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Research Article

EVALUATION OF *SALVIA LANATA* LEAF EXTRACT FOR ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITY

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ABSTRACT

Objective: The work is aimed to evaluate the anti-inflammatory and antioxidant activity of the ethanolic leaf extract of *Salvia lanata*.

Methods: Anti-inflammatory activity of the leaf extract of *S. lanata* at a dose of 100 mg/kg and 200 mg/kg against the standard drug indomethacin at a dose of 10 mg/kg i.p. was evaluated by carrageenan-induced rat paw edema and protein denaturation method. Antioxidant activity was determined by 1, 1 diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method, reducing power method, and nitric oxide scavenging assay.

Results: *S. lanata* leaf extract showed highly significant dose-dependent efficacy against carrageenan-induced paw edema at a dose of 200 mg/kg and lesser effect at 100 mg/kg. It inhibited heat-induced albumin denaturation with a maximum inhibition of 79.26% at 160 µg/ml. DPPH free radical scavenging activity of extract exhibited inhibition of 25.96%–87.74% within the concentration range of 10 µg/ml–160 µg/ml, nitric oxide assay from 12.26% to 79.22% in the same concentration range. In reducing power assay with an increase in concentrations, an increase in the absorbance of the reaction mixture was observed. Antioxidant activity was compared to standard drug ascorbic acid.

Conclusion: The leaf extract of *S. lanata* has potent anti-inflammatory and antioxidant activity.

Keywords: *Salvia lanata*, acute oral toxicity, anti-inflammatory, antioxidant, indomethacin, ascorbic acid.

Abbreviations: ROS: reactive oxygen species, RNS: reactive nitrogen species, IL-1β: Interleukin- 1 β, NK cells: Natural killer cells, TNF-α: Tumor Necrosis Factor-alpha, O2: Oxygen, $O2^{\cdot-}$: superoxide anion, OH: hydroxyl radical, 1O2: singlet oxygen, H2O2: hydrogen peroxide, SLEE: Salvia lanata ethanolic extract, i.v: intravenous, s.c: subcutaneous, h: hour, d: days, g: gram: n: number of animals, DPPH: 1, 1-diphenyl-2-picryl-hydrazil,

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INTRODUCTION

In recent years, plant-derived natural products, along with their therapeutic uses on body tissues, have attracted a huge population toward plant-based medications. This occurs due to side effects and produced by synthetic drugs [1]. Inflammation and oxidative stress are the reason for various chronic diseases worldwide. Synthetic drugs are chemically harmful to human health [2], along with re-emergence of the symptoms on drug discontinuation [3]. Therefore, consumers prefer natural therapies over synthetic drugs and consider natural medications superior to synthetic ones [4]. Inflammation is an array of processes which occur in a body as a protective response when it is injured by any foreign substance (antigen), mechanical injury, or chemical agent [5]. Inhibition of harmful stimuli, along with the healing of damaged tissue, is the part of the essential host defense process [6]. Survey being done reported incidence of rheumatoid arthritis from 0.5% to 3.8% in women and 0.15% to 1.37% in men in developed countries [7]. A range of immune cells such as mast cells, neutrophils, lymphocytes, and macrophages are involved in inflammation [8]. In the course of an inflammatory response, many mediators have also proved to play a key role, including leukotrienes [9], interleukin-1 β (IL-1β) [10], cytokines [11], NK cells, complement system, and tumor necrosis factor-alpha (TNF-α) [12].

Oxygen (O_2) is an essentiality of human life, and it is tolerable due to the various processes occurring in the human body and a range of enzymes which utilize it for fulfilling their activity [13]. Studies have reveled unfavorable effects by oxygen due to the generation of free radicals and high concentrations of some molecules called reactive oxygen species, reactive nitrogen species [14]. These species include superoxide anion ($O_2^{\cdot-}$), hydroxyl radical (OH), singlet oxygen ($^1O_2$), and hydrogen peroxide (H_2O_2) [15]. A free radical can be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital. Many radicals are unstable and highly reactive. They can either donate an electron to or accept an electron from other molecules, therefore behaving as oxidants or reductants [16]. Antioxidants delay or inhibit cellular damage mainly through their free radical scavenging property [17]. Natural antioxidants from plant materials have been shown to increase the antioxidant capacity of the plasma and reduce the risk of certain diseases such as cancer, heart diseases, and stroke [18]. Among the natural antioxidant substances such as phenolic compounds, diterpenes, and flavonoids, the pride of place was given to flavonoids, ubiquitously present in the plant kingdom, which exerts antioxidant, anti-inflammatory, and lipid-lowering effects [19]. Researches have shown that natural antioxidants such as Vitamin C, Vitamin E, carotene, isoflavonoids, anthocyanins, and xanthones have their effects on diseases such as diabetes, cardiovascular diseases, neurodegenerative diseases, various types of cancer, and some inflammatory conditions [20].

