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Antioxidant and Anti-inflammatory activity of methanol extract of *Salvia officinalis* flowers

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ABSTRACT

To evaluate the anti-inflammatory and antioxidant potential of methanol extract of flowers of *Salvia officinalis* Linn. (MESO). Antioxidant activity and free radical scavenging activity of MESO has been evaluated by employing various in vitro models including reducing power, Total antioxidant activity, DPPH radical, ABTS radical, superoxide radical ($O_2^{\bullet -}$), nitric oxide, hydrogen peroxide, hydroxyl radical scavenging activity and metal chelating activity. Anti-inflammatory activity has been evaluated by carrageenan induced rat paw edema animal model. The plant extract (MESO) revealed significant antioxidant activity with lower IC_{50} values. Metal chelating activity was found poor. In inflammation model, MESO demonstrated significant anti-inflammatory activity with maximum oedema inhibition at 3 rd hour post carrageenan injection. The results indicated that the plant could be a potential source of antioxidant and could find a use in the herbal therapy of inflammation also.

Keywords: Antioxidant, free radical, anti-inflammatory, Salvia

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INTRODUCTION

Salvia officinalis Linn. (Family: *Labiatae; Lamiaceae)* is a traditional ayurvedic ornamental medicinal plant commonly called as Sage. The plant is native to the Mediterranean region and also known as Salvia sefakuss in folklore. Traditionally the plant is astringent, anti-inflammatory, carminative, antispasmodic, and antiseptic. The leaves and flowers are cholagogue, hypoglycaemic, antiasthmatic (used for respiratory allergy), emmenagogue, antisudoriferous and antiseptic. Leaf of the plant is used as diaphoretic and antipyretic. The leaves are internally used for dyspeptic symptoms and excessive perspiration. The plant is also used for sore throat, laryngitis, tonsillitis, stomatitis. Externally the leaves are used for inflammations of the mucous membranes of nose and throat. Indigenously, the plant is utilized for inflammations and infections such as stomatitis, gingivitis, pharyngitis, and hyperhidrosis [1].

The leaves are reported to contain carnosolic acid; flavonoids including salvigenin, genkwanin, hispidulin, luteolin and its derivatives; phenolic acids including rosmarinic, caffeic, labiatic; a condensed catechin, salvia tannin. Literature also suggested that the roots contain diterpene quinonesroyleanone and its derivatives. Sage oil is used in perfumes as a deodorant and for the treatment of thrush and gingivitis. The herb is used in tooth powders, mouth washes, gargles, poultices, hair tonics and hair dressings [1]. Literature review suggested that there are plenty of traditional uses for this plant and several uses have already been proved pharmacologically thereafter. But, no study has been reported for anti-inflammatory and antioxidant activity of the flowers of the plant. Therefore, the aim of the present study is to evaluate the methanol extract of flowers of *Salvia officinalis* for anti-inflammatory and antioxidant activity.

MATERIALS AND METHOD

Plant material and preparation of the methanol extract

The flowers of *Salvia officinalis* were collected from Coimbatore district during late spring of the year. The plant was identified and authenticated by Dr. S. Sharma, Botanist, Research Institute in Indian System of Medicine (ISM), Himachal Pradesh. A voucher specimen (MS/2011/13) has been preserved for further utilization. The flowers were dried in shade followed by chopping and pulverization in a mechanical grinder. The powdered flowers (1.8 kg) were macerated thrice with petroleum ether (at room temperature; for 48 h) for defatting. The extract after air drying, was subjected to extraction with methanol. After exhaustive extraction, the methanol extract was collected and concentrated under reduced pressure at 45 – 50°C. A dark brownish concentrated methanol extract of flowers of *Salvia officinalis* (MESO) was obtained (yield 0.75%, w/w with respect to the dried starting material). The final product was then stored at 4°C prior to use.

