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Pharmacological Evaluation And Anti-Inflammatory Study Of Extract Of Salvia Officinalis L.

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Herbal medicines and traditional therapies are becoming more and more popular around the world. Consequently, numerous clinical and experimental studies on medicinal plants are being carried out, and the findings must be updated and incorporated. Based on existing literature, this plant has anticancer, anti-inflammatory, antinociceptive, antioxidant, antibacterial, hypoglycemic, hypolipidemic, and memory-enhancing qualities. S. officinalis has been shown in clinical trials to be an effective antinociceptive, hypolipidemic, and memory-boosting plant. Future studies must elucidate the possible therapeutic applications of S. officinalis's activities. The Lamiaceae family includes the fragrant perennial herb sage (Salvia officinalis), which is well-known for its culinary and therapeutic uses. Its potential to treat a range of illnesses, including as diabetes, cancer, Alzheimer's, and cardiovascular ailments, has been the subject of numerous studies. Numerous pharmacological actions for S. officinalis have been identified by these investigations. The current study focuses on the Pharmacological evaluation and Anti-Inflammatory Study of extract of Salvia officinalis L. Additionally, the chemical components that provide S. officinalis its pharmacological effects.

Key words: Pharmacological evaluation, Anti-Inflammatory Study, Herbal therapies, Medicinal Plants, Pharmacological screening, Salvia officinalis L.

INTRODUCTION

The term “traditional medicine” refers to ways of protecting and restoring health that existed before the arrival of modern medicine. As the term implies, these approaches to health belong to each country, and have been handed down from generation to generation. A traditional system requires to meet the needs of the local communities for many centuries. The traditional system of medicine is prevalent in India since the Vedic period and as early as the dawn of human civilization.

Ayurveda ancient sciences of life are believed to be prevalent for the last 5000 years in India. It is one of the most noted systems of medicine in the world. Ayurveda is based on the hypothesis that everything in the universe is composed of five basic elements viz. space, air, energy, liquid and solid. They exist in the human body in confined forms like vata (space and air), pitta (energy and liquid) and kapha (liquid and solid). Vata, pitta and

kapha together are called tridosha (three pillars of life). Imbalance in between these will cause pathological condition.

Today herbal medicine has just improved ancient secrets and brought them on the market. Many people turned to herbal medicine because they simply were disappointed with traditional medicine or surgery. Herbal remedies are still relatively popular today, mainly due to the fact that they are regarded as harmless because they are natural.

Crud extract mixtures of plant are better than pure isolated chemicals. Several biologically active compounds in a plant work together to produce greater effect then single chemical on its own. The mixture of chemicals found in herbs can be more potent than the single purified ingredient so beloved of drugs companies. Chemical partnerships explain why whole herbs can work better than single purified ingredients. In other words, the mixture has an effect greater than the sum of its parts.

PLANT PROFILE

SALVIA OFFICINALIS L.

Salvia officinalis L. (Sage) is a perennial round shrub in the family of Labiatae/Lamiaceae. Salvia is the largest genus of this family and includes near 900 species. Plants of this genus grow all over the world and the specie of S. officinalis is native to Middle East and Mediterranean areas.

Phytochemistry:

The major phytochemicals in flowers, leaves, and stem of S. officinalis are well identified. A wide range of constituents include alkaloids, carbohydrate, fatty acids, glycosidic derivatives (e.g.,cardiac glycosides, flavonoid glycosides, saponins), phenolic compounds (e.g., coumarins, flavonoids, tannins), poly acetylenes, steroids, terpenes/terpenoids(e.g., monoterpenoids, diterpenoids, triterpenoids, sesquiterpenoids), and waxes are found in S. officinalis.

MATERIAL & METHODS

Thin layer chromatography

Thin layer chromatography (TLC) was performed on precoated 0.2 mm and 2.0 mm thickness silica gel glassplates (EMD Chemicals Inc. Gibbstown, NJ) for analytical and preparative scales respectively. The plates were developed with solvent mixtures of methanol/dichloromethane 5:95. Plates were then examined under ultraviolet light at 254 nm and at 366 nm and were also sprayed with the anisaldehyde reagent for spots visualization

Quantification of bioactive marker compound by HPLC

Analysis:

Phenolic compounds in the samples were identified and quantified using a Smart line HPLC instrument equipped with a quaternary pump and a UV-VIS detector. Reverse phase chromatography separation was performed with a C18 Eurospher-100 (5 µm

particle, 125 mm × 4 mm). The chromatographic data were processed using Chrom Gate software (version 3.1). The flow rate used for column elution was 1 ml/min and peaks were monitored by UV detection at 280 nm. The sample injection volume was 20 µl. The solvent system was 0.2% (v/v) glacial acetic acid in water (solvent A) and acetonitrile (solvent B). The gradient elution program was as follows: 90-75% A (v/v) at 0-15 min, 75-20% A (v/v) at 15-40 min, 20-0% A (v/v) at 40-45 min, 0% A (v/v) at 45-50 min, 0-90% A (v/v) at 50-55 min. Samples and standard solutions were filtered through 0.45 µm hydrophilic PTFE membrane filters before injection. Identification of the compounds in the chromatograms was performed by comparison of their retention times with those of reference standards. Determination of each phenolic compound was performed using the corresponding calibration curve. Extract samples were injected three times to HPLC for analysis.

Method for Validation:

Linearity: The standard stock solutions were separately prepared in pure ethanol and diluted to appropriate concentration range for the establishment of calibration curves. The calibration curves were plotted on the basis of linear regression analysis of the integrated peak areas (y) versus concentrations (x, µg/mL) of the five authentic compounds at different levels (Table 1). Correlation coefficients were considered to confirm the significant linear calibration for the standard samples.

