Identification, Molecular Profiling, Docking Studies and determination of In-vivo Anti-Inflammatory Potential of Teucrium Stocksianum Bioss Fixed Oil (FO)

Syed Muhammad Mukarram Shah

University of Swabi

Syed Muhammad Hassan Shah

University of Nottingham

Rehman Zafar

Riphah Institute of Pharmaceutical Sciences, Riphah International University

Mohammad Ijaz Khan

University of Swabi

Fida Hussain

University of Swabi

Anwar Zeb

University of Swabi

Syed Wadood Ali Shah

University of Malakand

Muhammad Saeed Jan (✉ saeedjanpharmacist@gmail.com)

University of Swabi

Research Article

Keywords: Teucrium Stocksianum, Fixed oil (FO), GC-MS, Anti-inflammatory mechanism, Cyclooxygenase and Lipoxygenase

Posted Date: March 17th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-279456/v1>

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Abstract

Background: Considering the positive and valuable upshots of the naturally occurring ingredients as the complementary treatment for many ailments, this study investigates the *Teucrium Stocksianum* Bioss Fixed Oil (FO) for the treatment of inflammation.

Methods: Different plants of the *Teucrium* genera have been employed occasionally for the management of different disorders. *Teucrium stocksianum* (Lamiaceae) is traditionally use as antipyretic along with remedy of inflammation, diabetes and tumors. It also occupies the capacity to purify the blood. The aim of this study is to determine the chemical constituents and anti-inflammatory potentials of *Teucrium stocksianum* fixed oil (FO). Qualitative and quantitative analysis of fixed oil were performed via GC-MS (Gas Chromatography coupled with Mass Spectrometer). Preliminary inflammation antagonistic activity was calculated in mice using the carrageenan-induced paw edema model while the mechanism behind this inflammatory antagonistic effect was determined by employing various inflammogens including arachidonic acid, prostaglandins E₂ and leukotriene via paw edema model.

Results: The acute toxicity of fixed oil was determined at 3, 6 and 10 ml/kg body weight of the mouse. The GC-MS analysis documented 21 various unsaturated and saturated fatty acids elements. The methyl esters of octadecadienoic and linoleic acids were found predominantly at 22.60% and 23.84% respectively. In preliminary screening, FO demonstrated substantial anti-inflammatory capacity (67.87%, ** $P \leq 0.01$) in carrageenan induced paw edema model at 3 ml/kg body weight. The activity reached a peak value at the 3rd hour and continued persistent until the 5th hour of sample administration. FO displayed dose-dependent inhibition against all inflammogens at 3 ml/kg at 3rd h of the FO administration. These have shown marvelous protection i.e. 64.92%, 68.75% and 58.3% against arachidonic acid, prostaglandin E₂ and leukotriene accordingly, at 3 ml/kg. The arachidonic acid antagonist (Caffeic acid) demonstrated 69.43% activity at 100 mg/kg after 3rd h of its administration while in the acute toxicity test, FO has shown no gross lethality at 10 ml/kg dose.

Conclusion: According to our findings, the FO of *T. stocksianum* exhibits inflammation antagonistic potential through both lipoxygenase (LOX) and cyclooxygenase (COX) enzyme inhibition strategies that intensely sustenance the traditional practice of *T. stocksianum* in the management of multiple pathological inflammatory situations. The computed binding energies of the molecules exposed have synergistic potential to avert inflammation.

Background

Traditionally medicinal plants have been employed for many decades, especially in the emergent states. Currently, various herbal medicines are in practice for numerous ailments [1]. In developing countries, the utilization of medicinal plants in folk medicine as anti-inflammatory agents is very common [2]. According to the WHO report, round about 75% of the global population used herbal therapies for their fundamental health care system. Internationally, from 80 to 85% of the total medications for prime

healthiness are found from natural resources. Still, human beings have revealed enormous assistance of natural products research in the treatment and cure of almost all types of disease [3–4]. Seventy thousand different plant species are being investigated employing possible natural drugs for the prevention and control of several diseases. Both synthetic and natural materials can be used to produce drugs for different biological purposes [5]. The drugs which are obtained from natural products are invented to be more effective and safer as compared to synthetic drugs. Recently, for the natural product researcher, the anti-inflammatory agent who is attained from medicinal plants has increase magnetism [6–8].

