Phytochemical Profiles and Antifungal Activity of Essential oil from *Artemisia vulgaris* Grown at Different Altitudes of Nepal

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Abstract

Purpose: This study aimed to analyze the components of essential oil extracted from Artemisia vulgaris grown at different altitudes and evaluate its antifungal activity.
Methods: Essential oils were extracted by using hydro distillation method. The essential oil analyses were performed by Gas Chromatography-Mass Spectrometry technique. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were used for the multivariate analyses. The antifungal activity of essential oil was evaluated against Sclerotinia sclerotiorum by poisoned food technique. The collected data were entered into Microsoft Excel 2019 program and variables were statistically analyzed using ANOVA with the help of R studio.
Results: The highest essential oil yield percentage was observed in the plant samples collected from 1200 m altitude with an average yield of 0.95± 0.14 v/w %. Altogether 73 components of essential oil were visualized. Among these components Amorphene $< gamma- >$ showed the highest peak area (10.58 %) in the plant samples collected from 1800 m altitude. Similarly, Amorphene $< gamma- >$ was present at highest concentration (10.35 %) in 1500 m, (11.88%) in 900 m, (13.85 %) in 600 m, and (10.46 %) in 300 m altitude plant samples. PCA analysis gave rise to four different clusters and evaluation of HCA showed the amount closeness of components between the plant samples collected from different altitudes. The extracted essential oils showed highest antifungal activity against Sclerotinia sclerotiorum at 350 ppm (100 %) which was reduced with a decrease in the concentration of essential oil.
Conclusion: The phytochemical profiles of the essential oil showed 73 components and the essential oil of Artemisia vulgaris possessed antifungal activities against Sclerotinia sclerotiorum.

Keywords: Essential oil, Gas Chromatography-Mass spectrometry, Hydro-distillation Phytochemical profile

1 Introduction

Artemisia vulgaris is a perennial weed that is heavily invasive in the landscape, agronomic environment, waste sites, and along roadsides (Barney et. al, 2005). A. vulgaris, is typically recognized for its volatile oil and many studies have shown that essential oil of this species possess antioxidant, hypolipidemic, antibacterial and antifungal properties (Natividad et al., 2011, Khan, K. A., 2015). A. vulgaris is one of the traditional medicinal plants used in Nepal for the treatment of several diseases like mouth ulcer, fever, diarrhea etc. (Joshi et al., 2016).
Variation in the altitudes of plant growth plays an important role in the production of plant secondary metabolites. The formation of different plant metabolites depends on the differences in wind speed, snowfall, oxygen content, drying soils, frozen soil, atmospheric pressure, and some others of the regions where the plant is growing (Körner, 2003).
The fungal pathogens are becoming one of the major problems and challenges in cultivation of agricultural crops around the world. White mold caused by *Sclerotinia sclerotiorum* is one of the most common fungal diseases of several plant species and is capable of colonizing over 400 plant species found worldwide. The majority of these species are dicotyledonous, although a number of agriculturally significant monocotyledonous plants are also hosts (Pressete et al., 2019). Nowadays use of synthetic fungicide is being common practice to control such fungus and extensive applications of such synthetic fungicides have resulted in establishment of fungicide-resistant disease. Therefore, interest in essential oils and extracts of plant metabolites as bio-fungicides is being increased. Antifungal activities of essential oils of A. vulgaris from different countries have been studied but not much studied against plant pathogenic fungus *Sclerotinia sclerotiorum* in Nepal.
The objective of this study was to explore the chemical components of essential oil of A. *vulgaris* grown at different altitudes of Bagmati province of Nepal to correlate with its in vitro antifungal activity against *Sclerotinia sclerotiorum.*

2 Materials and methods

2.1 Plant materials and extraction of essential oil

Plant samples of A. vulgaris were collected during the mature vegetative stage from different geographical regions of Bagmati Province as shown in **Table 1.**
Essential oil was extracted by using hydro distillation method. To extract the essential oil, leaves of A. vulgaris were air dried at room temperature for 24 hours. These were then sliced into small pieces and subjected to extraction with water by hydro-distillation method for 3 hours using a Clevengers-type apparatus. The essential oil thus obtained was dried over anhydrous sodium sulphate and stored at 4 ºC until use. The yield percentage of the essential oil was calculated as follows:
$\text{Yield } \% = \frac{\text{Volume of the dry essential oil in ml}}{\text{Weight of the plant sample taken in grams}} \times 100$

