



**ANTI-INFLAMMATORY AND PHYTOCONSTITUENTS OF *TEUCRIUM
OLIVERIANUM* GING. EX. BENTH**

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ABSTRACT

Many species of *Teucrium* were used traditionally in treatment of inflammatory disorders. The present study aimed to assess the anti-inflammatory effect of methanolic extract of aerial parts of *Teucrium oliverianum* Ging. ex. Benth. (family, *Lamiaceae*). The extract was evaluated using the carrageenan-induced rat paw edema (Acute and sub-acute models), and turpentine oil-induced granuloma pouch bioassay. The phytoconstituents were also investigated. The plant extract of *Teucrium oliverianum* exhibited marked anti-inflammatory activity in the both phases of carrageenan induced acute edema test. Moreover, the activity in carrageenan induced sub-acute edema test for the extract of *Teucrium oliverianum* in a dose-dependent manner. The plant extract of *Teucrium oliverianum* exhibited potential inhibitory action on exudates formation. This study proved that this plant extract could be used for the treatment of rheumatism and other inflammatory disorders.

KEYWORDS: Anti-inflammatory, *Lamiaceae*, *Teucrium oliverianum*, Carrageenan, Turpentine oil.

1. INTRODUCTION

Anti-inflammatory drugs are currently used for medications, including pain, edema, inflammation, osteoarthritis, rheumatoid arthritis and skeletal muscle disorders.^[1] Prolonged use of these drugs commonly induced severe side effects.^[2] Therefore, alternative agents with less severe side-effects are needed and botanical products can be important candidates.^[3] Accordingly the need arise to develop new anti-inflammatory agents with minimum side effects.^[4]

Teucrium species (family *Lamiaceae*) are known for their medicinal utilization and exhibit interesting biological properties such as hypoglycemic, hypolipidemic, hepatoprotective, antipyretic, anti-inflammatory, antiulcer, antitumor, antibacterial and insect antifeedant activities.^[5,10] The genus *Teucrium* is one of the richest sources of diterpenes, with a neoclerodane skeleton and more than 220 diterpenes have been described.^[11] Also, essential oils have been reported from the aerial parts of several *Teucrium* spp. and the percentage of the major chemical constituents (mainly monoterpenes/ sesquiterpenes hydrocarbons and oxygenated sesquiterpenes) differs notably from species to species.^[12] *Teucrium polium* has been reported to possess antispasmodic, antimicrobial and anti-inflammatory^[13], hepatoprotective effect^[14] and analgesic properties.^[15] Also *Teucrium chamaedrys* has been used as

antimalarial, antispasmodic and for gastric pain, kidney disorders and heart diseases.^[16,17] Very little studies have been done about *Teucrium oliverianum* Ging. ex. Benth in spite of its distribution widely in the dry and stony places of the hills and deserts of almost all Mediterranean countries, South Western Asia, Europe and North Africa. The isolation of several compounds with a biologic significance^[18,20] and the alcoholic extracts of *Teucrium oliverianum* induced a potent antinociceptive activity.^[21] Ajabnoor *et al.*^[22] recorded its antidiabetic and hypoglycemic potentials and more recently, Shahat *et al.*^[23] found its activity against hepatocellular carcinoma and such effect could be attributed to hepatoprotective properties, antiproliferative activity and antiangiogenic potential. No available studies were recorded about its anti-inflammatory activity. So the present study was designed to explore the anti-inflammatory effects and the phytochemical constituents of methanol extract of *Teucrium oliverianum* using the carrageenan-induced rat paw edema (Acute and sub-acute models) and turpentine oil-induced granuloma pouch bioassay. Based on traditional use of some plants of this family in treating inflammatory disorders so, the present study decided to investigate the effects of methanolic extract of aerial parts of *Teucrium oliverianum* on different inflammatory models and the study was extended to determine their phytoconstituents.

2. MATERIALS AND METHODS

2.1. Animals

Wister albino mature male rats (180±20 g) were obtained from the Animal House of the College of Agriculture and Veterinary Medicine, Qassim University, Kingdom of Saudi Arabia and housed at a temperature of 22 - 28°C and relative humidity of 50–60%, with artificial light from 5.00 a.m. to 4.00 p.m. Animals had free access to tap water and standard rat chow, used for the study. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by US National Institutes of Health (NIH publication no. 85-23, revised 1996). The local ethics committee approved the study.

2.2. Chemicals

Analytical grade chemicals were purchased from Sigma Aldrich, St. Louis, MO, USA and were used for the bioassays in the laboratory.