The utilization of the NSAIDs (Non-Steroidal Anti-Inflammatory Drugs) holds its position as a core approach amongst curative selection for winning the battle against inflammation [9]. Existing studies exposed that inflammation has been related to cancer, diabetes mellitus, coronary disease, atherosclerosis, neurodegenerative disease, asthma and obesity, in addition to wounds, sickness, injuries and swelling [10]. Multiple possibilities of stimuli like Physical and biochemical stimulus canister can prognosis the inflammatory response. Swelling, pain, redness, heat and dysfunction are all common symptoms of inflammation [11]. NSAIDs can be used primarily for inflammation, but due to their prolonged-term use, they produce a lot of side effects which include prompt allergic reactions, gastrointestinal, immunosuppressed system, cardiovascular complications, urinary tract problems and hypersensitivity responses. Mainly NSAIDs are more likely to cause non-specific COX-2 and COX-1 enzyme inhibition [12–13]. Evidently, because of various side effects, potency, as well as efficacy of the present available NSAIDs and opioids, are not completely helpful [14]. Consequently, it is vital to investigate novel things and compounds for the management and treatment of inflammation.

The family Lamiaceae is one of the most significant herbal families, which integrated a broad diversity of the plants having medical and biological applications [15–16]. Lamiaceae included 240 genera and 3500 species around the globe. Reported literature shows that genera of this family exhibit diversified biologically active molecules [17]. Some of the species of this genus are also available in Pakistan, i.e. *Teucrium stocksianum*, *Teucrium royleanum*, *Teucrium scordium* and *Teucrium quadrifarium* [18–21]. Different floras of this genera have been used traditionally for different disease management. *Teucrium polium* belongs to this genera is utilized medicinally for management and treatment of flatulence, labor pain, analgesia, cough, jaundice, liver abnormalities and abortions [22–23]. *Teucrium stocksianum* have also occupied its space in folk remedies for the management of sore throats, cough, fever, foot-warming numbness, diabetes, blood purifier along body coolant [24–25]. Similarly, the *T. stocksianum* has been explored with antipyretic, analgesic and antiulcer properties [26–28]. Fixed and volatile oils have been isolated from this genus showing numerous pharmacological activities, i.e. antimicrobial, antioxidant, anti-inflammatory, antitumor and cytotoxicity [29–31]. Generally, different types of fatty acids obtained from plant sources bear diversified pharmacological activities like insecticidal activity of Lauric, oleic and linoleic acids [32], fatty acids of *Osmium sanctum* Linn with anti-inflammatory potential [33], antimicrobial activity of fixed oils of *Nigella sativa* seeds [34]. There are various molecules that have been previously secluded from various species of the *Teucrium montanins* [35], while from *Teucrium montanum* compounds A to E were previously isolated [36]. The *teucrins* A and *teucrin* E have acquired

from the *Teucrium chamaedrys* [37]. The molecules which were isolated from the *T. quadrivium* were teuvidin, teuflin, epi-teuvidin, teuvidin, teuquadrin B and teuspinin [38]. Coll *et al.* [9], have been explored the clerodanes, which is called as 11-hydroxyfruticolone, acetyl-hydroxyteucjaponin, β -hydroxy-fruticolone, 6-acetylteuc japonin and deacetyl-fruticolone from the *T. fruticans*. From the *Teucrium* species various biological potentials have been reported, like anti-seizure [39–40], anti-oxidant [41], anti-viral [42] antibacterial and antifungal potential [43] anti-cancer [44] hepato-protective, butarylcholine esterase and acetylcholine esterase [30] and anti-inflammatory potentials [45]. Similarly, Radhakrishnan *et al* [46] evaluated the anti-inflammatory potential of the aerial part of the crude ethanolic extract of *T. stocksianum*. Similarly, Mukarram Shah *et al* [47], explored the crude ethanolic extract and their sub-fractions for the anti-inflammatory potential and lastly subjected the main active fraction i.e. ethyl acetate for anti-inflammatory mechanism. Likewise, they were also evaluated the anti-inflammatory, analgesic and antioxidant potential of the same plant [38]. Based on the previous literature survey, and explored the crude and subsequent fraction along with essential oil for their anti-inflammatory activity we aimed that determine of chemical composition of fixed oil from *T. stocksianum* using GC-MS. Moreover, the FO extracted from *T. stocksianum* has not been evaluated yet for anti-inflammatory activity. Therefore, the current study has been arranged to evaluate the fixed oil of *T. stocksianum* for its anti-inflammatory activity and authenticate its traditional use against inflammation.

