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Chemical Composition, Antioxidant, Antifungal and Antifeedant Activity of the *Salvia reflexa* Hornem. Essential Oil

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Abstract

Background and Objective: The lamiaceae Martinov or Labiatae de Juss. is the large family of flowering plants, commonly known as the mint or deadnettle family. *Salvia reflexa* Hornem. is the important medicinal plant of this family. The objectives of this study were to determine the essential oil composition of the aerial part of the plant and to evaluate its antioxidant, antifungal and antifeedant properties. **Materials and Methods:** The plant essential oil was extracted by hydro distillation method and chemical composition was analyzed by gas chromatography–mass spectrometry. The antioxidant activity was evaluated in terms of DPPH radical scavenging, reducing power and metal chelating activity. The antifungal property was performed against two phytopathogenic fungi viz; *Curvularia lunata* and *Helminthosporium maydis* and antifeedant property was carried out against *Spilosoma obliqua* (Walker) larvae. **Results:** Nineteen compounds were identified which contributed 94.3% of the total oil. Palmitic acid (25.8%), phytol (24.0%), (E)-caryophyllene (12.4%) and caryophyllene oxide (10.7%) were identified as the major components. The antioxidant activity in term of DPPH radical scavenging, reducing power and metal chelating activity of the essential oil was found to be $IC_{50} = 71.82 \pm 0.45 \mu\text{g mL}^{-1}$, $RP_{50} = 23.6 \pm 1.05 \mu\text{g mL}^{-1}$ and $IC_{50} = 70.3 \pm 1.1 \mu\text{g mL}^{-1}$, respectively. The essential oil of *Salvia reflexa* showed strong antifungal and antifeedant activity in a dose dependent manner. **Conclusion:** The detailed study reveals that the essential oil of *Salvia reflexa* can be used as a good antioxidant, an excellent antifungal for inhibiting the growth of fungus and a very effective antifeedant for controlling the insects.

Key words: *Salvia reflexa*, essential oil, antifeedant activity, phytopathogenic fungi, palmitic acid, lamiaceae Martinov

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The medicinal plants have a great importance among human population and play a significant role in traditional herbal system besides a source of income in many developing countries¹⁻³. The Himalayan state, Uttarakhand treasures a number of valuable medicinal and aromatic plants in which a total of 964 species of medicinal plants have been reported by Chandra⁴. The Lamiaceae (Labiatae) also known as mint family is one of the most diverse and widespread plant family in terms of ethno medicine and its medicinal value is based on the volatile oil concentration⁵. *Salvia* is the largest genus of this family including about 986 species⁶, out of which seven species have been reported to grow in the central Himalayan region⁷. It is known to be one of the oldest medicinal plants used by human and commonly known as sage⁸. Plants belonging to this genus show high diversity in their secondary metabolites as well as in their pharmacological effects⁹. Many *Salvia* species and their essential oils are commonly used in the food, drug, cosmetic and perfumery industries. They are well known among people and widely used as flavourings or fragrance and for medicinal purposes in the several regions of the world¹⁰⁻¹². *Salvia* species, although bitter in taste are used traditionally to treat various conditions such as colic, diarrhoea, common cold, cough, flu, liver sickness, bacterial infections, febrile attacks, sores in the body and abdominal trouble and used as a purgative. Also used for alimentary and cosmetic purposes¹³⁻¹⁵. *Salvia reflexa* Hornem. a perennial subshrub commonly known as the sage mint or mint weed is the important medicinal plant native to the United States and Mexico. There are few reports in the literature, which showed diterpenes isolation¹⁶, chemical composition of essential oil¹⁷, antioxidant¹⁸ and allelopathic¹⁹ activity of *Salvia reflexa*. It was observed that literature search did not reveal any report on the essential oil of *Salvia reflexa* from India particularly from Uttarakhand. Hence the objective of the present investigation was to determine the chemical composition and antioxidant activity of the essential oil of *Salvia reflexa* and for the first time to evaluate the antifungal and antifeedant activity of the essential oil against two phytopathogenic fungi and a polyphagous pest, respectively.

