

# *In vivo* evaluation of the traditional uses of *Salvia veneris* Hedge and development of a topical formulation for localized pain and inflammation

Goksu Oduncuoglu\* and Emmanuel Mshelia Halilu

Department of Pharmacy, Faculty of Pharmacy, Cyprus International University, Nicosia, Turkey

**Abstract:** *Salvia veneris* is endemic to Cyprus and used for pain relief and infectious diseases by local people. This study aimed to evaluate folkloric uses of *Salvia veneris* and develop a topical formulation for analgesic and anti-inflammatory effects as an alternative to NSAIDs. The analgesic activity of *Salvia veneris* ethanol extract was studied using an acetic acid-induced writhing test in mice and formalin-induced paw licking in rats. For anti-inflammatory activity, the formalin-induced paw edema model was used. Five topical formulations were prepared by varying the concentrations of ingredients and evaluated. One-way analysis of variance was used at the  $P < 0.05$  confidence level for data analysis. The oral  $LD_{50}$  of *Salvia veneris* in rats was greater than 5000mg/kg. The extract showed significant ( $P = 0.008$ ) analgesic activity in the acetic acid-induced writhing test and significant ( $P < 0.01$ ) dose-dependent analgesic activity in both phases of the formalin test. In the formalin-induced paw edema test, 50 and 200mg/kg of extract significantly ( $P < 0.05$ ) suppressed inflammation. The extract showed dose-dependent antioxidant activity with 0.04559mg/mL  $IC_{50}$ . This study confirms the folkloric use of *Salvia veneris* and shows that a topical formulation of *Salvia veneris* can be used as an alternative to topical NSAIDs.

**Keywords:** Analgesic, anti-inflammatory, endemic, *Salvia veneris*, topical.

## INTRODUCTION

Inflammation is one of the defense mechanisms of the immune system that protects the body from harmful stimuli and initiates healing (Oronsky *et al.*, 2022). Pain is a sign of inflammation and is related to the presence of inflammatory mediators (Crespo-Pardo and Taboada-Iglesias, 2021). Therefore, it is crucial to reduce inflammation to relieve pain. Topical formulations with nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used for localized pain relief and to reduce inflammation and edema. Currently available topical NSAIDs have serious adverse effects, such as skin irritation and allergic contact dermatitis (Shi *et al.*, 2022). There is a need for a new topical formulation for pain relief and inflammation with fewer side effects as an alternative to topical NSAIDs. Plants are always important natural sources of novel bioactive compounds to develop pharmaceutical products (Nasim *et al.*, 2022). Because phytochemicals are inexpensive and have few side effects, the focus has shifted to developing plant-based drugs. According to the World Health Organization (WHO; 2022), 40% of approved pharmaceuticals are derived from natural substances, and more than 80% of people in the world rely on plant-based pharmaceuticals.

*Salvia* L. is the largest genus within the Lamiaceae family and includes more than 800 species spread all around the world. The genus name, *Salvia*, is derived from “salvare”, which means “to heal” in Latin and refers to the healing

properties of the plants (Mailis and Skaltsa, 2018). Therefore, species from *Salvia* are widely used in folk medicine for many medicinal purposes (Kamatou *et al.*, 2018). *Salvia* species have antioxidant and antimicrobial effects, wound healing, and spasmolytic effects (Güzel *et al.*, 2019; Remok *et al.*, 2023). A wide variety of secondary metabolites such as terpenoids, tannins, flavonoids, and phenolic compounds are found in *Salvia* plants. In recent studies, the analgesic and anti-inflammatory activities of the *Salvia* genus have attracted great interest. Therefore, many studies have confirmed that plants from this genus have significant potential as analgesic, anti-inflammatory, and antioxidant agents (González-Chávez *et al.*, 2018; Coffeen and Pellicer, 2019).

*Salvia veneris* Hedge, also known as Krythean Sage, is an endemic species in Cyprus. It is a perennial herb with hairy leaves, stems up to 40 cm long, and pale lilac flowers (Meikle, 1977). *S. veneris* grows in the Kyrenia Mountains region and produces flowers in March and April. This plant grows endogenously in the subtropical climate of Cyprus. Local people consume fresh leaves as an herb/spice and dry leaves as an herbal tea for common cold and infectious diseases. Although *Salvia veneris* has traditionally been used in Cyprus, no scientific study has reported its toxicity or validated its folkloric uses.

This study aimed to evaluate the toxicity profile and folkloric use of *Salvia veneris* and develop a topical formulation with an ethanol extract of *S. veneris* for analgesic and anti-inflammatory effects as an alternative

\*Corresponding author: e-mail: goksu.oduncuoglu@outlook.com

to NSAIDs. It should be noted that this is the first study to show the toxicological, analgesic and anti-inflammatory activities of *Salvia veneris* and develop a topical formulation from *Salvia veneris* extract for localized pain and inflammation.

## MATERIALS AND METHODS

### *Plant sample*

The leaves of *Salvia veneris* Hedge were collected from the mountains of Kyrenia, Cyprus, in March 2022. The plant was identified by Assist. Prof. Dr. Emmanuel Mshelia Halilu from the Faculty of Pharmacy, Cyprus International University. Specimen voucher with the number CIU/PHARM/LAMI/004 was kept at the herbarium in the Faculty of Pharmacy for future reference.

### *Preparation of extract*

*Salvia veneris* leaves were air dried and coarsely powdered. Fifty grams of the leaves were macerated with 200mL of ethanol at room temperature for 4 days. After filtration, the extract was concentrated at reduced pressure using a rotary evaporator.

### *Qualitative phytochemical screening*

Phytochemical screening was conducted to detect the presence of secondary metabolites, namely, alkaloids, steroids, terpenoids, tannins, flavonoids, and saponins, in *Salvia veneris* using the following standard methods (Halilu *et al.*, 2023).

### *Animals and ethical approval*

Albino mice (35-40g) and Wistar rats (100-150g) of either sex were provided from the animal facility of the Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto Nigeria. The experimental protocols were approved by the Ethical Committee of the Department of Pharmacology and Toxicology, Usmanu Danfodiyo University, Sokoto Nigeria, with ethical approval number PTAC/SVd/(Ae)Ta/55-23.