Table 1: Validation parameters of HPLC method for the reported phenolic compounds

Compounds	(%) Purity	Stock concentration (mg/ml)	Linear range (µg/ml)	Regression equation	R^2	% RSD
Rosmarinic acid	96 %	1	0-100	y = 42197x – 41350	0.9965	0.5
Salvianolic acid B	≥94 %	1	0-1000	y = 2055.1x + 54.199	0.9938	0.4
Salvianolic acid A	≥95 %	0.9	0-80	y = 20037x – 30584	0.9976	0.3
Carnosic acid	≥95 %	1	0-600	y = 4519.9x + 62174	0.9683	1.5

Caffeic acid	≥98 %	6	0-180	y = 7053.4x + 12476	0.9979	0.5

Precision and Recovery: The precision of the developed HPLC method was performed with different concentrations of five standard compounds. Twenty microliters of each standard solution was injected into the HPLC system continuously under the same chromatographic conditions for five times. The percent relative standard deviation (RSD %) for the peak area of each standard were calculated. To evaluate of the accuracy of the applied method, recovery experiments were performed after adding three varying quantities of the standards to the samples (S. officinalis). Recovery values of the five components were measured from the corresponding calibration curve, and RSDs were calculated.

PHARMACOLOGICAL SCREENING

The plants extract was selected base on literature review for antioxidant, antimicrobial, anti-inflammatory study. Animal studies were approved by Institutional Animal Ethics Committee (IAEC) of R.K.D.F college of Pharmacy, Bhopal, M.P. and carried out in accordance with the Guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Animals:

Healthy Wistar rats (200-250g) were used for the study. Rats were housed in small cages in environmentally controlled (25 ± 20C, 12h light and dark cycle, with free access to food and water ad libitum). Rats were fed with the standard laboratory chow diet during the period of study.

Evaluation of Antioxident Activity

In-vitro antioxidant potential of hydroalcohalic Extract of S. officinalis

Antioxidant is a substance which prevents the oxidation of other molecules by oxidized themselves. Oxidation is a process in which an electron is transferred from substances to an oxidizing agent and this reaction can cause the production of free radicals. And these free radicals start a number of reactions in the body and starts producing damage to cells. Antioxidants dismiss these reactions by removing these free radicals. They are able to do so because they oxidized themselves. Antioxidants can be defined as substances which can prevent oxidation of easily oxidisable compounds even when they are present in small amounts. In term of nutraceuticals, antioxidants can be defined as substances present in foods which can decrease or prevents the side effects caused by reactive species in human beings.

Not any single antioxidant assay can conclude the antioxidant potential of any drug. So, a number of in-vitro methods are used to screen antioxidant potential of sample extracts of plants. Amongst various methods, DPPH method is moreover quick, cheap and simple

when compared to other test methods. Several methods are used to examine the antioxidant effect which is explained as follows:

Determination of Total phenol content

The total phenolic content of the extract of hydroalcohalic S. officinalis leave was determined by Folin Ciocalteu method. 1 ml of extract (1.0 mg/ml) in alcohol and 1 ml of Folin - Ciocalteu reagent were mixed thoroughly and the mixture was allowed to stand for 15 minutes and then 2.0 ml of 20% (w/v) sodium carbonate solution was added. The mixture was further incubated for 60 minutes at room temperature. The total phenolic content will be determined spectrophotometrically at 650 nm. The Total Phenolic content was calculated from the calibration curve which was made by preparing 1 ml aliquots of 100, 200, 300, 400 and 500 µg/ml solutions of Gallic acid. The results were expressed as gallic acid equivalents per gram of the sample.

Determination of Total flavonoid content.

The total flavonoid content of the hydroalcoholic extract of S. officinalis was determined by aluminium chloride colorimetric method. 0.5 ml of extract of S. officinalis (1mg/ml) was mixed with 0.3 ml of 10% aluminium chloride, 0.3 ml of 5% NaNO2 solution and 4.0 ml of distilled water. Then this mixture was allowed to rest for 30 minutes at room temperature. The absorbance of all the mixtures was measured at 510 nm against blanck containing water instead of the sample. Catechin was used as a standard compound for measurement of total flavonoids. The total flavonoid content was calculated from a calibration curve and results were expressed as mg/g of Catechin equivalents of plant extract.

DPPH radical scavenging assay

Scavenging of DPPH free radicals is the base of a major antioxidant assay. DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical scavenging assay is an antioxidant method which works on the principle of an electron-transfer which yields a deep violet color, which is characterized by an absorption band in ethanol solution measured at 517 nm. In this method, DPPH radical accepts an electron to become stable and change in absorbance of DPPH radical caused by antioxidant is due to reaction between antioxidant molecule and radical, which results in scavenging of radical by hydrogen donation.

DPPH is a stable free radical which is purple in color and it turned yellow after scavenging takes place. The scavenging reaction between DPPH and an antioxidant will be written as follows:

$$(DPPH^*) + (H\text{-}A) \longrightarrow DPPH\text{-}H + (A^*)$$

(Purple) (Yellow)

Antioxidants present in the plants interact with DPPH and reduced it to DPPH-H ion. This change causes colour change from purple to yellow. The extent of colour change postulates the scavenging capability of the antioxidant complexes present in the extract.