MATERIALS AND METHODS

Collection of plant material: *Salvia reflexa* Hornem. was collected from village Harinagar (altitude 2100 m) of Nainital district (Uttarakhand) in the month of August-September, 2016. The plant material was taxonomically identified by

Dr. D.S. Rawat, Assistant Professor (Plant Taxonomist), Department of Biological Sciences, College of Basic Science and Humanities, Pantnagar.

Isolation of essential oil from *Salvia reflexa*: The essential oil of *Salvia reflexa* was isolated by hydro distillation method with the help of Clevenger-type apparatus²⁰ for 6-8 h. The essential oil so obtained was stored at a low temperature (4°C in refrigerator).

Chemical composition: The GC/MS analysis of the oil sample was carried out using GCMS-QP 2010 Plus equipment with DB-5 silica capillary column. Essential oil was injected into the system at injection temperature of 260°C. The flow rate of the carrier gas, helium, was maintained at 1.21 mL min⁻¹. The injector operating conditions were as follows: split ratio- 22.0, pressure- 69.0 kPa, total flow- 30.8 mL min⁻¹, linear velocity- 39.9 cm sec⁻¹ and purge flow- 3.0 mL min⁻¹. The oven temperature was initially programmed at 50°C for 2 min then raised at 3-210°C/min again raised at 8-280°C/min and held for 11 min at 280°C. The constituents of essential oil were identified by matching their mass spectra with those in NIST-MS, FFNSC Wiley Library and comparing with literature reports and GC retention indices and mass spectra²¹.

Antioxidant activity

DPPH Radical scavenging activity: The DPPH radical scavenging activity was evaluated according to the method developed by Blois²² and described by Liu *et al.*²³ and Lu *et al.*²⁴ with slight modification. The assay mixture contained 5 mL of 0.004% methanol solution of DPPH and essential oil of different concentrations (5-25 µmL). The absorbance of solution was measured at 517 nm in a UV-visible spectrophotometer using BHT (butylated hydroxytoluene) and catechin as the standard. The inhibition of free radical by DPPH in term of IC% was calculated by using the equation.

$$IC(\%) = \frac{A_0 - A_t}{A_0} \times 100$$

where, A_0 is absorbance value of control sample, A_t is absorbance value of test sample and IC is inhibitory concentration.

Reducing power: The reducing power of the essential oil was determined by the method developed earlier and are being practiced²⁵⁻²⁸. Varying concentrations of oil (5-25 µmL⁻¹) were mixed with 2.5 mL of phosphate

buffer (200 mM, pH = 6.6) and 2.5 mL of 1% potassium ferricyanide. After 20 min incubation, 2.5 mL of trichloroacetic acid was added to the mixtures, followed by centrifugation at 650 RPM for 10 min. Then after the upper layer (1 mL) was mixed with 5 mL of distilled water and 1 mL of 0.1% ferric chloride and the absorbance of the resultant solution were measured at 700 nm using UV-visible spectrophotometer. The BHT was used as the standard and the reducing power of samples was calculated using the formula:

$$RP (\%) = \frac{A_0 - A_t}{A_0} \times 100$$

where, A_0 is absorbance value of control sample, A_t is absorbance value of test sample and RP is reducing power.

Metal chelating activity: The metal chelating activity of essential oil on Fe^{2+} was examined by spectrophotometric method²⁹ which was recently used by Kunwar *et al.*³⁰. About 0.1 mL of 2 mM $FeCl_2 \cdot 4H_2O$, 0.2 mL of 5 mM ferrozine and 4.7 mL of methanol was added to different concentrations of oil (5-25 μ mL). The solutions were mixed and incubated for 10 min and the absorbance of test sample was measured at 562 nm in a UV-visible spectrophotometer. The EDTA (Ethylene diamine tetraacetic acid) was used as a standard and the metal-chelating activity of tested samples was calculated using the formula:

$$IC (\%) = \frac{A_0 - A_t}{A_0} \times 100$$

where, A_0 is absorbance value of control sample, A_t is absorbance value of test sample and IC is inhibitory concentration.