2.3. Preparation of plant materials

The plant was collected at the flowering stage, in and around Al-Gouf and Al-Qassim Districts, Kingdom of Saudi Arabia. The collected plant was identified and confirmed by Dr. A. Al- Sakeer, Department of Botany, College of Agricultural and Veterinary Medicine, Qassim University, Al Qassim, Kingdom of Saudi Arabia and a voucher specimen was deposited in the Department of Botany, for further reference. Shade dried and powdered plant materials were successively extracted as the following. About 300 gm of the powdered plant were soaked in 3000 ml methanol. It was left for 72 h. with intermittent shaking till obtain methanol extract. The methanol extract was filtered using Whatman No.1 filter paper and the residue was concentrated until obtaining paste under vacuum using the rotary evaporator (Rota vapor R-215, Büchi, Switzerland). The extract was used for evaluation of the anti-inflammatory activity and phytochemical constituents. Respectively, the voucher specimen (1021) for the methanolic extract of aerial parts of *Teucrium oliverianum* was deposited at herbarium unit at the Department of Veterinary Medicine, College of Agricultural and Veterinary Medicine, Al Qassim University, Buraydah, 51452 P.O. 6622, Kingdom of Saudi Arabia.

2.4. Phytochemical analysis: Total phenolics and total tannins content

The methods of Singleton and Rossi^[24] was applied using Folin-Ciocalteu's reagent, with some modifications, in which few amount of each residue (50 mg) was mixed with 2.5 ml of deionized water followed by the addition of 0.25 ml of Folin-Ciocalteu's reagent and allowed to react for 6 min. To measure the absorbance at 765 nm (using spectrophotometer, SPECTRO 22, USA) 2.5 ml of sodium carbonate 7% was added and allowed to stand for 1 hr. Measurements were calibrated to a standard curve of prepared Gallic acid solution and the total phenolic was expressed as mg Gallic acid equivalent per g of residue. Total tannin in the extract was determined

by a modification of the Folin-Ciocalteu method using polyvinyl poly pyrrolidone (PVPP) to separate tannin-phenols from non-tannin phenols.^[25] About 100 mg of PVPP was added to 1ml of sample extract diluted in 1ml of water and left 15 min at 4°C. After centrifugation 5000 rpm for 10 min, PVPP forms a precipitate with tannins and the supernatant has only simple phenols. Simple phenols were determined using the Folin-Ciocalteu reagent as previously described above. The difference between total and simple phenol values represents the total tannin content, expressed as mg Gallic acid equivalents g residue.

2.5. Total flavonoids

The method of Zhishen et al.^[26] was used to measure the total flavonoids content. A known weight of extract residue dissolved in 1 ml methanol was added to 5 ml distilled water. At zero time, 0.3 ml of 5% (w/v) sodium nitrite was added to the 15 ml capacity volumetric flask, then after 5 min, 0.6 ml of 10% (w/v) aluminum chloride (AlCl₃) was added and, after 6 min, 2 ml of 1M Na OH were added to the mixture, followed by the addition of 2.1 ml distilled water. Absorbance was read at 510 nm (using spectrophotometer, SPECTRO 22, USA) against the blank (water) and flavonoid content was expressed as mg quercetin equivalents/ g residue.

2.6. Evaluation of the anti-inflammatory activity: The carrageenan-induced rat paw edema model (Acute inflammatory Model)

The methods of Winter et al.^[27] and Adeyemi et al.^[28] were adopted. A total of five groups of rats having six animals each were used for the study. In the control group, only the vehicle, 0.5% carboxy methyl cellulose Na (CMC), was administered. Positive control group was treated with diclofenac Na (20 mg/kg body weight) as the reference drug. The extract was given in three doses level (200, 400 and 800 mg/kg b. wt.). The test extract/standard drug, diclofenac Na were suspended in 0.5% CMC and administered orally in a volume of 1 ml/100 g body weight of animal, once daily with the help of an oral gastric tube. Details of dose, time of administration and duration of treatment were mentioned for each experimental paradigm studied. After 1 h, the rats were challenged with subcutaneous injection of 0.1 ml of freshly prepared solution of 1% of carrageenan (Sigma Aldrich, St. Louis, MO, USA) in sterile 0.9% normal saline was injected into the sub plantar region of the left hind paw under light ether anesthesia. An equal volume of saline was injected into the right hind paw and served as internal control for the degree of inflammation in the left hind paw. The paw edema was measured Plethysmographically (Ugo Basile 7150, Varese, Italy Plethysmograph) and re-measured again 1, 2 and 4 h after injection of carrageenan. Edema was expressed as an increase in the volume of paw and the percentage of edema inhibition (or percent protection against inflammation) for each rat and each group was calculated according to the following equation:

$$\% \text{ Inhibition} = \frac{(\text{Vt}-\text{Vo}) \text{ control} - (\text{Vt}-\text{Vo}) \text{ test compound}}{(\text{Vt}-\text{Vo}) \text{ control}} \times 100$$

Where Vt is the mean volume of edema at specific time interval and Vo is the mean volume of edema at zero time intervals.

