate	16.093	0.60	1103	1098
14.	*Trans*-Caran,4,5-epoxi-	17.124	32.72	1127	1125
15.	Chrysanthenone	17.283	4.02	1131	1127
16.	Menth-2-en-1-ol <trans, para>	17.446	0.94	1134	1140
17.	Camphor	18.203	16.25	1150	1146
18.	Isoborneol	19.195	0.95	1171	1160
19.	4-Terpineol	19.970	2.32	1186	1177
20.	α-Terpineol	20.400	1.76	1195	1188
21.	Verbenone	21.175	0.44	1213	1205
22.	*Trans*-Piperitol	21.34	0.68	1216	1208
23.	Bornyl acetate	24.249	0.21	1280	1288
24.	Lavandulyl acetate	24.613	2.72	1287	1290
25.	α-terpinyl acetate	27.500	0.29	1353	1349
26.	α-Copaene	28.570	0.89	1378	1376
27.	(E)-Caryophyllene	30.431	0.61	1421	1419
28.	α-Humulene	31.918	0.10	1457	1454
29.	Germacrene-D	33.008	0.57	1483	1485
30.	γ-(Z)-Bisobolene	34.309	0.12	1512	1515
31.	δ-Cadinene	34.534	0.17	1519	1523
32.	Caryophyllenoid	37.057	0.18	1583	1582
33.	Epicedrol	38.669	0.06	1627	1619
34.	Agarospirol	39.505	0.17	1649	1648
35.	Eudesmol	39.827	4.24	1656	1650
36.	Valeranone	40.591	0.37	1676	1675

Monoterpene hydrocarbons	7.37
Oxygenated monoterpenes	77.97
Sesquiterpene hydrocarbons	2.64
Oxygenated sesquiterpenoids	4.84
Unidentified	7.18
Total identified	92.82
Yield	0.7%

R. $I._{cal.}$ = Retention indices experimental (calculated)
R. $I._{lit.}$ = Retention indices experimental (literature)
RT = Retention time

Antioxidant Activity

Phytochemical Composition and Chemotypic Diversity

The predominance of oxygenated monoterpenes in the floral oil of *A. indica* aligns with the broader phytochemical trends observed across the genus, where camphor, cineole, and artemisia ketone are frequently reported as major constituents. However, the relative abundance of *trans*-carane-4,5-epoxide (32.72%) is notable and distinguishes the present oil from previously studied chemotypes. For example, studies conducted in Kashmir[9] identified artemisia ketone (42.1%) and germacrene B (8.6%) as dominant constituents, Kumaun[12,13] unveiled *β*-caryophyllene (8.3%), germacrene B (6.3%), and caryophyllene

oxide (6.5%) as dominant components and Bhatt *et al.* (2025) identified 1,8-cineole (19.2%), camphor (15.7%), and *cis*-chrysanthenol (13.5%) in oils from Garhwal,[18] while oils from Nepal were characterized by the presence of ascaridole (15.4%) and isoascaridole (9.9%).