Table 1: Geographical regions and altitudes of collection site of plant materials of *A. vulgaris*

Province	Geographical Regions	Altitudes	No of samples
Bagmati	Siwaliks (Sub-tropical)	1800 m	3
		1500 m	3
		1200 m	3
		900 m	3
		600 m	3
	Terai (Sub-Tropical)	300 m	3

2.2 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The component of volatile oil samples extracted from the leaves of A. vulgaris were identified using Gas Chromatography–Mass Spectrometry (GC-MS) analysis. The analysis was performed using GC-2010 plus gas chromatograph coupled to a GCMS-QP2010 SE mass spectrometry detector and equipped with an AOC20i autoinjector. A capillary RTX-5MS column was used for separation. Helium (at a flow rate of 1.0 mL/min) was used as carrier gas. Temperature was kept at 60 ºC for 5 min and programmed to reach 240 ºC at the rate of 3 ºC per min. The samples were injected at the injected temperature of 250 ºC. The injection volume was 1.0 µL in 1:30 split ratio. The mass spectra were obtained with electron impact ionization (70 eV) at full scan mode (40 to 500 m/z), using an ion source at 200 ºC. The compounds were identified by comparing retention indices (RI) and mass spectra with data from the FFNSC 4.O, FFNSC 1.3 library, and the literature.

2.3 *In-vitro* antifungal assessment

Assessment of the antifungal activity of the essential oil against *Sclerotinia sclerotiorum* was carried out using the poisoned food technique as described by Euloge et al. (2012). Different concentrations (100 ppm, 150 ppm, 200 ppm, 250 ppm, 300 ppm, 350 ppm) were prepared by adding the appropriate amount of essential oil containing 0.5% (v/v) of Tween 80 to cooled molten PDA (45°C) followed by manual rotation in a sterile Erlenmeyer flask to disperse the oil in the medium. Twenty milliliters of the medium were dispensed into sterile petri dishes (9 cm in diameter). The medium was allowed to solidify at room temperature (23 ± 2°C) for about one hour. Agar discs with mycelia (6 mm in diameter) were cut from the periphery of actively growing regions of pure cultures using a sterile cork borer and aseptically inoculated at the center of the petri plates. Control plates (without the essential oil) were inoculated following the same procedure. Three replicates were maintained for each treatment and the plates were incubated at 28°C. The fungal colony diameter readings were taken after 24 hours and 48 hours of incubation. The percentage inhibition of the mycelial growth of the test fungi by the essential oil was calculated using the formula by Philippe et al. (2012).
Mycelia growth inhibition (%) $= \frac{\text{dc - dt}}{\text{dc}} \times 100$
Where:

$$\text{dc} = \text{mean diameter for control} - 6 \text{ mm},$$
$$\text{dt} = \text{mean diameter for treated mycelium} - 6 \text{ mm}.$$

2.4 Statistical Analysis

The collected data were entered into Microsoft Excel 2019 program and variables were statistically analyzed using ANOVA with the help of R studio. ANOVA was constructed to test the significant difference for each parameter at a 5% level of significance. Duncan's Multiple Range test was conducted for mean separation.

2.5 Multivariate Data Analysis

Principal component analysis (PCA) of essential oil was performed using XLSTAT 2024 with two principal component (PC) variables.

3 Results

3.1 Yield percentage of essential oil:

The yield percentage of essential oil from the collected plant samples are as shown in **Table 2**. The highest yield percentage was observed in the plant samples collected at 1200 m altitude and average yield was 0.95± 0.14 v/w %. However, lowest yield was observed in the samples collected from 600 m altitude (0.57 ± 0.04 v/w %).

Table 2: Yield percentage of essential oil extracted from *A. vulgaris* plant samples from different altitudes.

Altitude	Essential oil yield, v/w (%)
300 m	0.58 ± 0.06
600 m	0.57 ± 0.04
900 m	0.62 ± 0.04
1200 m	0.95 ± 0.14
1500 m	0.92 ± 0.06
1800 m	0.88 ± 0.05

Results are expressed as mean ± SD of triplicate measurements

3.2 Identification of essential oil components

GC–MS analysis of the essential oil extracted from the aerial part of the A. vulgaris showed the presence of different phytochemicals. The MS Library used for the comparison was FFNSC 1.3, NIST 2017, and from it, the oil components were identified based on their retention indices (RI) and by comparison of their mass spectra fragmentation patterns. Altogether 73 components of essential oil were visualized and among them 20 components (highest expression) with their peak area percentage are shown in Table 3 & 4.