Working procedure

The free radical scavenging activity of leave extract was evaluated by using DPPH assay. A decrease in the absorption of the DPPH solution when con. of extract was added was measured at 517 nm. Ascorbic acid was used as standard. DPPH radical scavenging activity of the S. officinalis extract was measured by decreasing the absorbance of methanol solution of DPPH . A DPPH stock solution (35 mg/l) was prepared using methanol and 5 ml of this stock solution was added to 1 ml of S. officinalis extract solution at different concentrations (10-100 µg/ml). After 30 minutes, absorbance was measured at 517 nm and compared with standard. Scavenging effect will be expressed as the percentage inhibition which was calculated using the following formula:

DPPH radical scavenging activity = [Abs control –Abs sample] / [Abs control] × 100

Where Abs control - absorbance values of the blanck sample

Abs sample - absorbance value of alcoholic root extract of S. officinalis

The antiradical activity was expressed in terms of the amount of antioxidant necessary to decrease the initial DPPH absorbance by 50% [IC50]

Nitric oxide scavenging activity

In the nitric oxide assay procedure, the first step is to convert nitrate to nitrite by means of a nitrate reductase. Then second step includes use of Griess Reagent to change nitrite to a deep purple azo compound. The quantity of the azochromophore precisely replicates the quantity of nitric oxide in extract samples. That is, sodium nitroprusside gets decomposed in aqueous solution at pH 7.2 to give nitric oxide. This nitric oxide reacts with oxygen in aerobic conditions to produce nitrate and nitrites and the amount of these can be evaluated using Griess reagent.

Free radical scavengers, if present in extract competes with oxygen to react with nitric oxide. Thus inhibits generation of nitrite. This results in quenching in absorbance.

Working procedure

For measurement of nitric oxide scavenging activity sodium nitroprusside in phosphate buffered saline was mixed with different concentrations of the extract (10-100 µg/ml) dissolved in methanol and incubated at 25°C for 30 minutes. In control, test compound was not added but same amount of methanol was taken. Then this solution was incubated for 30 minutes. 1.5 ml of this incubated solution was taken and mixed with 1.5 ml of Griess reagent (1% sulphanilamide, 2% phosphoric acid, and 0.1% N-1-naphthylethylenediamine dihydrochloride). The absorbance will be measured at 546 nm and compared with standards. The percentage scavenging activity was measured with reference to standard.The degree of scavenging activity will be calculated as a scavenging percentage. IC50 value was calculated

Inhibition (%) = [Absorbance Control –Absorbance Sample / Absorbance Control] ×100

Where Abs control - absorbance values of the blank sample

Abs sample - absorbance values of alcoholic extract of S. officinalis

Evaluation of Anti-inflammatory Activity

In-vitro Anti-inflammatory activity of Salvia officinalis by Human Red Blood Cell Membrane Stabilization Method:

Hydroalcohalic extract of salviya officinalis was investigated for In-vitro Anti-inflammatory activity by human red blood cell membrane stabilization method. Four different concentrations of extracts: 1mg/ml, 2mg/ml, 4mg/ml and 6mg/ml were used for anti-inflammatory study.

Preparation of drug

Standard drug (Indomethacin, 2.5 mg/ml) and extracts (1.0 -6.0 mg/ml) were prepared in isosaline (0.85% NaCl) to final concentration.

Preparation of Suspension (10% v/v) of Human Red Blood cell

The blood sample was collected from healthy human volunteer who has not taken any NSAID for 2 weeks prior to the experiment and transferred to heparinized centrifuge tube. Blood samples were centrifuged at 3000 rpm at room temperature for 15 min. The supernatant (plasma and leucocytes) were carefully removed while the packed red blood cells were washed with fresh normal saline (0.85% w/v NaCl). The process of washing and centrifugation was repeated five times until the supernatant was clear. Then, Human erythrocytes suspension (10% v/v) was prepared as reported by Oyedapo et al., 2004.

Assay of Membrane stabilizing activity

The HRBC membrane stabilizing activity assay was carried out as reported by Sadique et al., 1989; Oyedapo et al., 2004 using 10% (v/v) Human erythrocyte suspension while Indomethacin was used as standard drugs. The assay mixtures consisted of 2 ml of hyposaline (0.25% w/v) sodium chloride, 1.0 ml of 0.15 M sodium phosphate buffer, pH 7.4, 0.5 ml of 10% (v/v) human erythrocyte suspension, 1.0 ml of drugs (standard and extracts) and final reaction mixtures were made up to 4.5 ml with isosaline.

To determine the anti-inflammatory activity by HRBC membrane stabilization method, the following solutions were prepared[175-178].

a) **Test solution** (4.5ml) consists of 2ml of hypotonic saline (0.25%w/v), 1ml of phosphate buffer (pH7.4), and 1ml of test extract (1mg/ml – 6 mg/ml) in normal saline and 0.5ml of 10% w/v human red blood cells in isotonic saline.

b) **Test control** (4.5ml) consists of 2ml of hypotonic saline (0.25%w/v) 1ml of phosphate buffer (7.4pH) and 1ml of isotonic saline and 0.5ml of 10%w/v human red blood cells in isotonic saline.

c) **Standard solution** (4.5ml) consists of 2ml of hypotonic saline (0.25%w/v) 1ml of phosphate buffer (7.4pH) and 1ml of Indomethacin (2.5mg/ml) and 0.5ml 10%w/v human red blood cells in isotonic saline.

Drug was omitted in the blood control, while the drug control did not contain the erythrocyte suspension. The reaction mixtures were incubated at 37°C for 30 min and centrifuged at 3000 rpm for 20 min. The absorbance of the supernatant solution was measured spectrophotometrically at 560 nm. Each experiment was carried out in triplicate and the average was taken. The percentage inhibition of haemolysis or membrane stabilization was calculated using the following equation.

$$\% \text{ Inhibition of haemolysis} = 100 \times (A_1 - A_2/A_1)$$

Where:

A_1 = Absorption of hypotonic buffered saline solution alone

A_2 = Absorption of test sample in hypotonic solution.