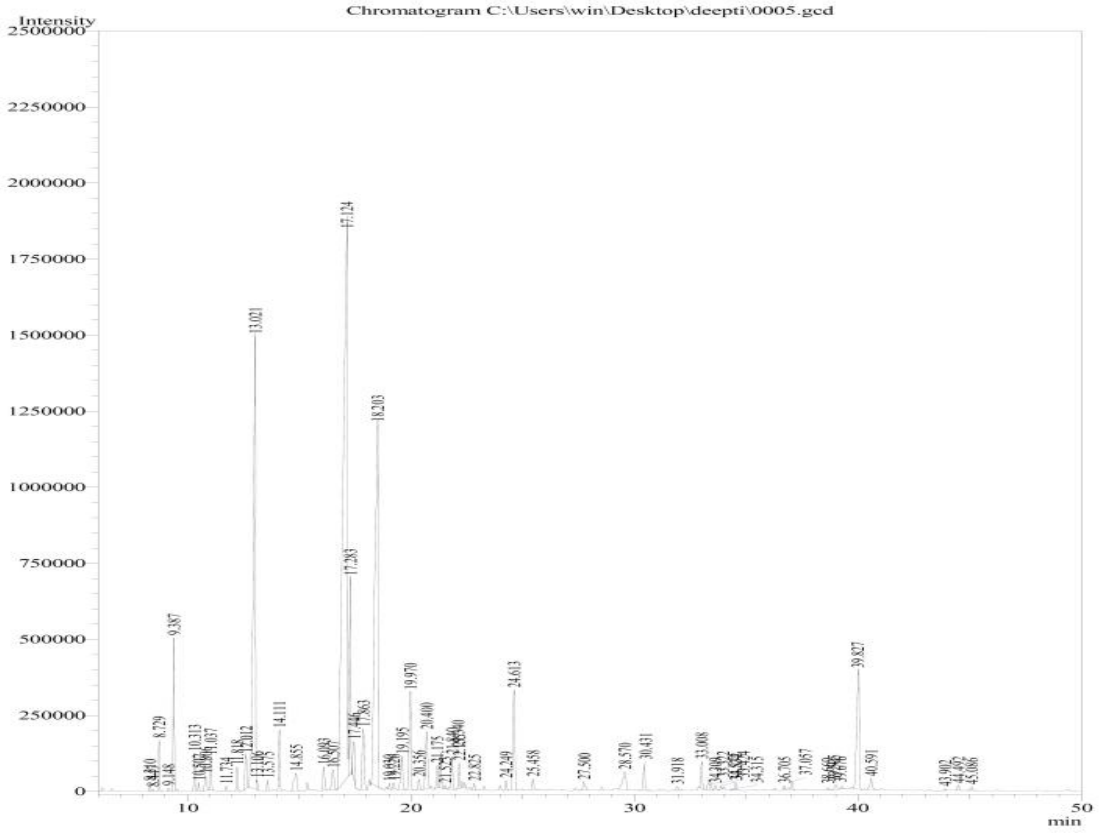

Fig.-1: GC Chromatogram of *Artemisia indica*

Similarly, chemotypes from Garhwal[17] were rich in davanone (30.8%), whereas oils from West Bengal[16] exhibited camphor (13.0%) and caryophyllene oxide (10.9%) as major components. These variations emphasise the significant role of geography, altitude, and plant part in shaping the essential oil profile of *A. indica*. The present findings therefore highlight the importance of documenting regional chemotypes, both for chemotaxonomic purposes and for optimising therapeutic applications.

Antioxidant Potential and Phytochemical Correlation

The antioxidant assays conducted in this study demonstrated potent free radical scavenging activity of *A. indica* flower oil, with DPPH and H_2O_2 inhibition rates of 90.51% and 84.58% at 10 mg/mL as compared to 93.48 % inhibition by ascorbic acid and 88.45% by BHT at 0.5 mg/mL (Fig- 2,3,4,5). The IC_{50} values were found to be 2.41 ± 0.042 mg/mL for DPPH and 3.63 ± 0.032 mg/mL for H_2O_2 were substantially lower than those reported for aerial-part oils of *A. indica* by Bhatt *et al.* (2025)[18], who observed IC_{50} values of 18.03 µL/mL and 29.50 µL/mL for DPPH and H_2O_2 assays, respectively. This indicates that the flower oil possesses 7-8 times greater radical scavenging efficiency compared to aerial-part oils. The pronounced activity is presumably due to the high concentration of oxygenated monoterpenes, primarily due to *trans*-carane-4,5-epoxide, camphor, and 1,8-cineole, all of which are known to contribute to redox balance and oxidative stress mitigation. Previous reports have also established the antioxidant potential of *A. indica* essential oils, but largely in the context of aerial plant parts. Rashid *et al.* (2013) documented moderate antioxidant capacity in oils from Kashmir[9], while Satyal *et al.* (2012)[15] reported bioactivity associated with volatile fractions of Nepalese *Artemisia* species. The markedly stronger activity observed in the present study suggests that floral tissues may be a superior source of bioactive oils, possibly due to their metabolic role in plant defence and pollinator attraction.