METHODS

Plant collection

Ghaniya (*S. lanata*) leaves collected from Ranikhet and Jageshwar region of Uttarakhand, India and identified in the N.B.P.G.R (National Bureau of Plant Genetics Resources) regional station, Niglat, Bhowali, Nainital, Uttarakhand (Ref No- LN-01). The leaves were cleaned and dried at room temperature. The dried leaves were grinded and powdered.

Preparation of extract

The 80 g of dried and powdered plant material of *S. lanata* were defatted with petroleum ether and further extracted with 250 ml of solvent ethanol using Soxhlet apparatus for 48 h. The liquid extract was reduced to a small amount using a rotary evaporator and further

concentrated to dryness. The obtained *S. lanata* ethanolic extract (SLEE) was further used for investigation.

Experimental animals
Female Wistar rats (150–200 g) were used in the experimental study. They were housed under the condition of 22±3°C, minimum 30% relative humidity and not exceeding 70%, 12 h light/12 h dark cycle, proper laboratory diet, and water. The ethical OECD guidelines 423 were followed throughout the acute oral toxicity study of SLEE. The animal study performed during this work was approved by the Institutional Animal Ethics Committee (Protocol No- KUDOPS/65).

Phytochemical screening
Qualitative phytochemical screening was performed to identify the different constituents. Tests for the identification of alkaloids, carbohydrates, amino acids, flavonoids, proteins, tannins and phenols, glycosides, sterols, and terpenoids were performed [21].

Acute oral toxicity study
Acute oral toxicity studies were carried out according to OECD guideline 423 using female Wistar rats. Different groups (n=3) were made, and fixed single doses of SLEE starting from 5, 50, and 300 up to 2000 mg/kg body weight were given gradually. Signs of toxicity or mortality were observed in each step of dosing. The animals were observed after 30 min and repeatedly within 24 h of dosing. A daily observation for 14 day was also done. The extract was safe up to 2000 mg/kg.

Carrageenan induced rat paw edema
Overnight fasted female Wistar rats (150–200 g) were taken. Both the paws were marked just beyond the tibiotarsal junction to ensure constant paw volume dipping each time. Wistar rats were divided into four groups having six animals in each group. Group 1 animals (control group) received vehicle, Group 2 animals (standard group) received standard drug indomethacin (10 mg/kg i.p), Group 3 animals (t1) received SLEE (200 mg/kg), and Group 4 animals (t2) received SLEE (100 mg/kg). The paw volumes (left and right) were measured initially using a plethysmograph (Inco). Thirty minutes before carrageenan injection, 10 mg/kg body weight (i.p.) indomethacin, 100 mg/kg and 200 mg/kg body weight SLEE, and vehicle were administered orally according to the groups. Thirty minutes later, the right paw was injected with carrageenan (0.1 ml, 1% suspension) s.c. into the subplantar region. The paw volume was measured at time intervals of 0 min, 30 min, 60 min, 90 min, and 120 min after carrageenan injection [22].

Inhibition of protein denaturation method
Initially phosphate buffer saline (pH 6.4) and different concentrations (10, 20, 40, 80, and 160 µg/ml) of SLEE were prepared. The 5 ml of reaction mixture consisted of 0.2 ml of egg albumin, 2.8 ml of phosphate buffer saline, and 2 ml of different concentrations of the extract. In the case of reference, acetylsalicylic acid was used in place of the extract. The mixture was incubated at 37°C±2 for 15 min and then heated for 5 min at 70°C. It was then left for cooling, and absorbance was measured using ultraviolet (UV)-visible spectrophotometer (Shimadzu) at 660 nm. Ethanol was used for blank [23].

The protein denaturation % inhibition was calculated by:

Percentage protein denaturation inhibition=Abs C-Abs T ÷ Abs C × 100.

Where,
Abs C = Absorbance of control.
Abs T = Absorbance of test.

1, 1-diphenyl-2-picryl-hydrazil (DPPH) free radical scavenging activity
The free radical scavenging activity of SLEE was measured by the DPPH method. 0.1 mM DPPH solution in ethanol and different concentrations (10, 20, 40, 80, and 160 µg/ml) of SLEE were prepared. One milliliter of DPPH solution was mixed with 3 ml of different concentrations of the extract. After 30 min, the absorbance of each solution was measured at 517 nm using UV-visible spectrophotometer (Shimadzu). Ascorbic acid was used as a standard for comparison. Ethanol was taken as blank. With an increase in the concentration of the reaction mixture, the absorbance decreases, indicating higher free radical scavenging activity [24].