Animals

Adult male Wister rats (weighing 150 – 170 g) were selected for the study. Polypropylene cages were used for the housing of the animals at 25 ± 2°C temperature with 12 h light and dark cycle. Animals were kept in the laboratory environment for a week prior to experiment for acclimatization. The animals were provided free access to standard pellet diet and water *ad libitum*. The care and use of laboratory animals were strictly in accordance with the guidelines prescribed by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

Drugs and chemicals

Carrageenan; 1, 1 – diphenyl – 2 – picryl hydrazyl hydrate (DPPH) and 2, 2′ – azinobis –3 – ethylbenzothiazoline – 6 – sulfonic acid (ABTS) were purchased from Sigma – Aldrich (St. Louis, MO, USA). Indomethacin and quercetin were arranged as gift samples from authentic reliable sources. All other chemicals and reagents were of reagent grade and available commercially (SRL Mumbai, E.Merck India).

Determination of total phenolic compounds

Folin – Ciocalteau method was used to assess the total soluble phenolic in the methanol extract and quercetin was used as a standard phenolic compound in the assay [2]. The concentration of total phenolic compounds in the extract was presented as gram of quercetin equivalent (QE) using linear regression equation of the standard quercetin graph (Figure 1):

$Y = 0.002 x + 0.136$, $r^2 = 0.8670$

Where, y was the absorbance and x was the concentration.

Antioxidant activity

Antioxidant activity was accessed by using various *in vitro* models such as reducing power assay [3], Total antioxidant activity [4], determination of DPPH (1 – 1 – diphenyl – 2 – picryl hydrazyl) radical scavenging activity [5], ABTS (2, 2′ – azinobis – 3 – ethylbenzothiazoline – 6 – sulfonic acid) radical decolorization assay [6], assay of superoxide radical ($O_2^{\bullet -}$) scavenging activity [7], assay of nitric oxide scavenging activity [8, 9], hydrogen peroxide scavenging activity [10], hydroxyl radical scavenging [11] and metal chelating activity [9, 12].

Carrageenan induced rat paw edema

In the experimental design, the animals were divided into five groups (n = 6). The grouping of the animals was done as follows:

- Group I as control group and received vehicle only (1%, CMC, 10 mL/kg; p.o.).
- Group II as standard group and received Indomethacin (5 mg/kg; p.o.).

- Group III as test group and received MESO 100 mg/kg, per orally
- Group IV as test group and received MESO 200 mg/kg, per orally
- Group V as test group and received MESO 400 mg/kg, per orally.

Anti-inflammatory activity of the methanol extract was evaluated by carrageenan induced rat paw edema model as described elsewhere[13, 14]. The animals were treated first with methanol extract (MESO) or indomethacin followed by injection of 0.1 mL of 1% carrageenan (in 1% CMC) solution in the sub-plantar region of the right hind paw just after 1 h of extract treatment. The paw volume was measured by using a volume displacement technique using a plethysmometer immediately and thereafter at 1, 3 and 5 h after the stimulus. The decrease in paw volume as compared to the control group (vehicle only treated) was considered as an anti-inflammatory response.

Statistical analysis

Results of the study were expressed as mean ± S.E.M. (n = 6). Statistical analyses were performed with one-way analysis of variance (ANOVA) followed by *post hoc* "Dunnett's Multiple Comparison Test" using GraphPad prism software package. "*P*" values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Determination of total phenolic compounds

The amount of total phenolic compounds present in the MESO was found to be 88.7 ± 2.6 mg (expressed as quercetin equivalents per g of extract) as depicted in figure 1.