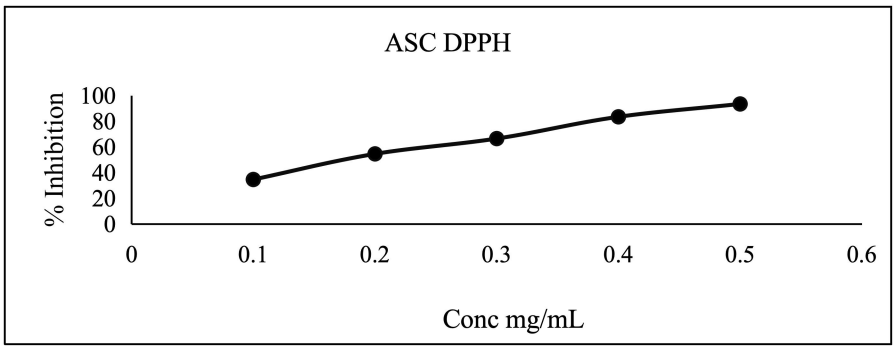

Fig.-2: A Curve of Percentage Inhibition Versus Different Concentrations of Ascorbic Acid

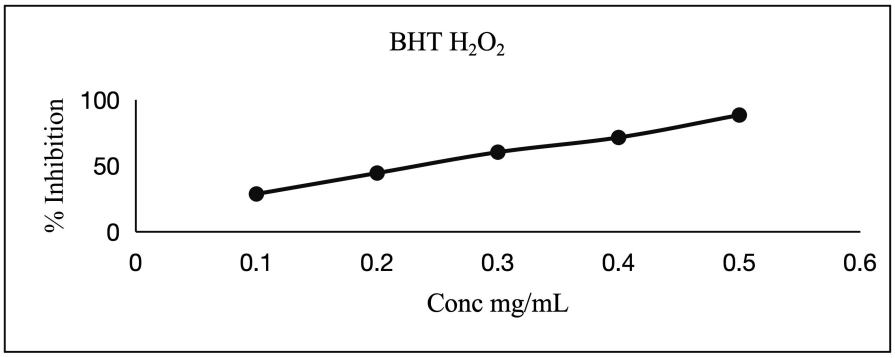

Fig.-3: A Curve of Percentage Inhibition versus Different Concentrations of BHT

Implications for Therapeutic and Industrial Application

The strong antioxidant potential of *A. indica* flower oil positions it as a promising candidate for diverse applications. Natural antioxidants play a critical role in countering oxidative stress-related disorders including cardiovascular and neurodegenerative diseases as well as in delaying the onset of cellular ageing. Beyond therapeutic applications, essential oils with high radical scavenging capacity are increasingly sought for use in food preservation and cosmetic formulations as alternatives to synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). Given its distinctive chemotype and potent bioactivity, the flower oil of *A. indica* could thus serve as a valuable natural ingredient in nutraceutical and cosmeceutical industries.