The percentage of free radical scavenging was determined as:

Percentage inhibition= Abs C- Abs T ÷ Abs C × 100.

Where,
Abs C=Absorbance of control.
Abs T=Absorbance of test.

Reducing power assay
The method started with the preparation of phosphate buffer (0.2 M, pH 6.6) and different concentrations (10, 20, 40, 80, and 160 µg/ml) of SLEE. One milliliter of the different concentrations of extract solution was mixed with 2.5 ml of buffer and 2.5 ml of 1% potassium ferricyanide. The same reaction mixture without extract served as control and with ascorbic acid served as standard. These mixtures were incubated at 50°C for 20 min. After incubation, 2.5 ml of 10% trichloroacetic acid was added to each solution and was centrifuged to 700 rpm for 10 min. After centrifugation, the upper layer of the solution (2.5 ml) was mixed with 2.5 ml distilled water and 0.5 ml (0.1%) of $FeCl_3$. The absorbance was measured at 700 nm using UV-visible spectrophotometer (Shimadzu). An increase in absorbance of the reaction mixture indicated an increase in reducing power [25].

Nitric oxide scavenging activity
Nitric oxide is produced by sodium nitroprusside in an aqueous solution and at physiological pH. The nitric oxide produces nitrite ions in the presence of oxygen which can be estimated by Griess reagent. Scavengers of nitric oxide compete with oxygen to inhibit the production of nitrite ions. Experimentally, sodium nitroprusside (10 mM) prepared in phosphate buffer saline was added to different concentrations (10, 20, 40, 80, and 160 µg/ml) of SLEE and incubated at room temperature for 150 min. Same reaction mixture without the extract served as control and with ascorbic acid served as standard. After incubation, 0.5 ml of Griess reagent (1% sulfanilamide, 2% H3PO4, and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) was added, forming a chromophore. The absorbance of chromophore was measured at 546 nm using UV-visible spectrophotometer (Shimadzu) [26].

Table 1: Anti-inflammatory activity of *Salvia lanata* leaf extract on carrageenan-induced rat paw oedema

Group	Dose	Paw volume (ml) measured by mercury displacement (Mean±SEM)				
		0 min	30 min	60 min	90 min	120 min
Control	Vehicle	0.2708±0.05017	0.4167±0.08333	0.6250±0.05590	0.7083±0.04176	0.5000±0.06455
Standard	10 mg/kg	0.1458±0.02083	0.3125±0.06250*	0.3958±0.06783**	0.4167±0.05270	0.2283±0.02167
Test T_1	200 mg/kg	0.2083±0.02635	0.3750±0.05590**	0.5417±0.1003***	0.6667±0.05270	0.4583±0.04167
Test T_2	100 mg/kg	0.1875±0.02795	0.3333±0.05270*	0.4167±0.05270**	0.5833±0.05270	0.2917±0.04167[1,2]

Data analysis by one-way ANOVA and values expressed in mean±SEM, p***<0.001, p**<0.01, p*<0.05 denotes the level of statistical significance as compared to control

RESULTS

The phytochemical screening of SLEE showed the presence of alkaloids, carbohydrates, flavonoids, tannins and phenols, glycosides, sterols, and terpenoids. In the acute oral toxicity study of SLEE, the extract was found safe up to 2000 mg/kg dose.

The result suggests the significant anti-inflammatory activity of SLEE at a higher dose of 200 mg/kg and lesser effects at a lower dose of 100 mg/kg. Significant reduction in the carrageenan-induced rat paw edema in a dose-dependent manner was seen, which is comparable to that of standard drug indomethacin.

Carrageenan induced rat paw edema

The paw volume of control, standard T1 and T2 groups (Table 1) expressed in mean±SEM. The graphical representation or change in paw volume observed with respect to time. A decrease in paw volume can be observed in case of test drug T1 and T2 (Fig.1). Although the standard drug showed better results as compared to the test, decrease in paw volume by test drugs shows its efficacy against inflammation.

Inhibition of protein denaturation method

In the present study for *in vitro* anti-inflammatory activity, the percentage inhibitions of protein denaturation by SLEE were found to be 2.32%, 27.06%, 49.31%, 53.51%, and 79.26% for the doses (Fig.2) of 10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml, and 160 µg/ml, respectively. The results reveal a significant ability of SLEE to inhibit the thermal denaturation of protein (egg albumin). Aspirin, an anti-inflammatory drug, was taken as standard, and it showed the maximum inhibition of 81.03%.