Antifungal activity: The antifungal activity of the essential oil of *Salvia reflexa* was tested by poisoned food technique³¹ against two phytopathogenic fungi *Curvularia lunata* and *Helmenthosporium maydis*. Potato dextrose agar, distilled water and chloramphenicol were used for the preparation of media. The fungal colony of *Curvularia lunata* and *Helminthosporium maydis* were transferred aseptically on the petri plates containing the media and incubated in inverted position in an incubator at $25 \pm 2^\circ C$ for one week. For testing the antifungal activity, seven days old culture of the test fungus was used for the preparation of inoculum discs. The prepared plates containing different concentrations of essential oil (10, 20, 40, 80 and 100 ppm) were inoculated

aseptically with assay discs of the test fungus and incubated for 3-7 days until the growth in the control plates reached at the edge of the plates. Clear zones of inhibition around the discs indicated the presence of antifungal activity which was recorded in millimetre. Percent inhibition was calculated by using following formula given by Mckinney³²:

$$\text{Inhibition (\%)} = \frac{X - Y}{X} \times 100$$

where, X is Radial growth in control and Y is Radial growth in treatment.

Antifeedant activity: Antifeedant activity of the essential oil of *Salvia reflexa* was evaluated by using leaf disc method in no-choice situation³³ against third instar larvae of *Spilosoma obliqua*. The fresh leaf discs of 25 sq.cm of soybean (*Glycine max*) were treated with different concentration (2, 4, 6, 8 and 10 ppm) of essential oil and kept in the petri dishes. After that a single third instar larva was introduced into each petri dish and allowed to feed until more than 75% of leaf disc were eaten away in control. The consumed area was measured graphically which were used for the calculation of various parameters like mean leaf area consumed (MLAC), feeding percentage, antifeedant activity, feeding inhibition, preference index and antifeedant category.

Statistical analysis: Data obtained in all experiments were performed in triplicate and data were analyzed as mean \pm SE subjected to one way ANOVA by using SPSS 16 (Statistical Package for the Social Science). Means were separated by the Turkey's multiple range test, when the analysis of variance (ANOVA) was significant.

RESULTS AND DISCUSSION

Chemical composition: The essential oil of *Salvia reflexa* was obtained as a light yellow liquid with a yield of 0.5% (v/w). The GC-MS analysis of the essential oil led to identification of 19 constituents which contributed 94.3% of the total constituents. Interestingly it was observed that the essential oil was completely devoid of monoterpenoids and the constituents contributing to the oil were sesquiterpenoids and higher terpenoids. In former study of the essential oil 27 constituents were identified¹⁷. The variation in present study could be because of different environmental conditions of the plant. Palmitic acid (25.8%) was found to be major constituent of the oil. This component had also been reported as major compound in previous studied of *Salvia reflexa*¹⁷. Other main

constituents were identified as phytol (24.0%), (E)-caryophyllene (12.4%), caryophyllene oxide (10.7%), hexahydrofarnezyllacetone (7.5%), phytol isomer (2.4%), β -asarone (1.7%), α -copaene (1.6%) and δ -cadinene (1.2%). The major classes of compounds were oxygenated diterpenes (34.7%) and fatty acids (27.7%). The detailed compositions have been presented in the Table 1 and the comparative class compositions have been graphically shown in the Fig. 1.