Table 3: Volatile constituents identified from the essential oil of the aerial parts of *A. vulgaris* collected from 1800 m, 1500 m & 1200 m altitudes.

S.N.	Components 1800 m	Area %	Components 1500 m	Area %	Components 1200m	Area%
1	Amorphene <gamma->	10.58	Amorphene <gamma->	10.35	Cadinene<gamma->	14.84
2	Camphor	9.5	Camphor	9.09	Caryophyllene (E)->	9.04
3	Eucalyptol	7.27	Eucalyptol	6.91	Camphor	8.68
4	Caryophyllene (E)->	6.91	Caryophyllene (E)->	6.56	Thujone<alpha->	6.52
5	Thujone <alpha->	6.22	Thujone <alpha->	5.96	Eucalyptol	6.41
6	Sabinene	3.34	Sabinene	3.41	Cadinene <de1ta->	5.09
7	Thujone <beta->	3.07	Thujone <beta->	3.02	Chrysanthenone	4.54
8	Maaliene <beta->	2.64	Maaliene <beta->	2.57	Thujone <beta->	4.15
9	Chrysanthenone	2.4	Bicyclogermacrene	2.47	Gurjunene<alpha>	3.78
10	Borneol	2.39	Chrysanthenone	2.33	Bicyclogermacrene	3.25
11	Bicyclogermacrene	2.38	Borneol	2.32	Humulene <alpha->	2.66
12	Cadinene <de1ta->	2.2	Camphene	2.24	Sabinene	2.61
13	Humulene <alpha->	2.13	Cadinene <de1ta->	2.15	Amorphene <epsylon->	2.28
14	Camphene	2.11	Humulene <alpha->	2.14	Cymene <para->	1.96
15	Cymene <para->	2.05	Curcumene <gamma->	2.12	Pinene <a1pha->	1.68
16	Terpinen-4-ol	2.04	Cymene <para->	2.02	Borneol	1.54
17	Curcumene <gamma->	2.03	Terpinen-4-ol	2	Caryophyllene oxide	1.43
18	Pinene <a1pha->	1.83	Pinene <a1pha->	1.95	Camphene	1.39
19	Sabinene hydrate <cis->	1.67	Sabinene hydrate <cis->	1.74	Copaene <a1pha->	1.37
20	Piperitone	1.56	Piperitone	1.56	Amorphene <gamma->	1.35

Table 4: Volatile constituents identified from the essential oil of the aerial parts of *A. vulgaris* collected from 900 m, 600 m & 300 m altitudes.

S.N.	Components 900 m	Area %	Components 600 m	Area %	Components 300 m	Area%
1	Amorphene <gamma->	11.88	Amorphene <gamma->	13.85	Camphor	13.64
2	Camphor	7.65	Camphor	8.54	Eucalyptol	10.59
3	Caryophyllene (E)->	6	Caryophyllene (E)->	7.41	Amorphene <gamma->	10.46
4	Chrysanthenone	5.43	Eucalyptol	5.26	Thujone <alpha->	9.14
5	Eucalyptol	5.26	Thujone <alpha->	5.03	Caryophyllene (E)->	7.76
6	Thujone <alpha->	4.33	Chrysanthenone	4.32	Thujone <beta->	4.5
7	Sabinene	3.27	Thujone <beta->	3.53	Sabinene	3.53
8	Bicyclogermacrene	2.87	Bicyclogermacrene	3.25	Chrysanthenone	3.3
9	Cadinene <de1ta->	2.86	Cadinene <de1ta->	2.93	Gurjunene<alpha>	2.76
10	Curcumene <gamma->	2.65	Sabinene	2.83	Cymene <para->	2.54
11	Verbenone	2.48	Maaliene <beta->	2.79	Camphene	2.42
12	Thujone <beta->	2.41	Humulene <alpha->	2.73	Cadinene <de1ta->	2.32
13	Cymene <para->	2.14	Cymene <para->	1.97	Terpinen-4-ol	1.93
14	Humulene <alpha->	2.12	Pinene <a1pha->	1.79	Borneol	1.89
15	Pinene <a1pha->	2.12	Borneol	1.79	Pinene <a1pha->	1.86
16	Borneol	2.05	Curcumene <gamma->	1.73	Humulene <alpha->	1.72
17	Maaliene <beta->	2.03	Camphene	1.6	Sabinene hydrate <cis->	1.7
18	Caryophyllene oxide	1.61	Verbenone	1.58	Muurolene<gamma->	1.56
19	Cubebene <beta->	1.51	Caryophyllene oxide	1.56	Bicyclogermacrene	1.46
20	Pinocarveol <trans->	1.45	Pinocarveol <trans->	1.34	Caryophyllene oxide	1.4