In-vivo Anti-Inflammatory Activity by Paw Edema method [87-97]

Formalin-induced Paw Edema model based upon the ability of test drug to inhibit the edema produced in the hind paw of the mice after injection of formalin. The nociceptive effect of formalin is biphasic, an early neurogenic component followed by a later tissue-mediated response. In the first phase there is release of histamine, 5-HT and kinin, while the second phase is related to the release of prostaglandins.

Animals

Healthy young adult albino (100-120 gm) of either sex and of approximate same age were used throughout the study were housed under standard laboratory conditions in polyacrylic cages, and were provided with pelleted food and water ad libitum. The animals were acclimatized to the laboratory condition for 1 week before starting the experiment. Animal studies were approved by Institutional Animal Ethics Committee (IAEC) of SRK University, Bhopal, M.P. and carried out in accordance with the Guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Formalin induced paw edema model

In-vivo Anti-inflammatory study of Salvia officinalis plant extract was conducted by formalin induced paw edema model using 12 albino rats and divided into three groups of four animal on each. In all groups, acute inflammation was induced by sub-planter injection of 0.1 ml of freshly prepared 1 % suspension of sterilized formalin in normal saline in left hind paw of the rats. The medicated formulations (0.3g) or base or standard were applied topically to the planter surface of hind paw with gentle rubbing with index finger to each rat of respective group one hour before and one hour after the formalin challenge. The paw edema volume was measured using plethysmometer at every 30 mint intervals for 4 hour after injection of formalin. The average paw edema volume of all the groups were calculated and compared with that of control. The percent inhibition of edema was calculated by using following formula.

$$\% \text{ Edema inhibition} = (1 - Vt/Vc)\ 100$$

Where, Vt = Mean edema volume of test, Vc = Mean edema volume of control

Eight groups of animals four each:

- Group I -Received gel base
- Group II -Received Extract
- Group III -- Received diclofenac (Voveran Emulgel)

Skin Irritation Study

In-vivo skin irritation study was conducted by 15 albino rats of either sex weighing between (100-120 g) was used. Animals were divided in to 3 groups of 5 animals on each. Hairs were depleted from the back of rats with the help of depilatories and area 2 cm^2 was marked on both the sides. One side served as control while the other as test and animals were used after 24 hrs. After hair depletion herbal gel was applied (500mg / rat) on test side and gel base was applied on control side once a day for 7 days and site was covered with cotton bandage and observed for any sensitivity and the reaction if any was graded as under :-

A – No reaction, B – Slight patchy erythema, C –Slight but confluent or moderate but patchy erythema, D – Moderate erythema, E – Severe erythema with or without edema.

RESULTS AND DISCUSSION

Thin layer chromatography

Thin layer chromatography (TLC) was performed on precoated 0.2 mm and 2.0 mm thickness silica gel glassplates (EMD Chemicals Inc. Gibbstown, NJ) for analytical and preparative scales respectively. The plates were developed with solvent mixtures of methanol/dichloromethane 5:95. Plates were then examined under ultraviolet light at 254 nm and at 366 nm and were also sprayed with the anisaldehyde reagent for spots visualization. The TLC patterns are shown in figure 1.

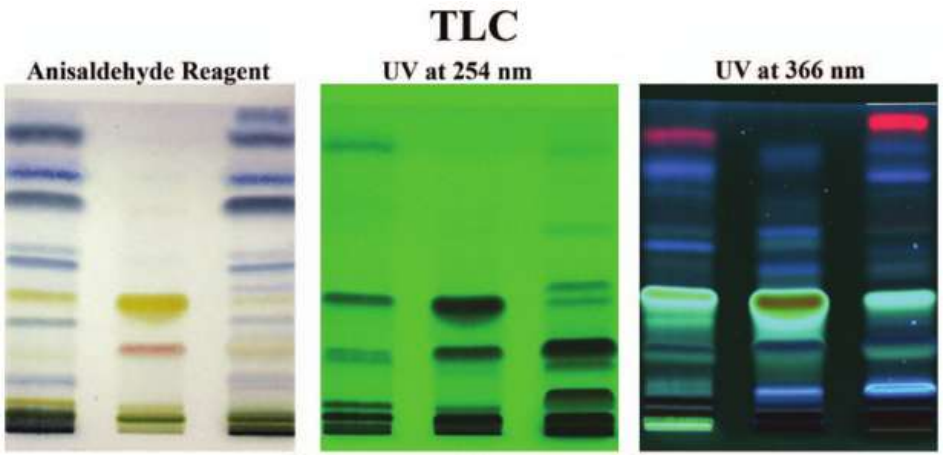

Figure 1: Thin Layer Chromatography result of S. officinalis extracts measured using (a) Anisaldehyde reagent, (b) measured at UV wavelength 254 nm, (c) measured at UV wavelength 366 nm.

HPLC Quantification

Optimizing of HPLC Condition:

In this survey, different HPLC parameters were tested and compared. The phenolic compounds were detected at 260, 280, 290 and 330 nm wavelengths, tested mobile phases were consisted of acetonitrile–water system and methanol–water system and mobile phase flow rates were 0.5, 0.7, 0.8, 1 and 1.2 mL/min. The binary mixtures of the acetonitrile–water system were more effective for the separation of the detected compounds. Addition of 2% (v/v) acetic acid to water improved peaks shape and separation efficiency. Due to greater baseline stability, mobile phase system of acetonitrile–2% aqueous acetic acid was selected. Also, the results showed that the most suitable elution flow rate was 1 mL/min and all the examined compounds were well detected at wavelength of 280 nm. Under these experimental conditions, all the five compounds were eluted within a run time of 60 min and the separation was adequate.