Material And Methods

Plant Extract

T. stocksianum plant was collected from District Swat, KPK Pakistan in May. Plant name was confirmed by plant taxonomist Dr. Nasrullah, Botany Department, University of Malakand (UOM), Chakdara Dir, Pakistan. This specimen was placed in the same department with Code (H.UOM.BG.199b) for future indication. Plant material was gloom dried and grounded to powder, using a cutter mill. The coarse powder was macerated in *n*-hexane at 25⁰C for 2-weeks with mild infrequent shaking. The extract was filtered and then concentrated in controlled conditions (40°C) using Soxhlet apparatus to get fixed oil.

Experimental animals

Swiss albino male mice weighed 25-30 gm were purchased from the NIH (National Institute of Health) Islamabad Capital Territory, Pakistan. They were kept in suitable cages at 25 ± 2°C and in light and dark for 12 hours. All the experimental animals *ad libitum* were provided with sufficient food and water during the accommodation phase. Animals were kept on fasting 18 h before the experiment but the water was available throughout the procedure. Mice were randomly distributed into different experimental groups as (n = 6). The Committee of animal ethics of the University of Malakand KPK accepted the protocols in strict compliance with internationally accepted principles for laboratory animal use and care after conforming to ARRIVE guidelines for the experimental animal studies through letter No; DREC/122 for Control and Supervision of Experiments on Animals standard [48]. After the experimental procedures, the animals were euthanized properly as per the standard procedures using the guideline of the American

Veterinary Medical Association (AVMA) for the euthanasia of the animals. Halothane vapors were given to the animals via vaporizer for anesthetic induction and overdose. The overdose and prolong time euthanized the animals [49].

Chemicals

Carrageenan, Histamine, Caffeic acid, Arachidonic acid, Prostaglandin E₂ and Leukotrienes were procured from Sigma-Aldrich Co. LLC, USA. Whereas Reckit & Colman, Pakistan was selected to purchase Aspirin (Acetylsalicylic acid).

Statistical analysis and calculations

Data were obtained from various experimental groups and Mean $\pm$ SEM was calculated as of six mice in every group. The analysis of One-way variance statistical analysis (ANOVA) pursued by post hoc Dunnett's multiple comparison test was applied. $P \leq 0.01$ differences and lower values between multiple groups were measured as significant values. Almost all inflammogens have shown peak inflammatory reaction at 3rd hour in right side paw of the control group, followed by decrease edema on 4th hour. Furthermore, the inflammatory reaction induced via arachidonic acid, prostaglandin E₂, Leukotrienes and carrageenan give non-significant values in presence of the carriers, normal saline, or 10% DMSO in animal, therefore the annotations were pooled [50].

Acute toxicity

Toxicity studied for FO was conducted at 3, 6 and 10 ml/kg body weight of the mouse. Mice were categorized into two sets on a random basis; each contained six animals of either sex and was treated with 3, 6, or 10 ml/kg FO. All experimental animals were under observation for the appearance of any disgusting effect initially for 4 hours. After 24 hours, dead animals were calculated [2].

Extraction of Fixed oil and preparation of FAMES

The 200 g powder of the plant was extracted with 350ml of the *n*-hexane in Soxhlet extraction equipment for 6 h. The solvent was regenerated, via an evaporator (rotary) at 42°C. Tri-floro boric acid in ethanol was employed for the generation of the volatile chemical constituent. About 1.6ml of NaOH mixture in 0.5 N methanol was further poured. It was sealed properly and heated for 07 minutes on a water bath instrument. Then compound was chilled and BF₃ solution (in methanol) 2.5 ml was transferred to it. This preserved mockup was warmed for 32 minutes on the water bath and then chilled. Concentrated 6ml NaCl solution was added to esterify solution and then extracted twice through 1 mL of *n*-hexane. The extracts get were filtered via a membrane filter. Afterward; the prepared samples (1 μ l) were injected into GC through an auto-injection system for analysis [51].