14 days prior to the experiments, all animals were acclimatized to laboratory conditions. Animals were allowed in plastic cages at room temperature (25±2°C) with controlled humidity (50±5%), ventilation, and a 12-h light/dark cycle. They easily accessed standard chow and tap water *ad libitum*.

Organization for Economic Development guideline no. 425 (OECD, 2001) and the Guide for Care and Use of Laboratory Animals were followed to conduct experiments.

### *Acute toxicity (LD<sub>50</sub>) test*

Lorke's method was used to assess the acute toxicity of *Salvia veneris* ethanol extract in rats (Lorke, 1983). In the

first phase, three groups of three rats each were administered increasing doses (10mg/kg, 100mg/kg and 1,000mg/kg body weight) of *Salvia veneris* ethanol extract. In the second phase, three different doses (1600mg/kg, 2900mg/kg and 5000mg/kg) of the extract were tested. Doses of 50mg/kg, 100 mg/kg, and 200 mg/kg were selected for further experiments. The LD<sub>50</sub> was calculated according to the following equation:

$$LD_{50} = \sqrt{a \times b}$$

a: Mean of the highest nonlethal dose

b: Mean of the least toxic dose

### *Analgesic activity*

#### *Acetic acid-induced writhing test*

Albino mice (35-40g) were divided into five different groups (n=5/group). Group 1 received orally distilled water (1ml/kg) (i.e., negative control), and Groups 2, 3, and 4 received plant extracts at 50, 100, and 200mg/kg, respectively. Inhibition of abdominal writhing in mice by *Salvia veneris* extract was compared with inhibition of abdominal writhing by piroxicam (10mg/kg), a reference analgesic, orally administered to Group 5. Forty-five minutes later, 1% glacial acetic acid (1ml/kg) was administered intraperitoneally (i.p.) to all animals to create pain. Five minutes after the acetic acid injection, the number of writhes was counted for 10 minutes (Whittle, 1964).

#### *Formalin test*

Rats were divided into five different groups (n=5/group). The first group received orally normal saline solution (10mg/kg) (i.e., negative control), and the second, third, and fourth groups received plant extracts at 50, 100, and 200mg/kg, respectively. Piroxicam (10mg/kg) was orally administered to the fifth group as a reference analgesic. Thirty minutes later, 0.05ml of 2.5% formalin solution was injected into the right paw of each rat to create pain. The licking time of the formalin injected paw was recorded at 0-5 minutes (early phase) and 50-60 minutes (last phase) (Hunskar and Hole, 1987).

#### *Anti-inflammatory activity*

Twenty-five rats were divided into five different groups (n=5/group). The volume of each rat paw was measured (0h). Group 1 received orally distilled water (5ml/kg) (i.e., negative control). Groups 2, 3, and 4 received oral *Salvia veneris* extract at 50, 100 and 200mg/kg, respectively. Group 5 (i.e., positive control) received piroxicam (10mg/kg). Thirty minutes after drug administration, edema was induced on the right paw of the rat in each group by injection of 0.05mL of 2.5% formalin. Edema of the formalin injected paw was measured hourly (1h, 2h, 3h, 4h, and 5h) after formalin injection (Domiaty *et al.*, 2022).

#### *Antioxidant activity*

The ability of the ethanol extract of *Salvia veneris* to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals

was assessed according to a previously described method (Kumarasamy *et al.*, 2007). In this assay, 0.1mM DPPH was prepared by dissolution in ethanol. The ethanol extract of the plant was prepared in six different concentrations (0.0195 - 0.625mg/ml) by serial dilution. Three milliliters of plant extract from each concentration were mixed with one milliliter of DPPH solution, and the mixture was incubated in the dark for 30 minutes at room temperature. Then, their absorbances were measured at 517nm. A control was prepared without the addition of the plant extract. Ascorbic acid at the same concentrations was used as a standard. The capability to scavenge DPPH was calculated using the following equation:

Scavenging activity (%) = [A(control)-A(sample)/A (control)]x100  
 A (control): absorbance of DPPH solution  
 A (sample): absorbance of plant sample with DPPH solution (Baliyan *et al.*, 2022)

#### **Preparation of the topical formulation**

The *S. veneris* topical formulation was prepared as outlined in the British Pharmacopoeia (BP) with slight modifications. The formulations of the emulsion-based cream of *S. veneris* are given in table 1. In the formulations, stearic acid was used as an emulsifier and was mixed with sodium alginate and cetyl alcohol. The mixture was melted by heating in a water bath at 80°C (oil phase). The preservative (sodium benzoate), triethanolamine, glycerin, and ethanol extract of *S. veneris* were dissolved in distilled water and heated to 80°C (water phase). Then, the water phase was slowly added to the oil phase while stirring in a water bath.

#### **Evaluation of the topical formulation**

##### **Organoleptic evaluation**

The organoleptic features, such as the odor, texture and color of the formulations, were examined, and the results are given in table 3.

##### **pH Determination**

The formulations (0.5g) were each dissolved in 50mL of distilled water to prepare solutions for pH measurements (Ilomuanya *et al.*, 2018). pH was measured by using an AE-PH813 pH meter.

##### **Centrifugation assay**

This assay was carried out by transferring two grams from each formulation into centrifuge tubes and then spinning at a speed of 5000 rpm for 15 minutes. Physical stability was evaluated by observing the presence or absence of phase separation (Valarmathi *et al.*, 2020).

##### **Spreadability**

The spreadability of the topical formulations was evaluated using the parallel-plate method (Bakhrushina *et al.*, 2022). One gram of the formulation prepared 48 h before the test was placed between two glass slides (20 x 10 cm). Then, the sample was compressed to obtain a

uniform thickness by placing a weight of 180 g on the glass slide for one minute. The diameter of the sample between the glass slides was measured. The given formula was used to calculate the spreadability:

$$S_i = d^2 \times \pi / 4$$

$S_i$ =spreading area (mm<sup>2</sup>)

$d$ =diameter (mm)

##### **Washability**

The topical formulations were applied on the skin, and the ease of removal was examined by washing with water.

##### **Homogeneity**

The homogeneity of the topical formulations was evaluated by judging their appearances and touch affinities.

##### **Microbial limit and growth tests**

Microbial analysis was performed for all formulations as outlined in the United States Pharmacopoeia (USP). The spread plate method was used to determine the total aerobic microbial count and the combined yeast and mold counts.