Amorphene $< gamma- >$ was present at higher concentration in all samples except in samples collected from 1200 m and 300 m altitude. At 1200 m altitude Cadinene¡gamma¿ showed the highest peak and Camphor showed the highest peak in the sample collected from 300 m altitude.

3.3 Multivariate Data Analysis Using Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA)

PCA and HCA were evaluated for the area percentage of the identified compounds. PCA analysis gave rise to four different clusters (Figure 1), where the variance was 40.8% for principle component 1 (PC1) and 28.6% for principle component 2 (PC2). Plants collected from 1800 m altitude and 1500 m altitude were in one cluster. Similarly, plants collected from 900 m altitude and 600 m altitude were in one cluster, while plants collected from 1200 m altitude and 300 m altitude were in different clusters.

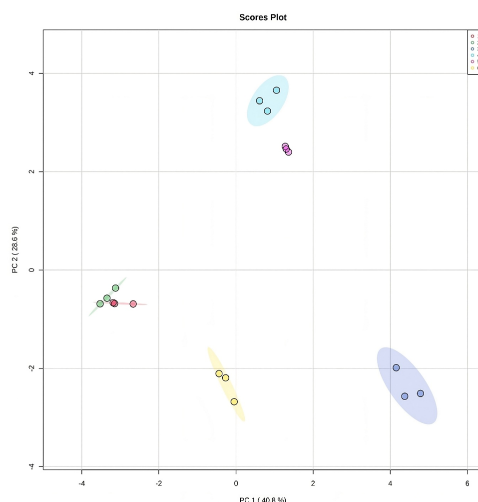

Figure 1: Score plot of PC1 versus PC2 of the volatiles obtained from the analyzed A. vulgaris essential oil (area % as a variable), where 1,2,3,4,5,6 represent samples collected from 1800m, 1500m, 1200m, 900m, 600m, 300m respectively.

Evaluation of HCA (Figure 2) using area percentage, showed the amount of closeness of components between the plant samples collected from different altitudes. The plant samples collected from 1200 m altitude were close with 300 m altitude. Similarly, 900 m altitude samples were close with 600 m altitude and 1800 m altitude were close with 1500 m altitude.

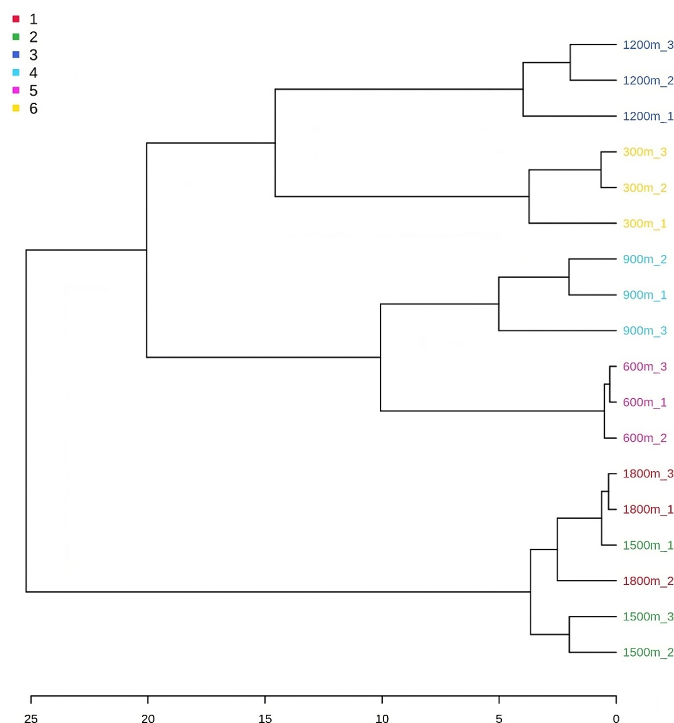

Figure 2: Dendrogram illustrating the clustering of A. vulgaris essential oil collected from different altitudes based on Euclidean distance where where 1,2,3,4,5,6 represent samples collected from 1800m, 1500m, 1200m, 900m, 600m, 300m respectively