Method Validation: The method was validated by the linearity, precision and reproducibility of the results. Regression equations were derived from the external standard method. The correlation coefficient of the equations (R^2) was over 0.9683, which indicated all the standard compounds showed good linearity in the relatively wide concentration. After determination, precision and repeatability (RSD of intraday) of the five authentic substances was below 1.5% (Table 2). The RSDs were taken as a measure of precision and their values indicated that the instrument was highly precise. The average recovery of the five components and RSDs were obtained (Table 2) and showed that the method had a good accuracy (Figure 2 and 3).

Table 2. A sample recovery data of the reported compounds in the leaves of S. officinalis

Compound	Sample contents (mg/g)	Added (mg/g)	Recovery (%)	Mean recovery (%)	RSD (%)
RA	15.02	7.50 11.30 18.00	99.84 100.10 100.30	100.08	1.48
Sal B	7.03	3.50 4.75 9.20	96.87 97.45 97.15	97.16	2.00

Sal A	4.55	2.30 3.35 5.50	98.20 97.65 99.50	98.45	2.80
CA	7.75	3.87 5.40 9.00	96.03 95.65 96.50	96.06	1.50
CAA	1.82	0.95 1.20 2.20	99.52 98.52 99.03	99.02	1.10

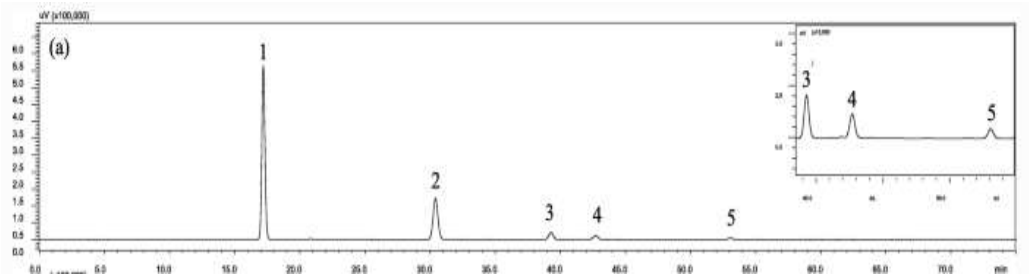

Figure 2: HPLC chromatograms of mixtures of standard

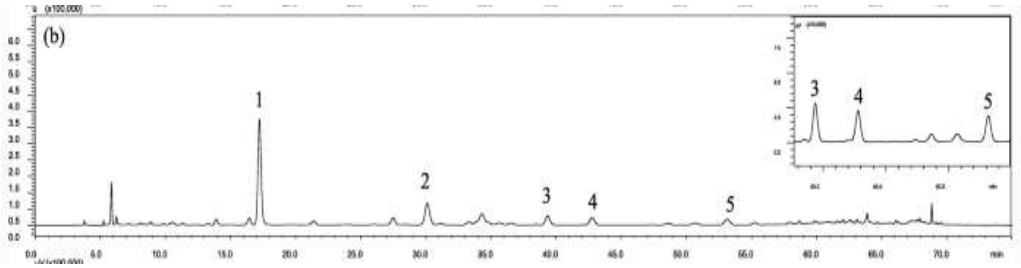

Figure 3: HPLC chromatograms of extract of s. officinalis

PHARMACOLOGICAL SCREENING

Evaluation of Antioxidant Activity

Total phenol and total flavonoid content

Phenolic and flavonoid compounds are widely distributed in plants, where they act as antioxidants and free radical scavenging agents. Phenolic compounds are most abundant class of secondary metabolitesin plants.

Total phenolic content was measured using the Folin–Ciocalteu reagent in the extract and expressed as Gallic acid equivalent (GAE), shown in Table .3 and Figure 4. Total flavonoids content in plant extract was determined using aluminium chloride colorimetric method and total flavonoids were expressed as mg of total Catechin content/g of sample, shown in Table 4 and Figure 5. The total phenolic content in hydroalcoholic extract was found to be 64.65 and total flavonoid content of hydroalcoholic extract was found to be 46.43, as compiled in Table 5.

Table 3: Calibration curve of standard Catechin for estimation of total flavonoids content

Concentration (µg/ml)	**Absorbance**
0	0
100	0.112
200	0.252
300	0.387
400	0.502
500	0.681

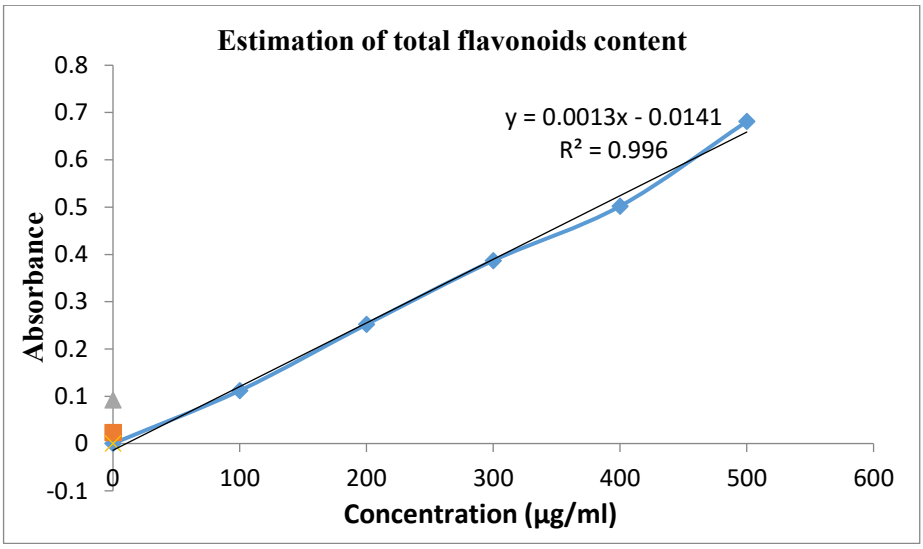

Figure 4: Standard Calibration Curve for estimation of Total Flavonoids

Table 4: Calibration curve of standard Gallic Acid for estimation of total phenolic content