##### **Accelerated stability test**

ICH guidelines were followed in the accelerated stability testing of the topical formulations. This test was conducted for at stable formulation at room temperature for seven days. The formulations were maintained at 40°C for 20 days and at room temperature for comparison. They were observed on the 0th, 5th, 10th, 15th, and 20th days for pH, appearance, spreadability, removal, phase separation, and homogeneity.

## **STATISTICAL ANALYSIS**

All experiments were triplicated, and the results are expressed as the mean ± standard error of the mean. IC<sub>50</sub> values were calculated by nonlinear regression. The differences between the obtained results were determined by one-way ANOVA and post hoc Tukey's multiple comparison test. For all statistical analyses, GraphPad Prism version 9.5.1 software was used. Values at P<0.05 were considered statistically significant.

## **RESULTS**

### **Qualitative phytochemical screening**

The different classes of phytochemicals present in *S. veneris* are shown in table 2.

### **Acute toxicity (LD<sub>50</sub>)**

In the first and second phases, mortality and toxicity signs were not observed in rats when the extract was administered orally. The LD<sub>50</sub> value was calculated and was found to be greater than 5000mg/kg.

**Table 1:** Composition of *Salvia veneris* extract-based topical formulation.

Ingredients	Category	F1	F2	F3	F4	F5
Ethanol extract	Active ingredient	1g	1g	1g	1g	1g
Sodium alginate	Emulsifier	0.3g	0.3g	0.3g	0.3g	0.3g
Cetyl alcohol	Thickener moisturizer	0.35g	1.5g	1.5g	2.5g	2.5g
Stearic acid	Thickener	0.8g	0.8g	1.2g	1.2g	1.2g
Triethanolamine	Surfactant	0.18g	0.18g	0.18g	0.18g	0.18g
Glycerin	Humectant	1.3g	1.3g	1.3g	1.3g	-
Sodium benzoate	Preservative	0.3g	0.3g	0.3g	0.3g	0.3g
Distilled water	Vehicle	q.s.	q.s.	q.s.	q.s.	q.s.

Key: q.s: sufficient quantity

**Table 2:** Phytochemicals of *Salvia veneris*

Phytochemicals	Presence
Alkaloids	-
Saponins	+
Tannins	+
Phenolic compounds	+
Flavonoids	+
Triterpenoids/steroids	+

Key: -: absent, +: present

**Table 3:** Evaluation of topical formulations

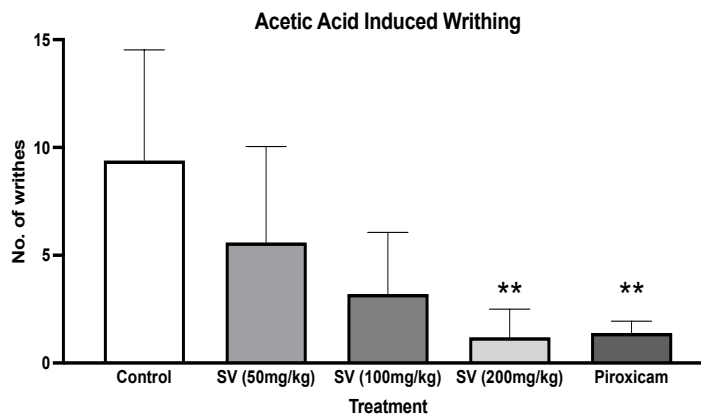
Parameters	Formulations				
	F1	F2	F3	F4	F5
Color	Light green	Bright green (shiny)	Bright green (shiny)	Bright green (shiny)	Bright green (shiny)
Texture	Smooth	Smooth	Smooth	Smooth	Smooth
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
Odor	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic
Phase separation	No separation (White precipitate at bottom)	No separation	No separation	No separation	No separation
Spreadability (mm <sup>2</sup> )	7088.22 mm <sup>2</sup>	4417.86 mm <sup>2</sup>	3525.65 mm <sup>2</sup>	2827.43 mm <sup>2</sup>	2206.18 mm <sup>2</sup>
Washability	+++	++	++	++	+
pH	8.60 ± 0.05	7.80 ± 0.05	7.85 ± 0.05	7.58 ± 0.05	7.67 ± 0.05

Key: +++ Excellent, ++ Good, + Satisfactory

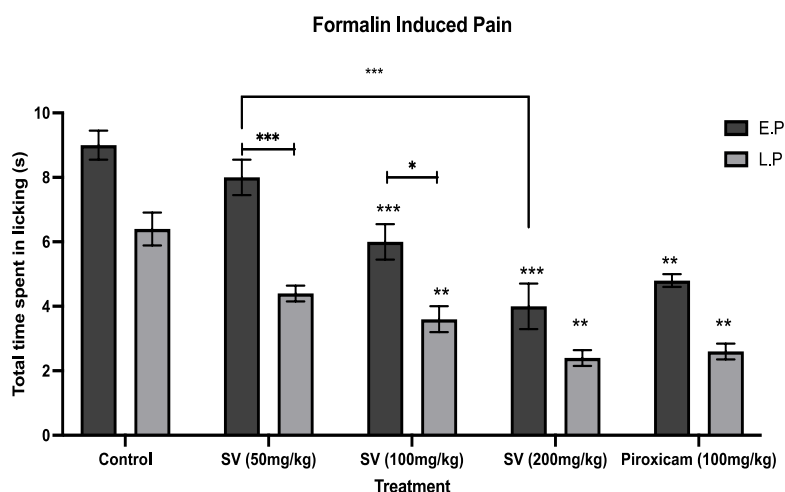
**Table 4:** Accelerated stability test of F1.