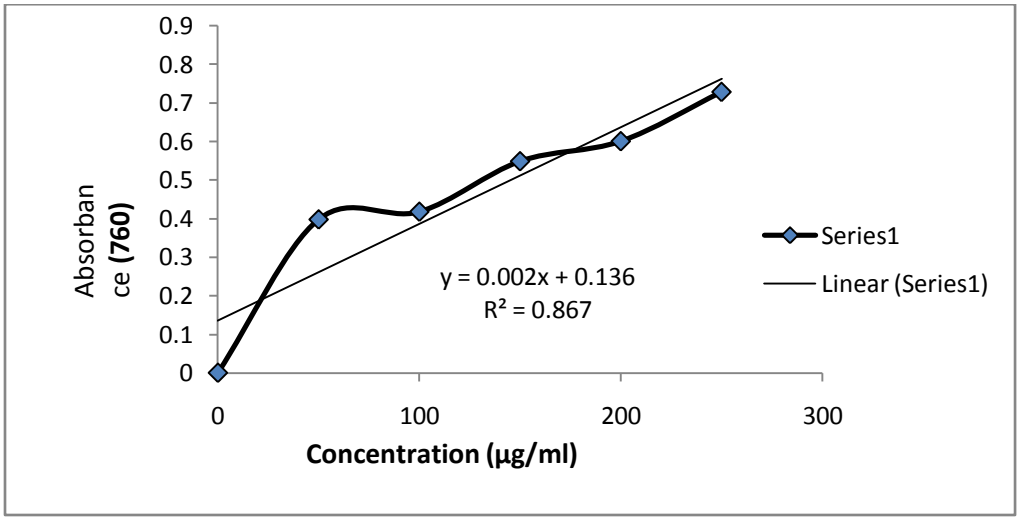

Figure 1. Total phenolic content assay

Reducing power

The results of the reducing power assay demonstrated MESO as a potential antioxidant as

compared to quercetin, sodium metabisulphite and ascorbic acid which is shown in figure 2. The reducing power of MESO was found to be concentration dependent.

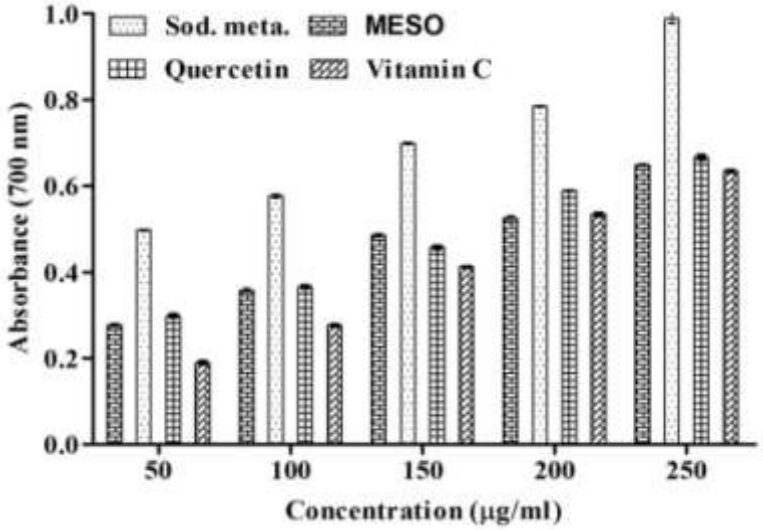

Figure 2. Reducing power assay of MESO and standard antioxidant compounds

Total antioxidant activity

The outcome of MESO on peroxidation of linoleic acid emulsion is shown in figure 3.

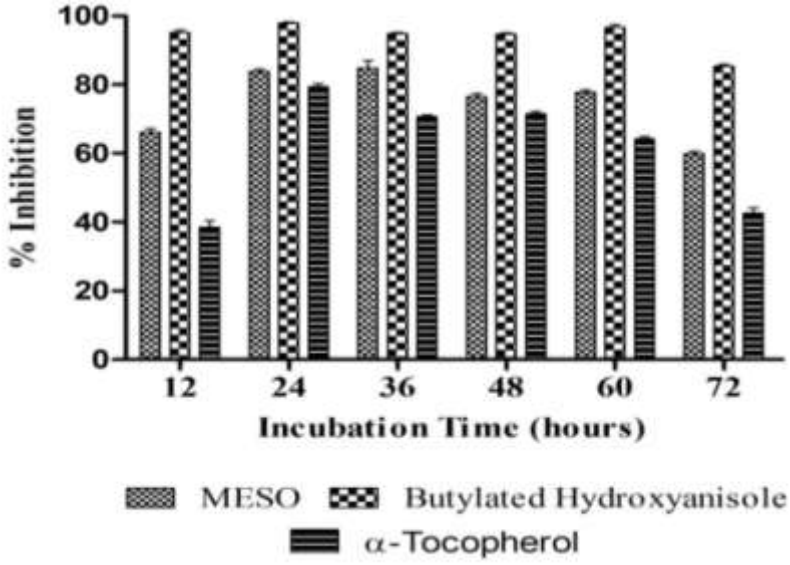

Figure 3. Total antioxidant activity of MESO and standard compounds

The antioxidant activity of MESO initially was increased with an increasing time of incubation up to 36 hours. MESO demonstrated higher antioxidant activity than 250 µg/ml concentration of α-Tocopherol but lower antioxidant activity than same concentration of BHA (Butylated hydroxyanisole). The percentage inhibitions of MESO, BHA and α-Tocopherol were found to be 66.12 ± 1.12 %, 95.13 ± 0.71 % and 38.58 ± 1.78 %, respectively.