Antioxidant activity: In present study, *in vitro* antioxidant activity of essential oil of *Salvia reflexa* was evaluated by DPPH radical scavenging activity, reducing power activity and metal chelating activity in comparison to standard antioxidant. The DPPH radical scavenging activity of the essential oil of *Salvia reflexa* was expressed in term of IC_{50} value which was found to be $IC_{50} = 71.82 \pm 0.45 \mu\text{g mL}^{-1}$ in

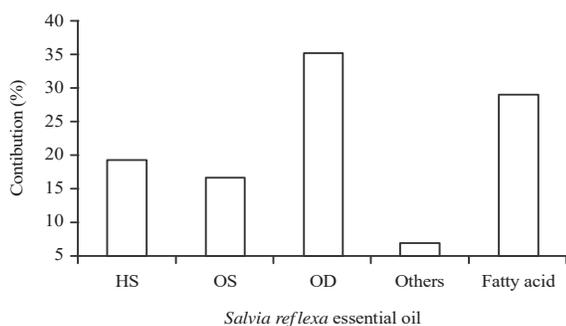


Fig. 1: Comparative class composition of the essential oil of *Salvia reflexa*

HS: Hydrocarbon sesquiterpenes, OS: Oxygenated sesquiterpenes, OD: Oxygenated diterpenes

comparison to BHT ($IC_{50} = 117.2 \pm 0.2 \mu\text{g mL}^{-1}$) and catechin ($IC_{50} = 61.84 \pm 0.07 \mu\text{g mL}^{-1}$). Generally the redox properties of phenolic compounds play a significant role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides³⁴. The essential oil of *Salvia reflexa* was found to have reducing power activity with $RP_{50} = 23.6 \pm 1.05 \mu\text{g mL}^{-1}$ in comparison to BHT ($RP_{50} = 102.2 \pm 1.05 \mu\text{g mL}^{-1}$). The reducing ability of a compound depends on the presence of reductones (antioxidants), which shows the antioxidant activity by breaking the free radical chain³⁵. The metal chelating activity of essential oil was recorded with $IC_{50} = 70.3 \pm 1.1 \mu\text{g mL}^{-1}$ in comparison to EDTA having $IC_{50} = 188.6 \pm 0.7 \mu\text{g mL}^{-1}$. As far as current literature survey could ascertain, no report is available on antioxidant activity of the essential oil of *Salvia reflexa*. Therefore, this study could be assumed as the first report on this topic.

Table 2 represented the antioxidant activity in terms of IC_{50} and RP_{50} of the essential oil of *Salvia reflexa*.

Antifungal activity: *In vitro* antifungal activity of essential oil of *Salvia reflexa* was tested against two phytopathogenic fungi, *Curvularia lunata* and *Helminthosporium maydis*. The percent fungal inhibition data are summarized in Table-3. It was observed that the antifungal activity shown by the oil of *Salvia reflexa* against both phytopathogenic fungi was equal to the standard antifungal above 40 ppm concentration by inhibiting 100% of mycelial growth. Essential oil can represent one of the most promising natural products for fungal inhibition^{36,37}. The antifungal potential of essential oil of