Concentration (µg/ml)	Absorbance
0	0
5	0.223
10	0.413
15	0.582
20	0.702
25	0.924

Figure 5: Standard Calibration Curve for estimation of Total Phenols

Table 5: Total phenolic content and total flavonoid content of hydroalcoholic extract

Extracts	Total Phenolic Content (mg of GAE/g)	Total Flavanoid Content (mg of Catechin /g)

S. officinalis	64.65 ± 1.2	46.43 ±1.32

Values represent mean ± SD (n=6)

DPPH radical scavenging activity

Antioxidant activity of hydroalcohalic extract of S. officinalis was evaluated by various in-vitro methods. DPPH radical was used as a substrate to evaluate free radical scavenging activity of hydroalcoholic extract. This process involves a reaction of specific antioxidant with a free radical DPPH and this causes a decrease in the concentration of DPPH by antioxidant, which decreases the absorbance of DPPH at 517 nm. Ascorbic acid was used as standard. The scavenging effect of extract of S. officinalis on the DPPH radical was found to be 82.65 % at a concentration of 100µg/ml as given in Table 6 and Figure 6. These results indicated that extract has a marked effect on scavenging the free radicals.

Table 6: DPPH radical scavenging activity of S. officinalis

Concentration µg/ml	**% Inhibition**	
	Ascorbic Acid	**S. officinalis**
10	45.54±0.05	40.76±0.04
40	54.765±0.03	49.65±0.02
60	66.86±0.02	65.76±0.05
80	76.97±0.04	73.65±0.04
100	89.32±0.06	82.65±0.02

Values are expressed as mean ± SD (n=6). Values are significant at $p<0.05$.

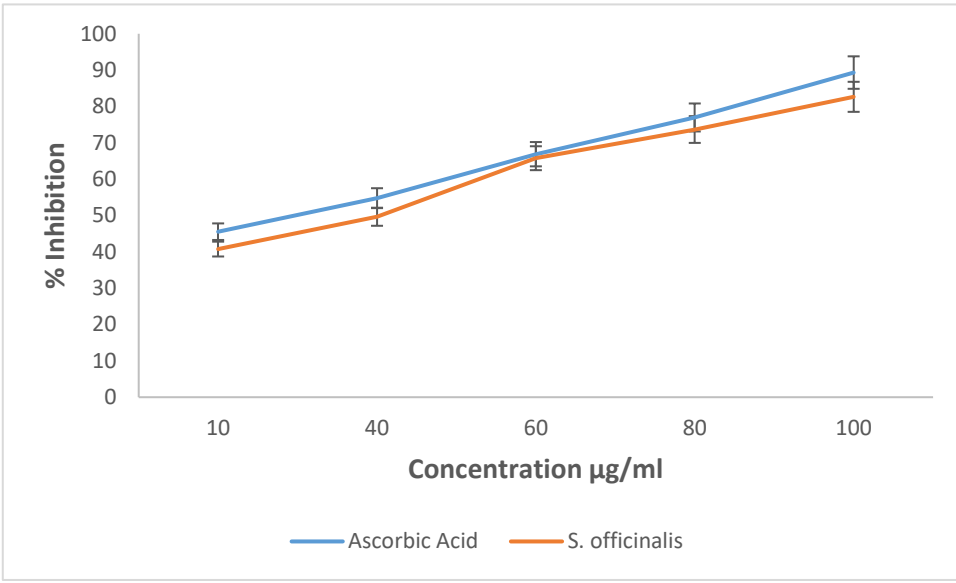

Figure 6: Representation of scavenging activity of S. officinalis on DPPH radicals

Nitric oxide scavenging activity

In the Nitric oxide scavenging assay, crude extract of the plant was evaluated for its inhibitory effect on nitric oxide production and ascorbic acid was used as standard. Alcoholic extract at 10, 40, 60, 80 and 100 µg/ml concentration produced 16.15±.12, 24.12±.02, 32.54±.10, 46.23±.21, 52.04±.30 percentage of inhibition as shown in Table 7 and Figure 7. Antioxidant activities (IC50) of the S. officinalis extract and ascorbic acid were calculated in all the methods.

Table 7: Nitric oxide scavenging activity of S. officinalis

Concentration µg/ml	**% Inhibition**	
	Ascorbic Acid	**S. officinalis**
10	28.43±0.15	16.15±0.12
40	36.11±0.07	24.12±0.02
60	43.89±0.12	32.54±0.13
80	55.97±0.22	46.25±0.21

100	67.92±0.11	52.28±0.04

Values are expressed as mean ± SD (n=6). Values are significant at $p<0.05$.