Determination of DPPH (1 – 1 – diphenyl – 2 – picryl hydrazyl) radical scavenging activity

The percent DPPH scavenging ability were found to be 98.98% (MESO), 98.99% (quercetin) and 97.13% (Ascorbic acid) at the concentration of 250 µg/mL. The results revealed the strong DPPH radical scavenging ability the plant comparable to standards. The IC_{50} values of MESO, quercetin and ascorbic acid were found to be 85.64 µg/ml, 77.72 µg/mL and 86.58 µg/mL, respectively (Table 2).

3.5. ABTS (2, 2′ – azinobis – 3 – ethylbenzothiazoline – 6 – sulfonic acid) radical decolorization assay

The scavenging ability of $ABTS^{\bullet+}$ radical for MESO and standard antioxidant compounds were found to be profound and concentration dependent . The calculated IC_{50} values of the MESO, quercetin and ascorbic acid were found to be 102.58 µg/mL, 78.54 µg/mL and 127.36 µg/mL, respectively (Table 2).

Assay of superoxide radical ($O_2^{\bullet-}$) scavenging activity

MESO demonstrated significant superoxide radical scavenging activity with lower activity than the quercetin and ascorbic acid. The IC_{50} values of MESO and ascorbic acid were found to be 150.16 ± 0.59 µg/mL and 87.91 ± 0.98 µg/mL, respectively (Table 2) [15, 16].

Assay of nitric oxide scavenging activity

The percentage inhibitions of MESO and standard compound were found to be 63.81 ± 0.19 and 73.96 ±0.40% at the concentration of 250 µg/mL, respectively. The IC_{50} values, calculated were found to be 167.02 ± 0.82 µg/mL and 130.59 ± 0.63 µg/mL for MESO and ascorbic acid, respectively (Table 2).

Hydrogen peroxide scavenging activity

MESO demonstrated potential H_2O_2 scavenging activity as compared to standard ascorbic acid. The percentage H_2O_2 scavenging activity at a concentration of 250 µg/mL of MESO and ascorbic acid were obtained as 84.19 ± 0.37 and 89.95 ± 0.64%, respectively. The IC_{50} values were calculated as 114.79 ± 0.97 µg/mL and 105.33 ± 0.48 µg/mL for MESO and ascorbic acid, respectively (Table 2).

Hydroxyl radical scavenging activity

In vitro, MESO demonstrated hydroxyl radical scavenging activity in a concentration-dependent manner (50 – 250 µg/mL). The IC_{50} values were found to be 140.34 ± 1.04 µg/mL, 76.90 ± 0.88 µg/mL and 112.20 ± 0.94 µg/mL for MESO, quercetin and ascorbic acid respectively (Table 2).

Metal chelating activity

The percentage metal chelating activity was found to be increased with increasing concentration of MESO and EDTA. The IC_{50} values were calculated from linear regression analysis and found as 337.75 ± 0.77 µg/mL and 112.17 ± 1.16 µg/mL for MESO and EDTA, respectively (Table 2).

Table 1. Inhibitory effects of MESO and Indomethacin on Carrageenan induced rat paw edema.

Drug	Dose (mg/kg)	Time after Carrageenan injection					
		1 h		3 h		5 h	
		Edema volume (mL)	Edema inhibition (%)	Edema volume (mL)	Edema inhibition (%)	Edema volume (mL)	Edema inhibition(%)
Control	-	0.540 ± 0.03	-	0.895 ± 0.063	-	1.173 ± 0.136	-
MESO	100	0.349 ± 0.016***	35.37	0.563 ± 0.018***	37.09	0.842 ± 0.025*	28.21
MESO	200	0.205 ± 0.015***	62.03	0.433 ± 0.013***	51.62	0.610± 0.031***	47.99
MESO	400	0.142 ± 0.017***	73.70	0.325 ± 0.031***	66.67	0.556± 0.036***	52.60
Indomethacin	5	0.114 ± 0.012***	78.88	0.140 ± 0.014***	84.35	0.302± 0.052***	74.25

MESO: Methanol extract of flowers of *Salvia officinalis*, Values are expressed as mean ± S.E.M. (n = 6).