Table 1: Chemical composition of the essential oil of *Salvia reflexa*

| Compounds | Contribution (%) | Formula | Methods of identification (MS) |
|-------------------------------|------------------|-------------------|----------------------------------|
| α -copaene | 1.600 | $C_{15}H_{24}$ | $M^+ = 204, m/e = 105, 119, 161$ |
| (E)-caryophyllene | 12.400 | $C_{15}H_{24}$ | $M^+ = 204, m/e = 41, 69, 93$ |
| α -humulene | 0.800 | $C_{15}H_{24}$ | $M^+ = 204, m/e = 80, 93, 121$ |
| Bicyclogermacrene | 0.400 | $C_{15}H_{24}$ | $M^+ = 204, m/e = 93, 107, 121$ |
| δ -cadinene | 1.200 | $C_{15}H_{24}$ | $M^+ = 204, m/e = 105, 119, 161$ |
| Caryophyllene oxide | 10.700 | $C_{15}H_{24}O$ | $M^+ = 220, m/e = 41, 79, 93$ |
| Humulene epoxide II | 0.400 | $C_{15}H_{24}O$ | $M^+ = 220, m/e = 43, 67, 109$ |
| β -asarone | 1.70 | $C_{12}H_{16}O_3$ | $M^+ = 208, m/e = 165, 193, 208$ |
| α -cadinol | 0.60 | $C_{15}H_{26}O$ | $M^+ = 222, m/e = 43, 95, 121$ |
| Hexahydrofarnezyllacetone | 7.500 | $C_{18}H_{36}O$ | $M^+ = 268, m/e = 43, 58, 71$ |
| Isophytol | 0.20 | $C_{20}H_{40}O$ | $M^+ = 296, m/e = 43, 57, 91$ |
| Palmitic acid | 25.800 | $C_{16}H_{32}O_2$ | $M^+ = 256, m/e = 41, 43, 73$ |
| Isopropyl palmitate | 1.300 | $C_{19}H_{38}O_2$ | $M^+ = 298, m/e = 43, 60, 102$ |
| Palmitaldehyde diallyl acetal | 1.000 | $C_{22}H_{42}O_2$ | $M^+ = 338, m/e = 41, 55, 84$ |
| Phytol | 24.0 | $C_{20}H_{40}O$ | $M^+ = 296, m/e = 57, 71, 123$ |
| Phytol isomer | 2.400 | $C_{20}H_{40}O$ | $M^+ = 296, m/e = 43, 57, 71$ |
| Ethyl linolenate | 0.600 | $C_{20}H_{34}O_2$ | $M^+ = 306, m/e = 67, 79, 95$ |
| Phytol, trimethylsilyl ether | 0.600 | $C_{23}H_{48}OSi$ | $M^+ = 368, m/e = 73, 123, 143$ |
| Pentacosane | 1.100 | $C_{25}H_{52}$ | $M^+ = 352, m/e = 43, 57, 73$ |
| Total | 94.3 | | |

Where, MS: Mass spectrometry

Table 2: Antioxidant activity of the essential oil of *Salvia reflexa*

| Sample Name | Mean \pm SE ($\mu\text{g mL}^{-1}$) | | |
|-------------|--|---|--|
| | DPPH radical scavenging activity (IC ₅₀) | Reducing power activity (RP ₅₀) | Metal chelating activity (IC ₅₀) |
| SREO | 71.82 \pm 0.45 | 23.6 \pm 1.05 | 70.3 \pm 1.1 |
| BHT | 117.20 \pm 0.2 | 102.2 \pm 1.05 | - |
| Catechin | 61.84 \pm 0.07 | - | - |
| EDTA | - | - | 188.6 \pm 0.7 |

SE: Standard error, DPPH: 1,1-diphenyl-2-picrylhydrazyl radical, IC₅₀: Half maximal inhibitory concentration, RP₅₀: Half maximal reducing power, SREO: *Salvia reflexa* essential oil, BHT: Butylated hydroxytoluene, EDTA: Ethylene diamine tetra acetic acid

Table 3: Percentage growth inhibition of the essential oil of *Salvia reflexa* against *Curvularia lunata* and *Helminthosporium maydis* at different concentration

| Essential oil concentration | Growth % inhibition against <i>Curvularia lunata</i> | Growth % inhibition against <i>Helminthosporium maydis</i> |
|-----------------------------|--|--|
| 10 ppm | 64.20 | 64.24 |
| 20 ppm | 92.50 | 82.42 |
| 40 ppm | 100.00 | 100.00 |
| 80 ppm | 100.00 | 100.00 |
| 100 ppm | 100.00 | 100.00 |
| Standard | 100.00* | 100.00** |

*Carbendazim (standard for *Curvularia lunata* fungi), **Mancozeb (standard for *Helminthosporium maydis* fungi)