Days	Temperature	Formulation	Parameters					
			pH	P1	P2	P3	P4	P5
0 <sup>th</sup>	RT	F1	8.60	NCC	**	ES	**	NPS
	40°C ± 1°C	F1	8.60	NCC	**	ES	**	NPS
5 <sup>th</sup>	RT	F1	7.50	CC	**	ES	**	NPS
	40°C ± 1°C	F1	8.01	CC	**	ES	**	NPS
10 <sup>th</sup>	RT	F1	7.45	NCC	**	ES	**	NPS
	40°C ± 1°C	F1	8.05	CC	**	ES	**	NPS
15 <sup>th</sup>	RT	F1	7.28	NCC	**	ES	**	NPS
	40°C ± 1°C	F1	8.02	NCC	**	ES	**	NPS
20 <sup>th</sup>	RT	F1	7.13	NCC	**	ES	**	NPS
	40°C ± 1°C	F1	8.18	NCC	**	ES	**	NPS

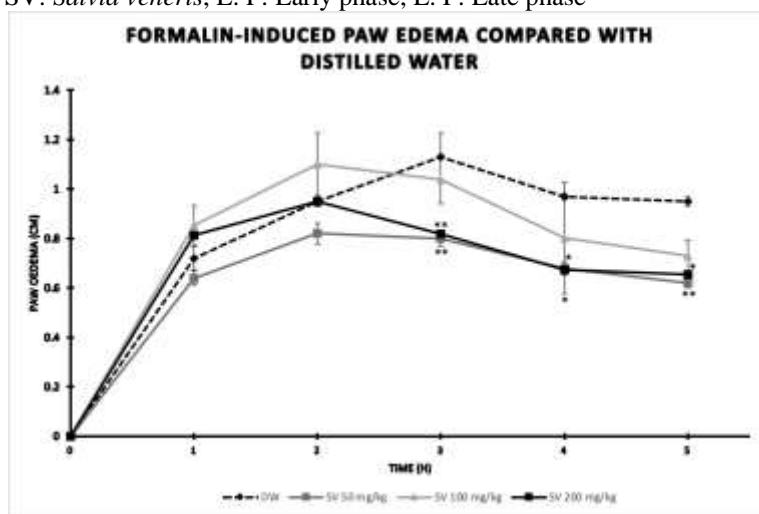
Key: P1: Appearance, P2: Spreadability, P3: Removal, P4: Homogeneity, P5: Phase separation, F1: Topical formulation, NCC: Not change in color, CC: Change in color, ES: Easy, NPS: No phase separation, \*\*: Good



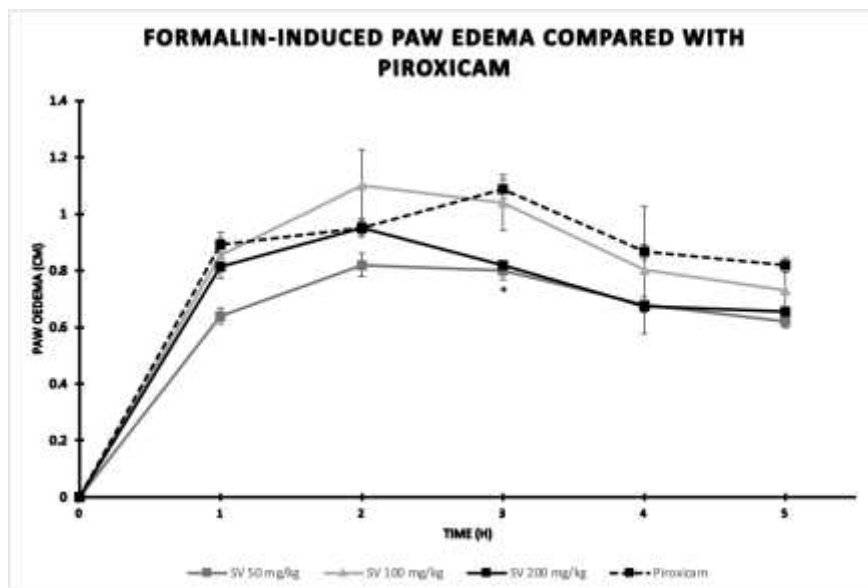
**Fig. 1:** Effect of *S. veneris* ethanol extract on acetic acid-induced writhing in mice. The results are given as the mean  $\pm$  SEM of five animals in each group; one-way ANOVA; \*\* $P < 0.01$  compared with the control. SV: *Salvia veneris*



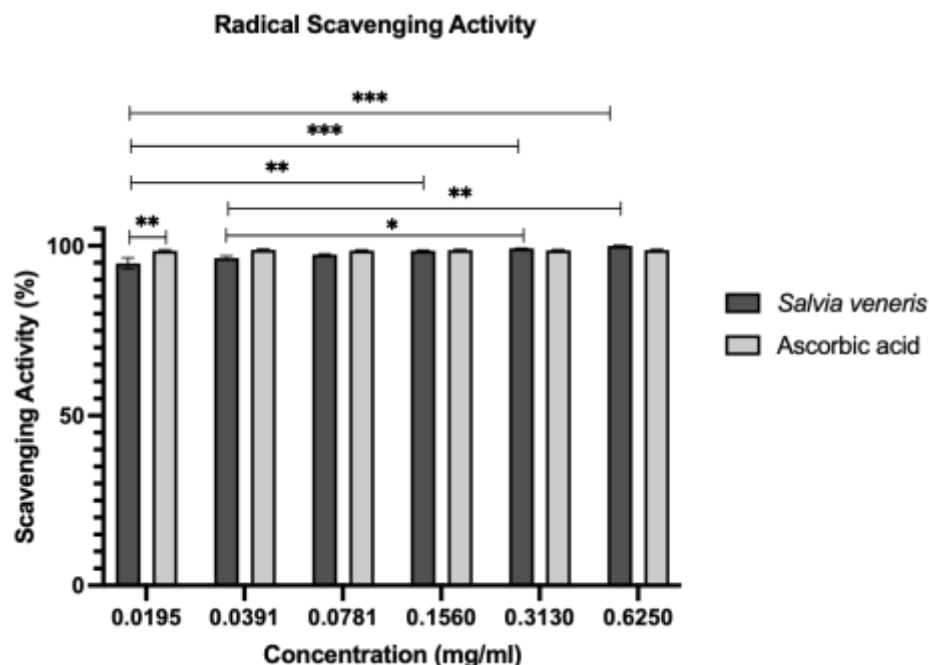
**Fig. 2:** Effect of *S. veneris* ethanol extract on formalin-induced pain in rats. The results are given as the mean  $\pm$  SEM of five animals in each group; one-way ANOVA followed by post hoc Tukey's multiple comparisons test, \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . SV: *Salvia veneris*, E. P: Early phase, L. P: Late phase



**Fig. 3:** Effect of *S. veneris* ethanol extract on formalin-induced paw edema in rats. The results are given as the mean  $\pm$  SEM of five animals in each group; one-way ANOVA, \* $P < 0.05$ , \*\* $P < 0.01$  compared to normal control (distilled water). SV: *Salvia veneris*, DW: Distilled water.