GC-MS analysis of FAMES

Fixed oil was analyzed using GC coupled to MS and auto-injector. Separation of the component was carried out in a column with a length of 35 m, 0.35 mm internal diameter, 0.240 µm thickness. Helium (He) was used as a carrier gas. Other parameters for chromatography were 250°C, 110 kilopascals and 1.8 min as temperature, pressure and solvent cut time of interface. 1 µl of sample and standard were administered at 250°C. After that, the column was operated primarily for 4 minutes at 85°C and then increased to 185°C, at an increased rate of 15°C/min. Column remained sustained at 185°C, for 15 min with a residual rise at the rate of 2.5°C/min to 220°C withhold there for 5 minutes. All the constituents got recognized by relating the spectrum acquired with instrument library standard spectra [52].

Fixed oil anti-inflammatory potential in mice

Initially, *stocksianum* fixed oil anti-inflammatory effect was determined in mice of both sexes, weighing 25-30 g. The animals have divided arbitrarily into five groups as 1- 5 with 06 (n = 6) mice of either sexing each group [53]. Normal saline as a negative control was utilized in Group 1 in 10 ml/kg of body weight. Group 2-4 were treated with 1, 2 and 3 ml/kg, the body weight of FO correspondingly, while group 5 treated with 100 mg/kg Aspirin (positive control), After 30 min of the treatment, all of the groups received 0.05 mL (1% w/v) subcutaneous single injection of carrageenan suspension in right-hand paw sub planar area of the test animals. An initial reading of the inflammation was taken immediately with Plethysmometer (LE 7500 plan lab S.L) after the injection of carrageenan after 01 to 05 hours. The mean volume of the paw of each group 2-5 was observed at different time intervals then comparing with the mean paw volume of group animals of negative control [54]. Edema Percentage inhibition was measured by the formula

$$\text{Inhibition} = (A_{\text{verage}} - T_{\text{ested}}) / A \times 100$$

Whereas “A” indicates the control group's average inflammation while “T” indicated the tested group's paw volume.

Fixed oil anti-inflammatory mechanism

Mice were randomly distributed in different experimental groups. Preliminary the animals received a single intraperitoneal injection of any vehicle like 0.9% normal saline or 10% DMSO or 100 mg/kg of caffeic acid that is a lipoxygenase inhibitor or 100 mg/kg of aspirin or fixed oil of *T. stocksianum* as 3 ml/kg. After half hour of the treatments, inflammation was injected in the right-hand paw of the mice with 0.1 ml of sub plantar injection of 0.5 % w/v arachidonic acid or 0.01 µg/ml of prostaglandin E₂ or 0.1 ml Leukotriene (0.1 µg/ml). Edema in terms of every animal paw bulk got determined before, instantly (0 h) and then for four hours (1, 2, 3 and 4 h) at 01 h interval after injection of the inflammogens [47].

Docking Studies

Computational studies were carried out by Autodock Vina 1.1.2 interrelated Pyrex-0.8. To get a better understanding of the mechanism by which fixed oils inhibit inflammation, the structures of enzymes were

downloaded from Protein data bank as reported in our previous studies [55]. Crystal structure of Cyclooxygenase enzyme COX-2 was obtained as PDB code 1CX2 and Crystalline structure of lipoxygenase enzyme 5-LOX was acquired as 3O8Y. The structures were modified and purified through Discovery studio visualizer and stored in PDB format which was further prepared for docking by converting them into PDBQT format.

All the components of fixed oils were assessed and those were selected for docking purposes which were obtained in good quantity after GC-MS studies. Palmitic acid attained in 2.77 % in the case of saturated oils and linoleic acid in 23.84% was nominated due to higher values. Structures of interested components ligands were obtained from PUB Chem and saved in PDB format.

Results And Discussions

Teucrium is a rich genus composed of 350 species. Multiple biological accomplishments have been described through this genus. *T. royleanum* exhibits antioxidant and antimicrobial potentials. The ethyl acetate extract is reported with excellent enzyme inhibition activity against butyrylcholine and acetylcholine. Numerous scientists have reported fixed oils and essential oils from different species of *Teucrium*. The essential oils of *T. polium* and *T. stocksianum* contain strong anti-nociceptive properties [26].