2.7. Sub-acute inflammatory model

Rats in the first experiment were given the same test compounds in three doses level (200, 400 and 800 mg/kg b. wt.) daily for 7 successive days. A solution of carrageenan (1%, 0.1 ml) was injected into the sub planter region of the left hind paw under light ether anesthesia 1 h after oral administration of the test material. A second injection of carrageenan (1%, 0.1 ml) was given on the third day. The changes in the volume of paw were measured Plethymographically at the first and eighth days.^[27,28]

2.8. Turpentine oil-induced granuloma pouch bioassay

This chronic inflammatory model was performed as previously described^[29] and modified using turpentine oil as irritant.^[30] In ether-anaesthetized rats subcutaneously dorsal granuloma pouch was made by injecting 2 mL of air, followed by injecting 0.5 mL of turpentine oil into it. As mentioned previously plant extract given orally (at a doses level 200, 400 and 800 mg/kg b. wt.) one h before turpentine oil injection and continued for seven consecutive days. On the eighth day, the pouch was opened under light ether anesthesia and the exudates were collected by a syringe. The volume (mL) of the exudates was measured and the percentage inhibition of inflammation relative to the control was determined as follows: % inhibition = (V control- V treated) / V control x 100.

2.9. Statistical analysis

Data were submitted to One Way ANOVA, $p \leq 0.05$. When a significant difference was found, the means were compared using Duncan, multiple range test.^[31] Calculations were carried out using the SAS system.^[32]

3. RESULTS AND DISCUSSION

In the present study, the plant extracts of *Teucrium oliverianum* exhibited marked anti-inflammatory activity in the both phases of carrageenan induced acute edema test in a dose-dependent manner (Table 1). *Teucrium oliverianum* was a potent anti-inflammatory activity against carrageenan induced sub-acute edema test also in a dose-dependent manner (Table 2). Inflammation is the integral part of body's defense mechanism. Acute inflammation is characterized by vasodilatation, exudation of plasma, release of various inflammatory mediators, cytokines, growth factors and emigration of leukocytes. While the features of chronic inflammation includes infiltration of mononuclear cells, proliferation of fibroblasts, blood vessels and increased connective tissue formation.^[33] Anti-inflammatory drugs inhibit different stages of inflammation.^[34]

The anti-inflammatory effect of methanol extract of aerial parts of *Teucrium oliverianum* was assessed in different inflammatory models. Carrageenan- induced inflammatory edema in the hind paw of rats. Inhibition of carrageenan-induced inflammation in rats is one the most suitable test procedures to monitor anti-inflammatory agents.^[35] Carrageenan, a mucopolysaccharide derived from Irish Sea moss Chondrus, was used to induce experimental arthritis. Carrageenan was non-antigenic and did not produce any systemic effects.^[36] Carrageenan produces acute inflammation believed to be biphasic; the early phase (1-2h) after carrageenan injection, in which the edema production is mediated by histamine and serotonin and the late phase (after 2nd h) the vascular permeability is maintained by bradykinin and prostaglandins.^[37] These mediators contribute in the inflammatory response and induce pain. Result showed that the second phase of carrageenan-induced edema is sensitive to clinically used anti-inflammatory drugs and commonly employed to assess the antiphlogistic effect of the natural products.^[38] Similarly non-steroidal anti-inflammatory drug (NSAID) diclofenac sodium produced significant ($p < 0.05$) anti-edematous effect which is consistent with the previous reports.^[39,40] Several studies have revealed the inhibitory effects of plant extracts and NSAIDs in similar animal models of pain and inflammation.^[41,43] Diclofenac and aspirin suppress inflammation and pain by inhibiting prostaglandin synthesis via inhibition of cyclooxygenase in arachidonic acid pathways.^[44]

In the present study, results depicted in Table 3 revealed that, the plant extract of *Teucrium oliverianum* exhibited potential inhibitory action on exudate formation. Turpentine oil can be used as an irritant. Therefore, turpentine oil-induced granuloma pouch offer a model for exudative type of inflammation which is widely used model for chronic inflammation occurred by means of development of proliferated cells in the form of granuloma. Inflammation involves proliferation of macrophages, neutrophils and fibroblasts, which are basic sources of granuloma formation.^[45] Kinin were the main mediator of granuloma, as it both vasodilate and increase vascular permeability in the early stages of inflammation.^[46] Keeping all these in view it may be said that the tested plant extracts may possess anti-kinin like activity. The test plant extracts inhibit the granuloma formation by preventing granulocyte infiltration, generation of collagen fibers, fibroblasts and suppressing mucopolysaccharides.^[47] A collective interpretation of the anti-inflammatory data of the test plant extract (Tables 2-4) revealed that *Teucrium oliverianum* demonstrated pronounced activities in the three animal models used in this study and the effect was equal in strength to that of the diclofenac sodium.