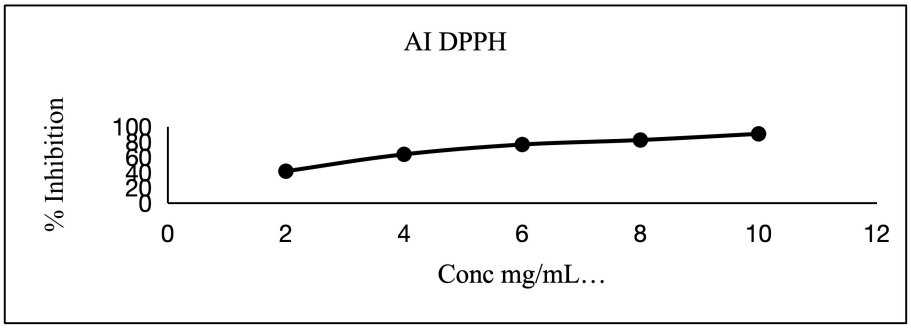

Fig.-4: A Curve of Percentage Inhibition Versus Different Concentrations of *A. indica* Flowers' Essential Oil

Limitations and Future Perspectives

The present findings highlight the antioxidant potential of *A. indica* flower oil, certain limitations must be addressed. The study relied solely on in vitro assays, which, though useful for initial screening cannot fully replicate the complexity of biological systems. Furthermore, the safety profile and pharmacological efficacy of the oil have not yet been evaluated in vivo. Another limitation is the absence of quantitative assays for total phenolic content (TPC) and total flavonoid content (TFC), which would provide additional support for the observed antioxidant activity. Future research should concentrate on mechanistic studies into the oil's mode of antioxidant action, cytotoxicity tests to determine safety margins, and in vivo assessments of the oil's efficacy.

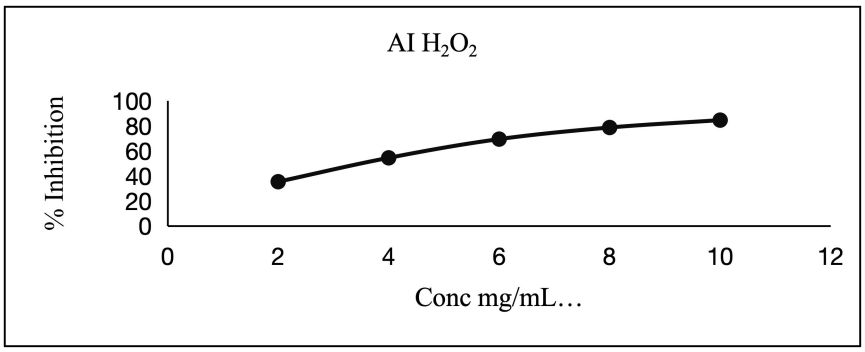

Fig.-5: A Curve of Percentage Inhibition Versus Different Concentrations of *A. indica* Flowers' Essential Oil

To further investigate chemotypic variance across geographic regions, chemometric techniques like principal component analysis (PCA) and hierarchical cluster analysis (HCA) could be used. Such multidimensional studies will not only enhance our understanding of *A. indica* but also facilitate its integration into standardised therapeutic and industrial formulations.

CONCLUSION

This study provides the first comprehensive characterisation of the essential oil from *Artemisia indica* flowers collected in the Kumaon Himalaya, revealing an epoxide-rich chemotype dominated by trans-carane-4,5-epoxide, camphor, and 1,8-cineole. The predominance of oxygenated monoterpenes (77.97%) distinguishes this floral oil from previously reported aerial-part chemotypes across different Himalayan and Indian regions, underscoring the strong influence of geography and plant part on phytochemical diversity. In vitro assays demonstrated potent antioxidant activity, with IC_{50} values for DPPH and H_2O_2 scavenging several-fold lower than those of aerial oils, suggesting that floral tissues may be a superior source of bioactive compounds. These results demonstrate *A. indica* flower oil's potential as a natural antioxidant with intriguing uses in food preservation, nutraceutical, and cosmetic formulations. Nonetheless, the present work is limited to in vitro evaluations. Future studies incorporating in vivo pharmacological assays, safety profiling, and mechanistic investigations are essential to validate its therapeutic relevance. Integration of chemometric tools to explore chemotypic variation will further strengthen the case for developing *A. indica* flower oil into standardised formulations.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

The present work is related to *SDG-3: Good Health and Well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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