3.4 Antifungal activity of essential oil

After both 24 and 48 hours of incubation, inhibition of mycelial growth was observed which was significantly affected by essential oil extracted from A. vulgaris obtained from different altitudes and its different concentrations (Table 5, 6, and 7). Least inhibition of mycelial growth was observed at 100 ppm of essential oil and inhibition was increased with the increase in concentration of essential oil of the plant samples collected from 300 m to 1800 m altitudes

After 24 hours of incubation highest inhibition of mycelial growth (100%) was observed at 350 ppm of essential oil collected from plant samples of all altitudes. At 100 ppm of essential oil least inhibition (28.07%) was seen in 300 m altitude plant sample which was statistically similar with 900 m altitude sample whereas highest inhibition (37.71 %) was seen in the essential oil extracted from 600 m altitude sample which was statistically similar with 300 m, 1500 m and 1800 m altitude plant samples. At 150 ppm least inhibition (42.10%) was seen in essential oil extracted from plant samples of 300 m altitude which was statistically similar with 1500 m altitude whereas highest inhibition (49.12 %) was seen in the essential oil extracted from 1800 m altitude which was statistically similar with 1200 m altitude plant sample. At 200 ppm least inhibition (50.00%) was seen in essential oil extracted from plant samples of 300 m altitude which was statistically similar with 600 m altitude whereas highest inhibition (60.52 %) was seen in the essential oil extracted from 1800 m altitude which was statistically similar with 900 m altitude plant sample. At 250 ppm least inhibition (63.15%) was seen in essential oil extracted from plant samples of 300 m altitude which was statistically similar with 1800 m altitude whereas highest inhibition (71.92 %) was seen in the essential oil extracted from 600 m altitude which was statistically similar with 1200 m altitude plant sample. At 300 ppm highest inhibition (91.22 %) was seen in the essential oil extracted from 1800 m altitude which was statistically similar with plant samples of all the altitudes.

After 48 hours of incubation highest inhibition of mycelial growth (100%) was observed at 350 ppm of essential oil collected from plant samples of all altitudes. At 100 ppm of essential oil inhibition of 23.33% was seen in 300 m altitude which was statistically similar with all other altitudes. At 150 ppm least inhibition (21.66 %) was observed in essential oil extracted from plant samples of 300 m altitude whereas highest inhibition (46.66 %) was seen in the essential oil extracted from 1800 m altitude. At 200 ppm least inhibition (27.77 %) was exhibited

by essential oil extracted from plant samples of 300 m altitude whereas highest inhibition (52.22 %) was seen in the essential oil extracted from 1800 m altitude which was statistically similar with 1500 m altitude plant samples. At 250 ppm least inhibition (45.55 %) was observed in essential oil extracted from plant samples of 300 m whereas highest inhibition (63.33 %) was seen in the essential oil extracted from 600 m altitude which was statistically similar with 300 m altitude plant samples. At 300 ppm highest inhibition (80.00 %) was observed in the essential oil extracted from 1800 m altitude and least inhibition (66.66 %) was seen in the essential oil extracted from 600 m altitude which was statistically similar with 300 m and 900 m altitude samples.

Table 5: Effect of different altitudes and concentration of essential oils of A. vulgaris on antifungal activity at 24 hours and 48 hours of incubation

Altitude	Mycelia growth inhibition (%) at 24 hours	Mycelia growth inhibition (%) at 48 hours
300 m	67.40^{a}	55.55^{a}
600 m	67.40^{a}	50.74^{b}
900 m	65.35^{ab}	52.03^{b}
1200 m	61.84^{c}	57.50^{a}
1500 m	64.18^{bc}	56.85^{a}
1800 m	66.81^{a}	55.37^{a}
LSD	2.44**	2.25**
C.V. (%)	5.61	6.2023
SEM (±)	0.90	1.10
Concentration of essential oil		
100 ppm	34.36^{f}	26.20^{f}
150 ppm	46.64^{e}	33.52^{e}
200 ppm	55.70^{d}	41.30^{d}
250 ppm	67.25^{c}	55.00^{c}
300 ppm	89.04^{b}	72.04^{b}
350 ppm	100.00^{a}	100.00^{a}
LSD	2.44**	2.25**
C.V. (%)	5.61	6.20
SEM (±)	10.24	11.24
Grand Mean	65.50	54.68

*Note: Mean separated by DMRT and values represented with the same letter(s) are non-significant at 5% level of significance; *Significant at 0.05 level of significance; ** Significant at 0.01 level of significance; NS, non-significant.*

Table 6: Effect of interaction between altitude and concentrations of essential oil on inhibition of fungal growth after 24 hours of incubation.