**Fig. 4:** Effect of *S. veneris* ethanol extract on formalin-induced paw edema in rats. The results are given as the mean  $\pm$  SEM of five animals in each group; one-way ANOVA, \* $P < 0.05$ , \*\* $P < 0.01$  compared to the positive control (Piroxicam). SV: *Salvia veneris*



**Fig. 5:** Radical scavenging activity of *Salvia veneris* ethanol extract and standard ascorbic acid. The results are presented as the mean  $\pm$  SEM; one-way ANOVA followed by Tukey's post hoc multiple comparisons test, \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

#### **Analgesic activity**

##### *Acetic acid-induced writhing test*

As presented in fig. 1, *Salvia veneris* ethanol extract (200mg/kg) showed a significant ( $P = 0.008$ ) decrease in the number of writhes with 87.2% inhibition compared with the control. A similar inhibition (85.1%) was observed with piroxicam. Maximum inhibition was recorded with 200mg/kg plant extract.

##### *Formalin test*

In the early and late phases, 100mg/kg and 200mg/kg of *Salvia veneris* ethanol extract produced a significant ( $P < 0.01$ ) decrease in the licking time of the formalin injected paw when compared with the control fig. 2. The licking time was significantly ( $P < 0.05$ ) lower in the late phase than in the early phase in *Salvia veneris* ethanol extract 50mg/kg, 100mg/kg and piroxicam-treated rats.

*Salvia veneris* extract showed a dose-dependent relationship with inhibition of formalin-induced pain. By increasing the dose from 50 to 200mg/kg, there was a significant decrease in the licking time of the paw injected with formalin in the early phase ( $P<0.001$ ).

#### **Anti-inflammatory activity**

*Salvia veneris* extract significantly decreased ( $P<0.05$ ) the edema of formalin injected paw after 3h at 50 and 200mg/kg doses compared with the negative control, which received distilled water fig. 3. Maximum reduction in paw edema was observed at doses of 50 and 200mg/kg of *Salvia veneris* extract. As shown in fig. 4, 50mg/kg *Salvia veneris* extract significantly ( $P<0.05$ ) reduced formalin-induced paw edema after 3h compared with piroxicam. *Salvia veneris* extract (200mg/kg) produced a significant ( $P<0.05$ ) reduction in paw edema from 2h to 4h and 5h.

#### **Antioxidant activity**

The DPPH radical scavenging activity of the ethanol extract of *Salvia veneris* was assessed using a spectrophotometric assay. The extent of the color change of the DPPH radical from purple to yellow was measured at 517 nm. The ethanol extract of *S. veneris* showed remarkable antioxidant activity with an  $IC_{50}$  value of 0.04559mg/ml in comparison with the standard ascorbic acid ( $IC_{50}=0.000928$ mg/ml). The *S. veneris* ethanol extract exhibited scavenging activity against DPPH in a concentration-dependent manner. As depicted in fig. 5, there was a statistically significant increase in the radical scavenging activity of *Salvia veneris* extract when the dose was increased. Only the lowest concentration (0.0195mg/ml) of ascorbic acid showed significantly higher DPPH scavenging activity than the lowest concentration of *Salvia veneris* ethanol extract ( $P=0.003$ ), and at the other concentrations, scavenging activity did not differ significantly.

#### **Evaluation of topical formulations**

The formulations were found to be homogenous because they had a uniform distribution of plant extract. Moreover, different color spots or different touch affinities were not observed in the formulations. The ingredients were not separated when force was applied, and the structural integrity was stable. In addition, they were easily spread with a small amount of shear. After application of the topical formulations on the skin, the touch was smooth, and it did not form a greasy smear on the skin. The results are presented in table 3.

#### **Microbial limit test**

All formulations were tested at four different concentrations. Concentrated (without dilution), 1/5, 1/10, and 1/100 diluted. The total bacterial count (TBC) was 30 CFU/g for concentrated F1, whereas fungal growth was

not detected. Growth of bacteria and fungi was not detected in F2, F3, F4, and F5.

#### **Accelerated stability test**

The results of the accelerated stability test of the topical formulations are presented in table 4. F1 was stable at room temperature and at an accelerated temperature (40°C) for 20 days.

## **DISCUSSION**

Topical formulations with NSAIDs are used for the treatment of localized inflammation, edema, and pain. There is a need for a new topical formulation as an alternative to the available topical NSAIDs with fewer side effects. The study results confirmed the safety, analgesic, anti-inflammatory, and antioxidant activities of *Salvia veneris* ethanol extract. Therefore, these results suggest that *Salvia veneris* ethanol extract is a novel candidate in topical formulations for analgesic and anti-inflammatory effects.

*Salvia veneris* was found to be rich in secondary metabolites such as phenolic compounds, flavonoids, and terpenoids. Similar to other members of the Lamiaceae family, alkaloids were not detected in *Salvia veneris*. The main role of secondary metabolites in plants is defense. They protect plants from environmental stress through their free radical scavenging properties (Hasanuzzaman *et al.*, 2020).

Acute toxicity studies revealed that *Salvia veneris* ethanol extract is practically nontoxic since it has an  $LD_{50}$  greater than 5000mg/kg (Erhirhie *et al.*, 2018). Therefore, this extract is considered relatively safe. In addition, *Salvia veneris* showed a better acute toxicity profile than some other *Salvia* species, such as *S. verbenaca* ( $LD_{50}=2000$ mg/kg) and *S. officinalis* ( $LD_{50}=3000$ mg/kg) (Mrabti *et al.*, 2022; Rhaimi *et al.*, 2023). In addition,  $LD_{50}$  was used to determine the concentration of *Salvia veneris* ethanol extract for analgesic and anti-inflammatory models.