Statistically significant from control group: *p < 0.05, **p < 0.01, ***p < 0.001. (One way ANOVA followed by "Dunnett's Multiple Comparison Test" as *Post hoc*)

Table 2. IC_{50} values of MESO against different *in vitro* assays including DPPH, ABTS, superoxide and hydroxyl radicals (values are expressed as mean ± S.E.M; n = 3)

Drugs	Inhibitory concentration (IC_{50}) (µg/mL)						
	DPPH radical	ABTS radical	Superoxide radical	Hydroxyl radical	H_2O_2 scavenging	NO scavenging	Metal chelating activity
MESO	85.64 ± 0.72	102.58 ± 0.44	150.16 ± 0.59	140.34 ± 1.04	114.79 ± 0.97	167.02 ± 0.82	337.75 ± 0.77
Quercetin	77.72 ± 0.43	78.54 ± 0.86	-	76.90 ± 0.88	-	-	-
Ascorbic acid	86.58 ± 0.38	127.36 ± 0.83	87.91 ± 0.98	112.20 ± 0.94	105.33 ± 0.48	130.59 ± 0.63	-
EDTA	-	-	-	-	-	-	112.17 ± 1.16

Carrageenan induced rat paw oedema

MESO significantly inhibited carrageenan – induced paw oedema as stated in the table 1. The maximum of the paw inflammation was observed at 3 rd hour after the administration of carrageenan. The anti-inflammatory effect of MESO was found to be dose-dependent.

Therapies with conventional NSAIDs pose the patients to various toxic manifestations along with unwanted side effects. Sometimes due to the undesirable side effects, patients need to switch to alternative therapies. Research in the field suggested newer approaches to the management of pain and inflammation to avoid serious toxic outcomes due to NSAIDs [17-19]. Carrageenan – induced paw oedema is a one of the most popular and well established animal model for screening of anti-inflammatory agents or medicines [20]. Inflammation induced by carrageenan can be linked with the inter-play of various chemical mediators of inflammation such as histamine, prostaglandins, leukotrienes, PAF (Platelet Activating Factor), and other cyclooxygenase & lipooxygenase products along with the involvement of migration of Leukocytes and neutrophils [20]. Free radical along with reactive oxygen and nitrogen species have also been involved in the pathophysiology of inflammation. In the present study, the results suggested significant inhibition of oedema formation at 3 rd hour following carrageenan injection. MESO demonstrated significantly effective anti-inflammatory activity in the said animal model. Due to migration of leukocyte and macrophages to the site of injury superoxide (O_2^-) radicals are generated as evident from the understanding of the pathophysiology of inflammation [19, 21, 22]. As a very highly toxic species, superoxide radical initializes the generation of hydrogen peroxide (H_2O_2) leading to the formation of hydroxyl radical in the presence of suitable transitional elements [17]. The radical generated, can directly damage the physiological environment by attacking the biomolecules and initiate the process of inflammation. These radicals can also act as second messengers in the production of various inflammatory mediators [18, 19, 22]. MESO has also been screened *in vitro* in various models, for antioxidant activity including ABTS, DPPH, superoxide, hydrogen peroxide, hydroxyl radical and nitric oxide scavenging. Results of the present investigation clearly revealed potent antioxidant activity of MESO in the *in vitro* models as specified above. MESO was also found to own significant nitric oxide, superoxide radical, hydrogen peroxide and hydroxyl radical scavenging ability along with significant reductive ability. In ABTS and DPPH radical scavenging ability determination, a concentration dependent scavenging activity was observed. But metal chelating activity was found to be poor with higher IC_{50} values. In the conclusion, it is clearly indicated that the methanol extract of flowers of *Salvia officinalis* (MESO) has demonstrated significant anti-inflammatory activity *in vivo* with potent free radical scavenging & antioxidant ability.

CONCLUSIONS

Methanol extract of flowers of *Salvia officinalis* (MESO) revealed significant anti-inflammatory activity along with potent antioxidant activity and can be suggestive of alternative herbal therapy

for the management of inflammation.

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DECLARATION OF INTEREST

The authors declare no conflict of interest in this study.

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