Concentration of essential oil	**100 ppm**	**150 ppm**	**200 ppm**	**250 ppm**	**300 ppm**	**350 ppm**
Altitude						
300 m	28.07^{n}	42.10^{kl}	50.00^{ij}	63.15^{efg}	87.71^{b}	100.00^{a}
600 m	37.71lm	52.63^{hi}	52.63^{hi}	71.92^{c}	89.47^{b}	100.00^{a}
900 m	31.57^{mn}	45.61^{jk}	60.52^{fg}	68.42^{cde}	85.96^{b}	100.00^{a}
1200 m	35.96^{lm}	48.24^{ijk}	57.89^{gh}	71.05^{cd}	91.22^{b}	100.00^{a}
1500 m	36.84^{lm}	42.10^{kl}	52.63^{hi}	64.91^{def}	88.59^{b}	100.00^{a}
1800 m	35.96^{lm}	49.12^{ij}	60.52^{fg}	64.03efg	91.22^{b}	100.00^{a}
LSD	5.98*					
C.V. (%)	5.60					
SEM (±)	3.92					
Grand Mean	65.49					

Note: Mean separated by DMRT and values represented with the same letter(s) are non-significant at 5 % level of significance; *Significant at 0.05 level of significance; ** Significant at 0.01 level of significance; NS, non-significant.

Table 7: Effect of interaction between altitude and concentrations of essential oil on inhibition of fungal growth after 48 hours of incubation.

Concentration of essential oil	**100 ppm**	**150 ppm**	**200 ppm**	**250 ppm**	**300 ppm**	**350 ppm**
Altitude						
300 m	23.33^{op}	21.66^{p}	27.77^{op}	45.55^{jk}	68.33^{d}	100.00^{a}
600 m	26.66^{op}	28.33^{o}	36.66^{mn}	58.33^{ef}	66.66^{d}	100.00^{a}
900 m	23.88^{op}	25.55^{op}	$38.88l^{mn}$	51.66^{ghi}	68.33^{d}	100.00^{a}
1200 m	28.88^{o}	36.11^{n}	42.22^{klm}	54.44^{fgh}	74.44^{c}	100.00^{a}
1500 m	26.66^{op}	42.77^{kl}	50.00^{hij}	56.66^{fg}	74.44^{c}	100.00^{a}
1800 m	27.77^{op}	46.66^{ijk}	52.22^{ghi}	63.33^{de}	80.00^{b}	100.00^{a}
LSD	5.51**					
C.V. (%)	6.20					
SEM (±)	4.36					
Grand Mean	54.67					

Note: Mean separated by DMRT and values represented with the same letter(s) are non-significant at 5% level of significance; *Significant at 0.05 level of significance; ** Significant at 0.01 level of significance; NS, non-significant.

4 Discussion

The present study was conducted to compare the phytochemicals and antifungal activities of essential oil extracted from A. vulgaris obtained from different altitudes of Nepal. A. vulgaris growing in high altitude of Nepal is the rich source of secondary metabolites, mainly alkaloids, flavonoids, terpenes, steroids, essential oils, etc.

The yield of the essential oils obtained from different altitudes ranged from 0.58 ± 0.06% to 0.95 ± 0.14.9% volume by weight basis. The highest yield percentage was observed in the plant samples collected from 1200 m altitude with an average yield 0.95 ± 0.14.9 v/w%. Thereafter, highest yield was found with the samples collected from 1500 m altitude (0.92 ± 0.06 v/w%) followed by 1800 m (0.88 ± 0.05 v/w %), 900 m (0.62 ± 0.04 v/w%) and 300 m (0.57 ± 0.04 v/w%). However, lowest yield percentage was found in plant samples collected at 600 m altitude with average 0.57 ± 0.04 v/w%. Our study showed that yield of essential oil varies with different altitudes. At 874 m altitude of Manipur the yield of essential oil was found up to 0.75 v/w% (Singh et al., 2023). Similarly, (Misra & Singh, 1986) observed the yield up to 1.3 v/w% extracted from the plant samples of Nilgiri hills at 2000 m height. However, Han et al. (2023) found the yield up to only 0.31 v/w% at 2051 m altitude. These results showed that the yield of A. vulgaris essential oil vary at different altitudes which can be due to stress, environmental conditions and other factors.