The acetic acid-induced writhing test is a sensitive procedure for evaluating the analgesic effects of compounds. The abdominal constriction response is related to local peritoneal cells and is mediated by prostaglandin pathways (Yam *et al.*, 2020). A significant reduction in abdominal constriction indicates that *Salvia veneris* ethanol extract has an analgesic principle and may be mediated by prostaglandin pathways. To determine whether the analgesic effect results from central or peripheral actions or both, a formalin test was performed. Injection of formalin causes a biphasic licking response, known as the early phase and late phase. The early phase is related to acute neurogenic pain since formalin directly stimulates sensory nerve fibers, and the late phase is related to inflammatory pain due to the presence of

inflammatory mediators (Valle-Dorado *et al.*, 2022). *Salvia veneris* ethanol extract significantly reduced pain in both phases. The time for licking the formalin injected paw was inhibited by 33.3% in the early phase and by 43.8% in the late phase by 100mg/kg extract. A dose-dependent effect was observed since 200mg/kg ethanol extract inhibited the licking time of the formalin injected paw by 55.6% in the early phase and 62.5% in the late phase. It suppresses acute and inflammatory pain similar to central nervous system analgesics (e.g., morphine), whereas indomethacin suppresses only inflammatory pain since it acts peripherally and inhibits the late phase (Niibori *et al.*, 2020). Therefore, these results suggest the analgesic activity of *Salvia veneris* extract on the central nervous system.

A formalin-induced paw edema model was used to screen for the anti-inflammatory effect of *Salvia veneris* ethanol extract in rats. The study results showed that the ethanol extract of *Salvia veneris* significantly reduced formalin-induced paw edema 3 h after injection. Therefore, it shows anti-inflammatory activity by inhibiting inflammatory mediators such as prostaglandins. According to the study results, the analgesic and anti-inflammatory activities of the *S. veneris* ethanol extract may be due to the inhibition of the cyclooxygenase enzyme, which leads to prostaglandin synthesis.

The radical scavenging activity of the *Salvia veneris* ethanol extract significantly increased with increasing concentration. It had the highest antioxidant activity (99.98%) at 0.625mg/ml, which was significantly higher than the antioxidant activity recorded at 0.0195mg/ml ( $P<0.001$ ) and 0.0391mg/ml ( $P=0.004$ ). A significant difference in antiradical activity was observed only at the lowest concentrations of *S. veneris* and ascorbic acid, which was used as a standard. In addition, the *S. veneris* ethanol extract has a lower IC<sub>50</sub> for radical scavenging than the ethanol extract of *S. officinalis*, which is reported as 0.2523mg/ml (Vieira *et al.*, 2020). In other studies, the radical scavenging activity of different *Salvia* species was evaluated, and their IC<sub>50</sub> values ranged from 0.0235-0.163mg/ml (Nickavar *et al.*, 2007, Iravani *et al.*, 2020). Therefore, *Salvia veneris* shows better antioxidant activity than *S. hypoleuca*, *S. limbata*, *S. officinalis*, *S. reuterana*, *S. syriaca*, *S. verticillata*, and *S. virgata*. In addition to its strong antioxidant activity, *Salvia veneris* extract showed antimicrobial activity. *S. veneris* extract is effective against gram-positive bacteria and has a strong inhibitory effect (Toplan *et al.*, 2017). Therefore, *S. veneris* extract can be used as an antiseptic to protect the skin barrier and prevent bacterial growth on the skin when applied topically.

Leukocytes are immune system cells and they protect the body against infection. In the damaged regions, leukocytes increase oxygen uptake and enhance reactive

oxygen species (ROS) production (Chelombitko, 2018). Therefore, the release of ROS increases and exacerbates inflammation in the damaged regions. Antioxidants are important compounds that neutralize free radicals and ROS in damaged cells (Martemucci *et al.*, 2022). Thus, they provide protection against the development of inflammation. The good antioxidant activity of *Salvia veneris* ethanol extract also improves its anti-inflammatory activity by reducing free radicals and ROS.

As previously reported, *Salvia veneris* contains the monoterpenes  $\alpha$ -pinene, 1,8-cineole, camphor, borneol and thujone (Toplan *et al.*, 2017). Monoterpenoids exert their analgesic effects by inhibiting transient receptor potential (TRP) channels. 1,8-Cineole, camphor, borneol and thujone inhibit TRPV3, and camphor also inhibits TRPA1 (Chacon *et al.*, 2022). 1,8-Cineole inhibits the TRPM8 receptor and shows strong analgesic activity similar to that of morphine (Pishgahzadeh *et al.*, 2019). In addition, monoterpenoids are good anti-inflammatory agents. Therefore, the anti-inflammatory activity of *Salvia veneris* can be linked with the monoterpenoids in the extract. Borneol and  $\alpha$ -pinene have been reported to possess anti-inflammatory activity by inhibiting lipoxygenase, cyclooxygenase enzymes, and nitric oxide synthase (Zuzarte *et al.*, 2018; Salehi *et al.*, 2019). Due to their lipophilic structures and sizes, monoterpenoids can easily penetrate the skin to reach their side of action. This makes them good candidates for use in topical formulations.

Different topical formulations from the ethanol extract of *Salvia veneris* were prepared and evaluated to assess their characteristic properties. All formulations were green, which was mainly due to the presence of chlorophyll. They are well homogenized, smooth, and perfectly consistent. Neither phase separation nor cracking in the phase was observed.

According to Bureau of Indian Standards (BIS), the microbial content of skin formulations should not be more than 1000 CFU/g. The microbial content of all formulations was recorded within an acceptable range. The antimicrobial activity of *Salvia veneris* has been previously described. Therefore, it may prevent the growth of microorganisms in the formulation, and provide self-preservation.

The therapeutic efficacy of topical pharmaceutical products is related to their skin absorbability (Iyer *et al.*, 2021). If topical products can be applied easily as a thin layer on the skin, they can be better absorbed. Therefore, spreadability is one of the important properties of topical products for exerting their effects. F1 has better spreadable and washable properties than the other formulations due to its higher water content. The least spreadable and washable formulation is F5 since it does not contain glycerin. As reported in BIS, skin



formulations should have pH values of 4.0-9.0. The pH of all formulations was recorded between 7.58 and 8.60, which is in the range and good for skin pH.