The tested plant extract found to possess respectively 12.24 ± 0.93 , 27.25 ± 2.12 and 12.55 ± 0.78 mg/g ($n=5$), high concentrations of tannins, polyphenols and flavonoids. These phytoconstituents could be responsible

for anti-inflammatory activity. Flavonoids were known to inhibit the enzyme prostaglandin synthetase, more specifically the endoperoxidase and reported to produce significant anti-inflammatory effect due to inhibition of chemical mediators of inflammation.^[48] Cyclooxygenases as COX-1 and COX-2 catalyze the biosynthesis of prostaglandin H₂ from the arachidonic acid substrate. The inhibition of COX-1 results in some undesirable side-effects, whereas COX-2 inhibition provides therapeutic effects in pain, inflammation, cancer, glaucoma, Alzheimer's and Parkinson disease.^[49] In a similar study, phenolic compounds were shown

potent anti-inflammatory activity.^[50] Many polyphenolics, tannins and flavonoids were found to inhibit COX-1 and COX-2.^[50] The anti-inflammatory effect of extracts may be due to the presence of flavonoids and tannins. It became clear that the mechanism of anti-inflammatory action is assumed to be mediated through the inhibition of Cyclooxygenases, which need to be measured in a future study. The study also signifies that phytoconstituents (total phenols and flavonoids) could be responsible, at least in part, for its anti-inflammatory.

Table 1: Anti-inflammatory Activity of Some Methanol Extract of *Teucrium oliverianum* (Acute Inflammatory Model).

Treatment	Volume of edema (mL)			
	0	1h	2h	4h
Control	1.22±0.11	2.59±0.23	3.63±0.18	3.77±0.30
TO (200 mg/kg)	1.21±0.12	1.82±0.20 (55)*	2.15±0.16 (60)*	2.42±0.14 (52)*
TO (400 mg/kg)	1.15±0.10	1.79±0.15 (53)*	2.02±0.15 (63)*	2.20±0.30 (58)*
TO (800 mg/kg)	1.18±0.11	1.63±0.25 (67)*	1.91±0.12 (69)*	2.03±0.12 (66)*
DE(20mg/kg)	1.21±0.14	1.65±0.20(67)*	1.85±0.13(73)*	1.97±0.20(70)*

*Significantly different compared to corresponding control $p \leq 0.05$.

Number of animals = 6. Values are expressed as Mean \pm SEM. TO, *Teucrium oliverianum* DE, Diclofenac Na.

Table 2: Anti-inflammatory activity of some methanol extract of *Teucrium oliverianum* (Sub-acute inflammatory model).

Treatment	Volume of edema (mL)		
	0 day	1 st day	8 th day
Control	1.22±0.11	3.77±0.30	6.15±0.30
TO (200 mg/kg)	1.21±0.12	2.42±0.14 (52)*	3.40±0.30 (55)*
TO (400 mg/kg)	1.15±0.10	2.20±0.30 (58)*	3.32±0.20 (55)*
TO (800 mg/kg)	1.18±0.11	2.03±0.12 (66)*	2.88±0.30 (65)*
DE (20mg/kg)	1.21±0.14	1.97±0.20 (70)*	3.02±0.30 (63)*

*Significantly different compared to corresponding control $p \leq 0.05$. Number of animals = 6. TO, *Teucrium oliverianum*. DE, Diclofenac Na.

Table 3: Anti-inflammatory Activity of Some Methanol Extract of *Teucrium oliverianum* in the Turpentine oil – induced Granuloma Pouch Model.

Treatment	Volume of exudates	% of inhibition
Control	2.23±0.30	-----
TO (200 mg/kg)	1.84±0.15*	17.48
TO (400 mg/kg)	1.65±0.17*	26.00
TO (800 mg/kg)	1.42±0.12*	36.32
DE (20 mg/kg)	1.40±0.15*	37.21

*Significantly different compared to corresponding control $p \leq 0.05$. Number of animals = 6. TO, *Teucrium oliverianum*. DE, Diclofenac Na.

4. CONCLUSION

Methanolic extract of *Teucrium oliverianum* possesses potential anti-inflammatory activity, which was comparable to diclofenac sodium. It is recommended that the current study verified the traditional use of this plant extract for the treatment of rheumatism and other inflammatory disorders. This study discovers the possible use of the methanolic extract of *Teucrium oliverianum* in the treatment of inflammatory disorders. This study will help the researcher to uncover and purify the active ingredients of this extract to become available commercially.

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