Essential oil extracted from different altitudes exhibited 73 compounds. The concentration of these constituents varied with the altitudes in which the plant is growing. Among these components, Amorphene $< gamma- >$ showed the highest peak area (10.58%), in the plant samples collected from 1800 m altitude. Similarly, Amorphene $< gamma- >$ was present at highest concentration 10.35% in 1500 m, 11.88% in 900 m, 13.85% in 600 m altitude, and 10.46% in 300 m altitude plant samples. However, its presence was least (1.35%) in essential oil obtained from plants collected from 1200 m altitude. Camphor exhibited second highest peak area which ranged from 13.64% to 7.65% followed by Eucalyptol from 10.59% to 5.26%. According to Jiang et al. (2019), eucalyptol was found out to be one of the major components of essential oil in A. vulgaris. Likewise, GC-MS analysis by Sharma & Adhikari (2023) showed presence of many compounds like camphene, cyclohexane, eucalyptus, borneol, and caryophyllene present in A. vulgaris grown in different altitudes of Nepal. Analysis of essential oil from the aerial parts of A. vulgaris from Nepal revealed sabinene (11.29%) as the most prominent compound (Pandey et al., 2017).

Although our study showed Amorphene $< gamma- >$ as one of the major components of essential oil, the composition pattern for any essential oil is influenced by many factors, which may be related to the plant, viz. genetics, plant part, sampling season, growth stage, and plant physiology or related to the environment such as climate, cultivation conditions, soil, temperature, humidity and many other factors. Differences in the prominent compound between the authors could be clarified by variables such as rainfall and season of collection, plant ontogeny, as well as geographic location, plant parts, and extraction techniques (Labarrere et al., 2019). Such type of variation in components of essential oil may be due to harvesting seasons, extraction methods, geographical sources and adaptive metabolism of plants, etc. (Kamazeri et al., 2012).

After PCA and HCA analysis our study revealed that plants collected from 1800 m altitude and 1500 m altitude were in one cluster. Similarly, plants collected from 900 m altitude and 600 m altitude were in one cluster, while plants collected from 1200 m altitude and 300 m altitude were in different clusters. This shows presence of similar types of essential oil components among plants of 1800 m and 1500 m altitude. Similarly, essential oil of 900 m altitude and 600 m altitude plant samples also have similar components. However, 300 m and 1200 m altitude

plant essential oil have different components separate them remain in different clusters.
A. vulgaris has been shown to have antimicrobial properties and significant fungicidal activity, inhibiting the mycelia growth of fungi (Munda, S et.al., 2019). At 24 hours of incubation, highest inhibition was recorded from essential oil of plant sample from 1800 m (66.81%) and at 350 ppm of essential oil (100%). Least inhibition effect was observed in 1200 m (61.84%) and 100 ppm (34.36%). But at 48 hours of incubation least inhibition effect was observed in 600 m (50.74%). This result revealed that essential oils had antifungal activity in a dose-dependent manner, highest at 350 ppm at which fungal growth was completely inhibited and its effect declined with decrease in concentration and time. Moreover, antifungal activity also varied between altitudes. Such type of antifungal activity can be attributed to the major compounds present, which possess the antifungal properties. Eucalyptol has antifungal activity against the candida spectrum (Malik et.al, 2019). Caryophyllene also has antifungal properties, and caryophyllene oxide works as a broad-spectrum antifungal in plants and inhibits Fusarium moniliforme (Jassal et al., 2021).

Conclusion

The phytochemical profiles of the essential oil from the aerial parts of A. vulgaris collected from different altitudes were extracted using hydro distillation method and evaluated using GC-MS analyses. A total of 73 components were identified. PCA and HCA results clustered different plant samples from various altitudes together due to their very close chemical composition. The essential oil of A. vulgaris possessed antifungal activities against S. sclerotiorum. From this study, we can observe the presence of different phytochemicals in the essential oil of A. vulgaris that can be used as a bio-fungicide against fungal pathogen S. sclerotiorum.

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Conflict of interest

The author declares that there is no conflict of interest.

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