Due to the better physical appearance, spreadability, and washability of F1 among the other formulations, it was optimized as a final formulation. Further evaluation of accelerated stability was performed using F1. Formulation at room temperature and formulation at an accelerated temperature are stable for 20 days. Phase separation was not observed, and the formulations were homogenous. In addition, deterioration or mold formation was not recorded. Color change was observed in the topical formulation at room temperature and at an accelerated temperature. *Salvia veneris* extract contains chlorophyll and other organic compounds that tend to change color. The main reason for the color change is the oxidation of natural compounds in the extract, which is common in commercial formulations (Lourenço *et al.*, 2019). Thus, it is not a sign of instability. A decrease in pH was observed, but the pH value of the formulations was still within the range.

## CONCLUSION

This study demonstrates that the ethanol extract of *Salvia veneris* has remarkable analgesic, anti-inflammatory, and antioxidant activities, confirming the traditional use of this plant in Cyprus. The ethanol extract of *Salvia veneris* possesses anti-inflammatory and analgesic activities by suppressing acute and inflammatory pain. Therefore, *Salvia veneris* ethanol extract may be a novel drug candidate for pain and inflammation. This study showed the consistency and quality of the topical formulations. They were validated and evaluated as safe and suitable for the skin. Future studies are needed to isolate and characterize bioactive phytochemicals from *Salvia veneris* extract.

## REFERENCES

- Bakhrushina E, Anurova N, Zavalniy S, Demina B, Bardakov I and Krasnyuk II (2022). Dermatologic gels spreadability measuring methods comparative study. *Int. J. Appl. Pharm.*, **14**(1): 164-168.
- Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP and Chang CM (2022). Determination of antioxidants by dpph radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*. *Molecules*, **27**(4): 13-26.
- Chacon FT, Raup-Konsavage WM, Vrana KE and Kellogg JJ (2022). Secondary Terpenes in *Cannabis sativa* L.: Synthesis and Synergy. *Biomedicines*, **10**(12): 31-42.
- Chelombitko MA (2018). Role of Reactive Oxygen Species in Inflammation: A Minireview. *Moscow Univ. Biol. Sci. Bull.*, **73**(4): 199-202.
- Coffeen U and Pellicer F (2019). *Salvia divinorum*: from recreational hallucinogenic use to analgesic and anti-inflammatory action. *J. Pain Res.*, **12**: 1069-1076.
- Crespo-Pardo L and Taboada-Iglesias Y (2021). Inflammatory mediators: Its connection with chronic pain and associated problems. Review. *Rev. Soc. Esp. Dolor.*, **28**(1): 37-46.
- Domiaty S, El Galil K and Ragab H (2022). Molecular mechanics simulations and experimental investigation of the effect of tadalafil on various inflammatory pain mediators. *ACS Omega*, **7**(48): 43330-44484.
- Erhirhie EO, Ihekwereme CP and Ilodigwe EE (2018). Advances in acute toxicity testing: Strengths, weaknesses, and regulatory acceptance. *Interdiscip. Toxicol.*, **11**(1): 5-12.
- González-Chávez MM, Alonso-Castro AJ, Zapata-Morales JR, Arana-Argáez V, Torres-Romero JC, Medina-Rivera YE, Sánchez-Mendoza E and Pérez-Gutiérrez S (2018). Anti-inflammatory and antinociceptive effects of tilifodiolide, isolated from *Salvia tiliifolia* Vahl (Lamiaceae). *Drug development research*, **79**(4): 165-172.
- Güzel S, Özey Y, Kumaş M, Uzun C, Özkorkmaz EG, Yıldırım Z, Ülger M, Güler G, Çelik A, Çamlıca Y and Kahraman A (2019). Wound healing properties, antimicrobial and antioxidant activities of *Salvia kronenburgii* Rech. F. and *Salvia euphratica* Montbret, Aucher & Rech. F. var. *euphratica* on excision and incision wound models in diabetic rats. *Biomed. Pharmacother.*, **111**: 1260-1276.
- Halilu E, Chinenye J, Ugwah-Oguejiofor C, Oduncuoglu G and Gamde M (2023). Physicochemical, toxicity and antioxidant activity of *Terminalia catappa* kernel oil in mice. *Pharmacognosy Res.*, **15**(1):119-127.
- Hasanuzzaman M, Bhuyan MHMB, Zulfiqar F, Raza A, Mohsin SM, Mahmud JA, Fujita M and Fotopoulos V (2020). Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator. *Antioxidants (Basel, Switzerland)*. **9**(8): 681.
- Hunskar S and Hole K (1987). The formalin test in mice: Dissociation between inflammatory and noninflammatory pain. *Pain*, **30**(1): 103-114.
- Ilomuanya M, Ajayi T, Cardoso-Daodu I, Akhimien T, Adeyinka O and Aghaizu C (2018). Formulation and evaluation of polyherbal antioxidant face cream containing ethanol extracts of *Psidium Guajava* and *Ocimum Gratissimum*. *Nig. J. Pharm. Res.*, **14**(1): 61-68.
- Iravani M, Mahinpour R, Zahraei Z, Toluei Z, Asgari F, and Haghighipour N (2020). Comparison of cytotoxic and antioxidant activities and phenol content of four *Salvia* L. species from Iran. *J. Med. Plants.*, **19**(76): 59-68.
- Iyer A, Jyothi VGSS, Agrawal A, Khatri DK, Srivastava S, Singh SG and Madan J (2021). Does skin permeation kinetics influence efficacy of topical