Table 4: Antifeedant action of essential oil of *Salvia reflexa* on third instar larvae of *Spilosoma obliqua*

| Concentration | MLAC (cm ²) | Feeding percent | Antifeedant activity (%) | Feeding inhibition (%) | Preference index | Antifeedant category |
|---------------|-------------------------|-----------------|--------------------------|------------------------|------------------|------------------------|
| 10 ppm | 0.41 | 1.64 | 97.74 | 95.57 | 0.04 | Extremely antifeedant |
| 8 ppm | 6.19 | 24.77 | 65.99 | 49.25 | 0.51 | Moderately antifeedant |
| 6 ppm | 11.65 | 46.61 | 36.02 | 21.96 | 0.78 | Slightly antifeedant |
| 4 ppm | 11.91 | 47.65 | 34.59 | 20.91 | 0.79 | Slightly antifeedant |
| 2 ppm | 16.33 | 65.32 | 10.34 | 5.45 | 0.95 | Slightly antifeedant |
| Control | 18.21 | 72.85 | - | - | - | - |
| f-value | ** | | | | | |
| SEM | 0.297 | | | | | |
| Cd at 1% | 1.284 | | | | | |
| Cd at 5% | 0.916 | | | | | |
| CV | 4.776 | | | | | |

MLAC: Mean leaf area consumed, SEM: Standard error of the mean, Cd: Critical different, CV: Coefficient of variation, **: Statistically significant

Salvia reflexa may be attributed to their major components such as, palmitic acid (25.8%) and phytol (24.0%) or the synergic effect of minor constituents as shown in Table 1.

Antifeedant activity: Plant essential oils have been suggested as alternative sources for insect control because they are selective, biodegrade to nontoxic products and have minimal effects on nontarget organisms and the environment³⁸. Antifeedant activity of the essential oil of *Salvia reflexa* was evaluated on the basis of leaf area consumed by the larvae of *Spilosoma obliqua*. The mean leaf area eaten by the third instar larvae is given in the Table 4 along with other parameters. In this study mean leaf area consumption at 10, 8, 6, 4 and 2 ppm concentration were recorded 0.41, 6.19, 11.65, 11.91 and 16.33 cm², respectively in comparison to the control (18.21 cm²). At maximum concentration (10 ppm) due to antifeedant property of the essential oil, the larvae could not consume the leaf disc properly so the MLAC was reduced to such an extent as the maximum antifeedant activity was recorded at 10 ppm (97.74%) and least was recorded at 2 ppm (10.34%).

CONCLUSION

On the basis of present investigation it can be concluded that essential oil of *Salvia reflexa* exhibited good to moderate antioxidant property thus used as a natural antioxidant, to preserve the food stuff and prevent them from oxidative deterioration. The strong antifungal activity in a dose dependent manner showed that the plant might be a source of natural fungicide to protect the agricultural crops, foods etc. in place of synthetic fungicides. Highest antifeeding ability at 10 ppm concentration against third instar larvae of *Spilosoma obliqua* has indicated that the plant might be a source of natural antifeedant to protect the agricultural crops without having any adverse effect on the environment for the management of lepidopteran insect pest. Thus the future study obviously needs the isolation of active ingredients (compounds) followed by their spectral analysis for quality control of the compounds responsible for antioxidant, antifungal and antifeedant activity which could possibly facilitate the new formulations for their bio-activity at lower concentrations and could be used as natural fungicide and antifeedant.

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SIGNIFICANCE STATEMENT

This study discovered that the essential oil of *Salvia reflexa* is reported from India for the first time that can be beneficial for generation of data for chemical and taxonomical studies as well as the judicious exploitation of plant in future. This study will help the researchers to uncover the critical areas of this plant as it is the new record from Uttarakhand Himalaya region that many researchers were not able to explore. Thus a new theory on chemical composition, antioxidant, antifungal and antifeedant property of essential oil of *Salvia reflexa* may be arrived at.

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