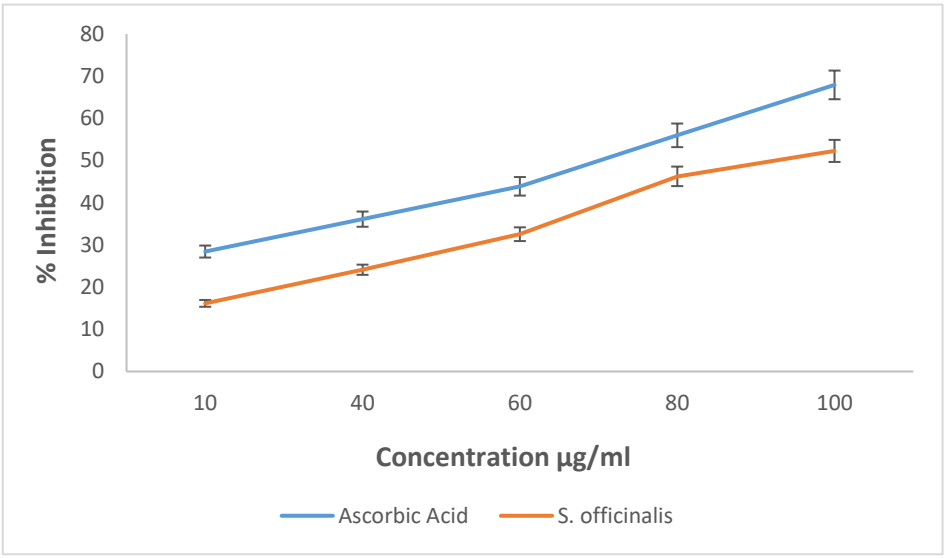

Figure 7: Representation of scavenging activity of S. officinalis on Nitric oxide radicals

Evaluation of Anti-Inflammatory Activity

In-vitro Anti-inflammatory activity of different Extracts of Salvia officinalis by Human Red Blood Cell Membrane Stabilization Method

During inflammation, lysosomal hydrolytic enzymes are released into the sites which cause damages of the surrounding organelles and tissues with attendance of variety of disorders (Sadique et al., 1989). Various methods were employed to screen and study drugs, chemicals, herbal preparations that exhibit anti-inflammatory properties or potentials.

The study also provides a strong evidence for the use of the stem Salvia officinalisin folkloric treatment as anti-inflammatory agent. The plant therefore could be regarded as a natural source of membrane stabilizers and was capable of providing an alternative remedy for the management and treatment of inflammatory related disorders and diseases.

Table 8: In-Vitro anti-inflammatory activity of extract of Salvia officinalis by membrane stabilization method

Concentration	**Salvia officinalis Extract**	**Standared Drug (indomethacin)**

(µg/ml)	Absorbance (560nm)	% of Inhibition	Absorbance (560nm)	% of Inhibition
100	0.189±0.23a	2.4	0.143±0.21a	62.81
200	0.178±0.12 a	35.7	0.135±0.51a	68.21
300	0.159±0.14 a	40.51	0.109±0.26 b	71.11
400	0.145±0.18 b	46.87	0.139±0.16 b	69.91
600	0.103±0.31 b	62.1	0.145±0.17 b	65.34

Values are expressed as X (Mean) +SEM, n=3. (One-way ANOVA followed by Student t-test). Statistically significance of [a]P < 0.05, [b]P<0.01, [c]P<0.001 and [d]NS in comparison to respective control (0.250±0.21 absorbance at 560nm).

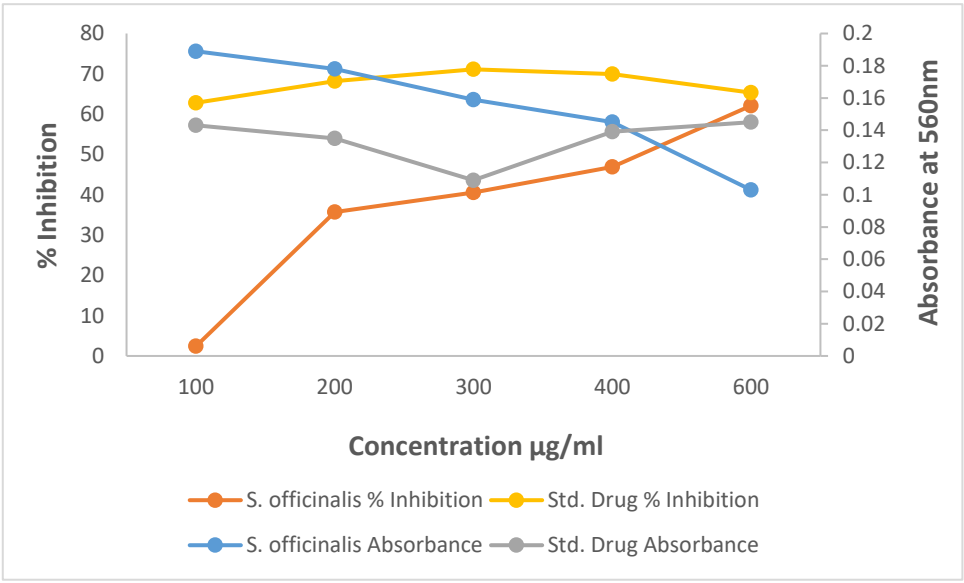

Figure 8: In-Vitro anti-inflammatory activity by HRBC membrance stabilitsation

In-Vivo Anti-Inflammatory Study

Percentage inhibition of edema by extract containing hydroalcohalic extract in rat's left hind paw was observed to be- 15.42% at 1 hr. and 25.17% at 4 hr. All the results were

compared with standard voveran emulgel gel, as shown in Table 9 and Table 10 and graphically represented in Figure 9 and 10.

The result revealed that hydroalcohalic extract of Salvia officinalishas anti-inflammatory action.

Table 9: Mean Paw edema Volume of the albino rats

Hind Paw (Mean+SEM)

Formulation	**Mean Paw edema Volume**			
	1 hr	**2hr**	**3hr**	**4hr**
Control	1.75+0.03	1.9+0.05	1.75+0.05	1.69+0.58
Hydroalcohalic extract of salvia officinalis	1.58+0.04*	1.48+0.13*	1.46+0.50*	1.35+0.05*
Standard Drug (Voveran Emulgel)	1.2±0.05	0.84±0.04	0.78±0.03	0.74±0.024

Values are expressed as X (Mean) +SEM, n=3. (One-way ANOVA followed by Student t-test). Statistically significance of $^{*}P < 0.05$, $^{**}P<0.01$, in comparison to control.