- dermal drug delivery system? Assessment, prediction, utilization, and integration of chitosan biomacromolecule for augmenting topical dermal drug delivery in skin. *J. Adv. Pharm. Technol. Res.*, **12**(4): 345-355.
- Kamatou GP, Makunga NP, Ramogola WP and Viljoen AM (2018). South African *Salvia* species: a review of biological activities and phytochemistry. *J. Ethnopharmacol.*, **119**(3): 664-672.
- Kumarasamy Y, Byres M, Cox PJ, Jaspars M, Nahar L and Sarker SD (2007). Screening seeds of some Scottish plants for free radical scavenging activity. *Phytother Res.*, **21**(7): 615-621.
- Lorke D (1983). A new approach to practical acute toxicity testing. *Arch. Toxicol.*, **54**(4): 275-287.
- Lourenço SC, Moldão-Martins M and Alves VD (2019). Antioxidants of Natural Plant Origins: From Sources to Food Industry Applications. *Molecules*, **24**(22): 4132.
- Mailis T and Skaltsa H (2018). Polar Constituents of *Salvia willeana* (Holmboe) Hedge, Growing Wild in Cyprus. *Plants (Basel, Switzerland)*, **7**(1):18.
- Martemucci G, Costagliola C, Mariano M, D'andrea L, Napolitano P and D'Alessandro AG (2022). Free Radical Properties, Source and Targets, Antioxidant Consumption and Health. *Oxygen*, **2**(2): 48-78.
- Meikle RD (1977). Flora of Cyprus. Version 1. Bentham Moxon Trust, Royal Botanic Gardens.
- Mrabti HN, El Menyiy N, Charfi S, Saber M, Bakrim S, Alyamani RA, Rauf A, Ali AMH, Abdallah EM, El Omari N, Bouyahya A and Assaggaf H (2022). Phytochemistry and Biological Properties of *Salvia verbenaca* L.: A Comprehensive Review. *Biomed. Res. Int.*, **2022**(3): 787-818.
- Nasim N, Sandeep IS and Mohanty S (2022). Plant-derived natural products for drug discovery: current approaches and prospects. *Nucleus (Calcutta)*, **65**(3): 399-411.
- Nickavar B, Kamalinejad M and Izadpanah H (2007). *In vitro* free radical scavenging activity of five *Salvia* species. *Pak. J. Pharm. Sci.*, **20**(4): 291-294.
- Niibori M, Kudo Y, Hayakawa T, Ikoma-Seki K, Kawamata R, Sato A and Mizumura K (2020). Mechanism of aspirin-induced inhibition on the secondary hyperalgesia in osteoarthritis model rats. *Heliyon*, **6**(5): e03963.
- Oronsky B, Caroen S and Reid T (2022). What exactly is inflammation (and What Is It Not?). *Int. J. Mol. Sci.*, **23**(23): 14905.
- Pishgahzadeh E, Shafaroodi H and Asgarpanah J (2019). Analgesic and anti-inflammatory activities of the essential oil from *Artemisia sieberi* Besser. *Braz. J. Pharm. Sci.*, **55**(2): e17011
- Remok F, Saidi S, Gourich AA, Zibouh K, Maouloua M, Makhoukhi FE, Menyiy NE, Touijer H, Bouhrim M, Sahpaz S, Salamatullah AM, Bourhia M and Zair T (2023). Phenolic content, antioxidant, antibacterial, antihyperglycemic, and  $\alpha$ -amylase inhibitory activities of aqueous extract of *Salvia lavandulifolia* Vahl. *Pharmaceuticals* (Basel, Switzerland), **16**(3): 395.
- Rhaimi S, Brikat S, Lamtai M and Ouhssine M (2023). Acute oral toxicity and neurobehavioral effects of *salvia officinalis* essential oil in female wistar rats. *Adv. Anim. Vet. Sci.*, **11**(4): 654-662.
- Salehi B, Upadhyay S, Erdogan I, Kumar A, Jayaweera S, Dias D, Sharopov F, Taheri Y, Martins N, Baghalpour N, Cho W and Sharifi-Rad J (2019). Therapeutic potential of  $\alpha$ - and  $\beta$ -pinene: A miracle gift of nature. *Biomolecules*, **9**(11): 738.
- Shi C, Ye Z, Shao Z, Fan B, Huang C, Zhang Y, Kuang X, Miao L, Wu X, Zhao R, Chen X, Zhang B, Tong R, Hu X, Fu Z, Lin J, Li X, Sun T, Liu G, Dai H, Guo C, Zhang B, Xu T, Wen A, Zuo X, Liu J, Chen X, Li H, Wang J, Luo M, Fan T, Qian Y, Li X, Qiu W, Lin X, Pang Y, Hou Y, Yao D, Kou W, Sun B, Hu C, Xia Y, Zhao M, Zhu C, Li Q and Zhang Y (2022). Multidisciplinary guidelines for the rational use of topical non-steroidal anti-inflammatory drugs for musculoskeletal pain. *J. Clin. Med.*, **12**(4): 1544.
- Skin creams - specification (second revision) (2004). Bureau of Indian Standards; New Delhi: IS 6608
- Toplan G, Kurkcuoglu M, Goger F, Iscan G, Duymus H, Mat A, Baser KH, Koyuncu M and Sariyar G (2017). Composition and biological activities of *Salvia veneris* Hedge growing in Cyprus. *Ind. Crops Prod.*, **97**: 41-48.
- Valarmathi S, Kumar MS, Sharma V and Imran M (2020). Formulation and evaluation of herbal face cream. *Res. J. Pharm. and Tech.*, **13**(1): 216-218.
- Valle-Doradomg, Hernández-León A, Nani-Vázquez A, Ángeles-López GE, González-Trujano ME and Ventura-Martínez R (2022). Antinociceptive effect of *Mansoa alliacea* polar extracts involves opioid receptors and nitric oxide in experimental nociception in mice. *Biomed. Pharmacother.*, **152**: 113253.
- Vieira SF, Ferreira H and Neves NM (2020). Antioxidant and anti-inflammatory activities of cyto-compatible *Salvia officinalis* extracts: A comparison between traditional and soxhlet extraction. *Antioxidants* (Basel, Switzerland), **9**(11): 1157.
- Whittle BA (1964). The use of changes in capillary permeability in mice to distinguish between narcotic and nonnarcotic analgesics. *Br. J. Pharmacol.*, **22**(2): 246-253.
- World Health Organization. (2022). "WHO establishes the Global Centre for Traditional Medicine in India" Retrieved from <https://www.who.int/news/item/25-03-2022-who-establishes-the-global-centre-for-traditional-medicine-in-india> Accessed 25 May 2023
- Yam MF, Loh YC, Oo CW and Basir R (2020). Overview of neurological mechanism of pain profile used for animal "Pain-Like" behavioral study with proposed analgesic pathways. *Int. J. Mol. Sci.*, **21**(12): 43-55.

Zuzarte M, Alves-Silva JM, Alves M, Cavaleiro C, Salgueiro L and Cruz MT (2018). New insights on the anti-inflammatory potential and safety profile of *Thymus carnosus* and *Thymus camphoratus* essential oils and their main compounds. *J. Ethnopharmacol.*, **225**: 10-17.