DPPH free radical scavenging activity

The percentage inhibitions (Fig.3) of DPPH free radicals by the extract were found to be 25.96%, 37.74%, 44.02%, 71.6%, and 87.74% for the doses of 10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml, and 160 µg/ml. The standard antioxidant drug ascorbic acid showed the highest inhibition of 97.37%.

Reducing power assay

The *in vitro* reducing power assay was performed for SLEE. Significant results were obtained in a concentration-dependent manner. With increase in the concentrations (Fig.4) 10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml, and 160 µg/ml, the absorbance gradually increased (0.2163, 0.5608, 0.6123, 0.6704, and 0.7318). Ascorbic acid was taken as standard which showed the maximum reducing power activity with 0.932 as its absorbance.

Nitric oxide scavenging activity

Nitric oxide scavenging activity observed in SLEE (Fig.5) showed significant effects with the percentage inhibitions of 12.26%, 33.11%, 39.86%, 64.34%, and 79.22% with the same concentration range as specified in the above method. Ascorbic acid was taken as a standard for comparison, and it showed the maximum percentage inhibition of 87.06%.

DISCUSSION

SLEE showed the presence of some phytoconstituents, namely, flavonoids, phenol, terpenes, alkaloids, steroids, and glycosides. The presence of a pentacyclic triterpenoid 3-epi-ursolic acid [27] and some diterpenoid quinones specifically royleanone, 20-Hydroxy-7α-acetoxyroyleanone [28], horminone, desacylnemorone, and 7α-acetoxy-royleanone [29] has also been reported earlier in *S. lanata*. Ursolic acid is reported to produce anti-inflammatory and antiproliferative effects by the suppression of nuclear factor-kappa-light chain enhancer of activated B cells activation and blocking the expression of inducible nitric oxide synthase and cyclooxygenase-2 [30]. Based on the presence of these constituents, the anti-inflammatory and antioxidant activity was conducted. There is no clear evidence, but it might be 3-epi

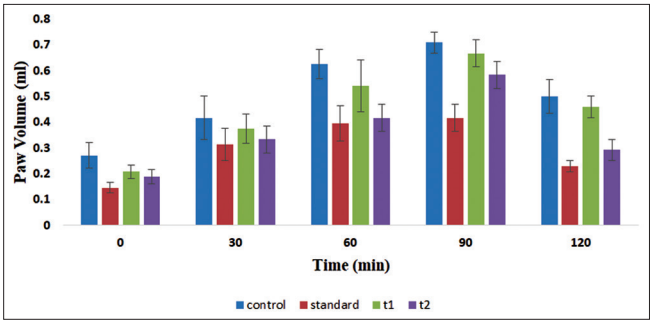

Fig. 1: Graphical representation of different groups (control, standard t1, and t2) on carrageenan-induced rat paw edema

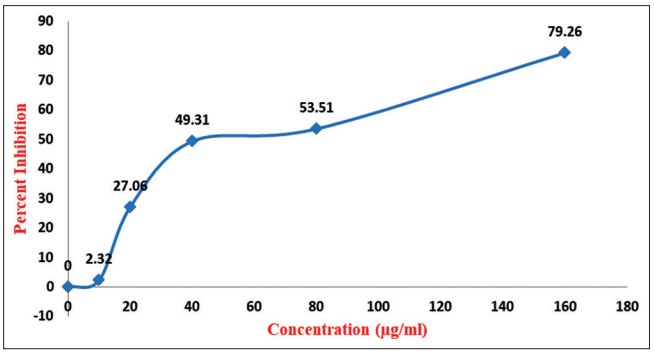

Fig. 2: Anti-inflammatory activity of *Salvia lanata* ethanolic extract for different concentrations using inhibition of protein denaturation method

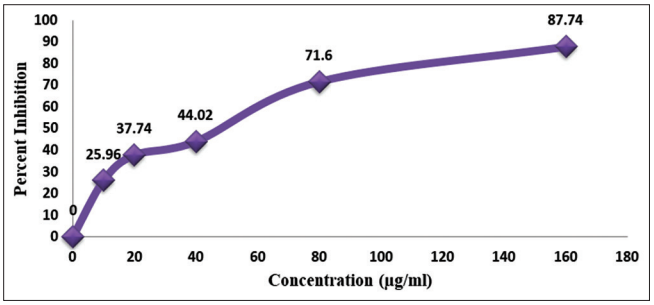

Fig. 3: Antioxidant activity of *Salvia lanata* ethanolic extract by 1, 1-diphenyl-2-picryl-hydrazil free radical scavenging activity