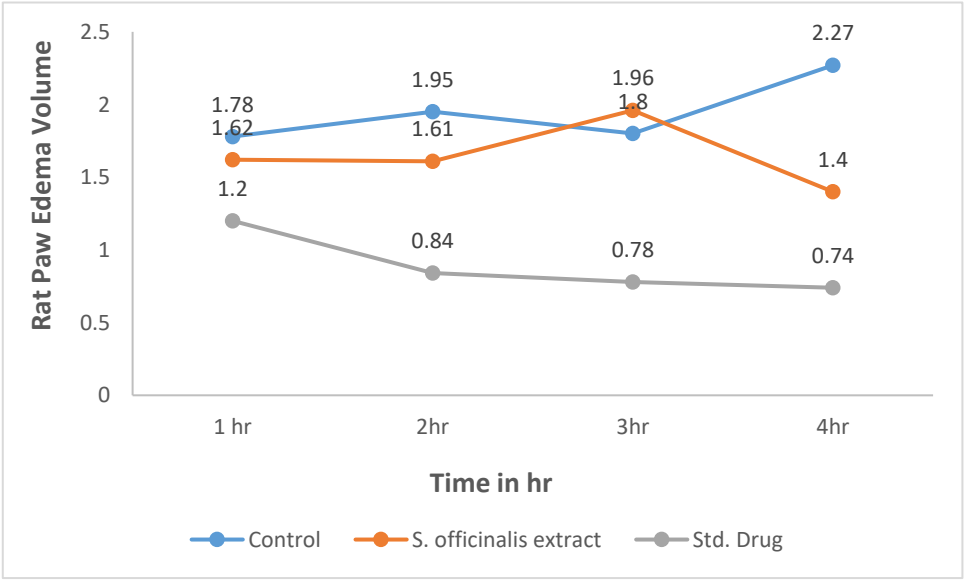

Figure 9: Trend of Paw Edema Volume wrt Time

Table 10: Percentage inhibition of edema

Group	Percentage inhibition of edema			
	1 hr	2hr	3hr	4hr
Control	-	-	-	-
Hydroalcohalic extract of salvia officinalis	15.92+0.12	17.67+0.14	21.26+0.14	24.93+0.16
Standard Drug (Voveran Emulgel)	41.32±0.20	48.54±0.16	51.53±0.19	55.51±0.32

Values are expressed as X (Mean) +SEM, n=3. (One-way ANOVA followed by Student t-test). Statistically significance of $^{*}P < 0.05$, $^{**}P<0.01$, in comparison to control.

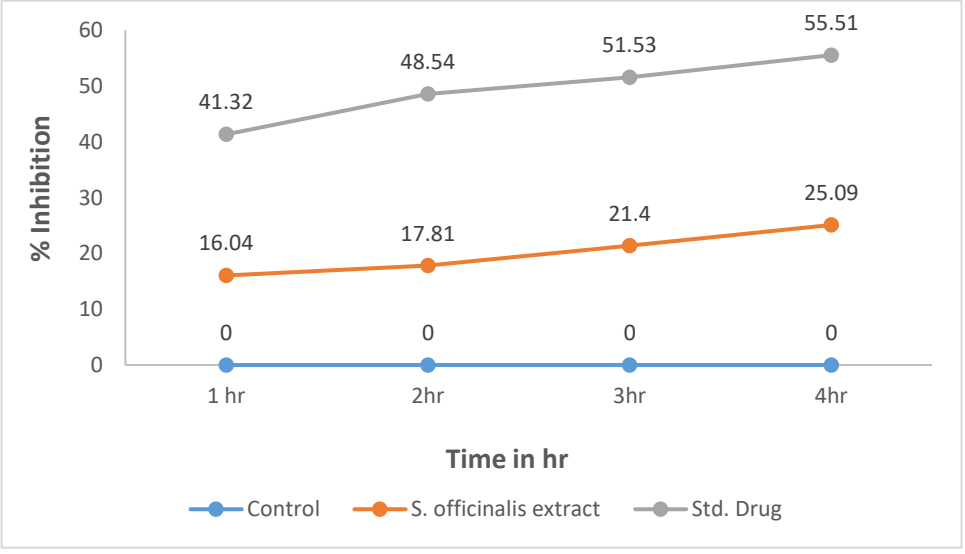

Figure 10: Trend of % Inhibition of Paw Edema

Skin Irritation Study

The skin irritation test was conducted for a period of seven days and the results are tabulated in Table 11. The results indicated that the control preparation, Extract and marketed products did not cause any skin reaction. It can be assured that Hydroalcohalic extract of salvia officinalis did not cause any skin irritation and can be used in the gel formulation.

Table 11: Skin irritation study

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	A	A	A	A	A	A	A
Hydroalcohalic extract of salvia officinalis	A	A	A	A	A	A	A
Voveran emulgel	A	A	A	A	A	A	A

A – No reaction, B – Slight patchy erythema, C –Slight but confluent or moderate but patchy erythema, D – Moderate erythema, E – Severe erythema with or without edema.

CONCLUSION

In this study the anti-Inflammatory activity of extract of Salvia officinalis L. is performed and revealed that extract of Salvia officinalis has anti-inflammatory activity. Percentage inhibition of edema by extract containing hydroalcohalic extract in rat's left hind paw was observed to be- 15.42% at 1 hr. and 25.17% at 4 hr. All the results were compared with standard voveran emulgel gel. The result revealed that hydroalcohalic extract of Salvia officinalis has anti-inflammatory action. The study also provides a strong evidence for the use of the Salvia officinalis as anti-inflammatory agent. The plant therefore could be regarded as a natural source of membrane stabilizers and was capable of providing an alternative remedy for the management and treatment of inflammatory related disorders and diseases.

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