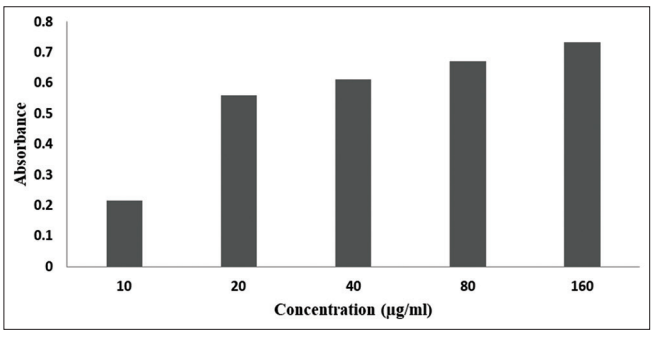

Fig. 4: Concentration-dependent effect of *Salvia lanata* ethanolic extract on reducing power assay

1 Table 1- Anti-inflammatory activity of Salvia lanata leaf extract on Carrageenan induced rat paw oedema.

2 There were four group (6 female Wistar rats in each group) Group 1- control group, Group 2- standard group, Group 3- (test 1 group- 200mg/kg), Group 4- (test 2 Group- 100 mg/kg) i.p. route.

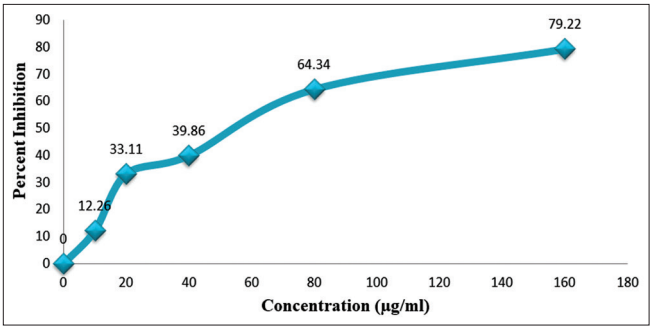

Fig. 5: Antioxidant activity of *Salvia lanata* ethanolic extract by nitric oxide scavenging activity

ursolic acid involved in the anti-inflammatory and antioxidant activity. Carrageenan-induced rat paw edema is a distinct acute inflammatory model used to evaluate the anti-inflammatory effects of various synthetic and natural products. Carrageenan is said to produce a biphasic event. The early phase includes the production of histamine, platelet-activating factors, serotonin, and leukotrienes [31], whereas the later phase is involved in neutrophil infiltration and edema due to the release of substances like prostaglandin [32]. Carrageenan also leads to the release of some proinflammatory cytokines such as IL-β and TNF-α. Free radical, along with oxidative stress, has also been found to be involved in the inflammatory response generated by carrageenan. Due to the increasing side effects of synthetic drugs, the focus has gone toward plants-based products. Evidence has shown flavonoids, triterpenoids, and phenolic acids involved in antioxidant and anti-inflammatory activity. The dose-dependent effects of DPPH free radical scavenging activity, nitric oxide assay, and concentration-dependent effects of reducing power shown by SLEE could be related to it [33].

The present study has focused only on ethanolic leaf extract, which showed significant effects in reducing edema as well as inhibition of free radicals, thereby acting as an antioxidant. *Salvia* species carry a wide range of therapeutic values, and triterpenes like ursolic acids have their active role in anti-inflammatory and antioxidant activity. As there are not many studies reported for *S. lanata;* hence, it creates hope for a more advanced form of study to be done. *S. lanata* can be studied more thoroughly by including various parts of the plant and a range of extracts. This will come up with more knowledge about the plant, as well as it might show better anti-inflammatory and antioxidant activity.

CONCLUSION

The ethanolic extract of *S. lanata* leaves exhibited significant anti-inflammatory and antioxidant activity at different levels in all the methods used in the study, and the results support the use of *S. lanata* leaves for various inflammatory conditions and as an antioxidant in human health. However, further studies need to be done to identify the actual mechanism of action for both activities.

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AUTHORS' CONTRIBUTIONS

Ms. Lipi Nogai made a substantial contribution to the acquisition, testing the collected data, and drafting the manuscript. Mr. Pankaj Lohumi contributed to a collection of plant species and extraction. Dr Tirath Kumar supervised the interpretation of data and revising it for ensuring critical academic content to be published. Finally, all the authors reviewed the drafted manuscript.

CONFLICTS OF INTEREST

The authors declared that there are no conflicts of interest related to this study.

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