Composition of Fixed oil

In the current study, the chromatogram obtained from GC-MS analysis highlighted the presence of twenty-one (21) different fatty acids; out of which 14 are saturated. Details of saturated and unsaturated acids are given in **Table 1** and **Table 2** respectively. Results of all data collected from GC-MS.

Palmitic acid was originated in the highest quantity (2.77%) amongst the saturated fixed oil, followed by tetracosanoic acid, stearic acid, myristic acid, lauric acid and margaric acid showing 0.73%, 0.43%, 0.38%, 0.26% and 0.11% correspondingly. The percentages of other saturated fatty acids were less than 0.1% as shown in Table 1. The unsaturated fatty acids found in this analysis were 3.80%, ω -7 as oleic acid, 0.46%, ω -6 as palmitoleic acid, 23.84%, ω -6 as *cis*-linoleic acid, 4.70%, ω -7 as elaidic acid, 22.60% as octadecadienoic acid, 0.13%, ω -6 as γ -linoleic acid and 21.16%, ω -3 linolenic acid as shown in **Table 2**. The pharmacological activities of the fixed oil differ, for example, linoleic acid is a potential COX-2 inhibitor [56]. Unsaturated fatty acids, particularly ω -6 γ -linolenic acid (GLA) and ω -3 α -linolenic acid (ALA) produce malignant cell lines to be inhibited and cytotoxic [57]. The GC-MS characterization of *T. stocksianum*FO exposed that linoleic (23.84%) and linolenic (21.16%) acid both were found in high concentrations (**Table 2**).

Acute toxicity study

During the acute toxicity study, there was no evidence of mortality and behavioral change in the experimental mice. Considering the results of acute toxicity, a dosage regimen of 1, 2 and 3 ml/kg from

fixed oil could be nominated as the safe dose. Detail of the dosage regimen to animals are given in **Table 3** [58].

Preliminary Anti-inflammatory Potential via carrageenan induction in paw edema

Carrageenan is a non-specific inflammogen, commonly employed for preliminary evaluation of anti-inflammatory potential of the test samples. It develops inflammation in two phases, in the early phase chemical mediators, histamine and serotonin are released while the later phase is mediated by another class of mediators that mainly includes prostaglandin E₂ and leukotriene [59]. In preliminary anti-inflammatory screening, the FO of *T. stocksianum* has shown marked anti-inflammatory activity in a dose-dependent manner. The results are shown in **Table 4**. Test sample displayed, 23.07%, 38.91% and 67.87 % inhibition against carrageenan induced paw edema test, at a dose of 1, 2 and 3 ml/kg at 3rd h of the test sample administration as elaborated in **Figure 1**.

Possible anti-inflammatory Mechanism of Fixed oil

There are multiple causes of inflammation like it might be initiated by trauma, infection, pollutants, or burn [60]. When any sort of damage occurs to the cytoplasmic membrane, it initiates a cascade of biological reactions, initially, it releases phospholipids which are enzymatically (Phospholipase A₂) converted to arachidonic acid. Arachidonic acid is converted to prostaglandins and leukotriene (metabolites), by cyclooxygenase and lipoxygenase respectively, which are strong pro-inflammogens [61].

To determine the involvement of the actual mediator in the anti-inflammatory activity of the fixed oil, paw edema in mice was induced with arachidonic acid, prostaglandin E₂ and leukotriene mediators (**Table 5**). FO (3 ml/kg, I/p) and caffeic acid (100 mg/kg) exhibited profound protection, 64.92, and 69.43% respectively at 3rd h against arachidonic acid induced paw edema. While the reference standard drug aspirin remained non-significant and could only produce 08.2% protection against edemogen shown in **Table 6** and **Figure 2**.

The test sample also exhibited significant 68.75 and 58.3% anti-inflammatory activity against PGE₂ (prostaglandin E₂) and LT (leukotriene) correspondingly, with a calculated dose of 3 ml/kg of body weight of the mouse at 3rd h of test sample administration, depicted in **Table 7** and **Figure 3**.

A literature survey revealed that linolenic acids exhibit strong anti-inflammatory activity (Singh et al., 1997). It is a precursor for Gamma Linolenic acid (GLA), while GLA is metabolized to dihomogamma linolenic acid (DGLA). DGLA is further metabolized by lipoxygenase and cyclooxygenases to produce leukotriene of series 3 and prostaglandins of series 1 (eicosanoids)(Singh et al., 1997). The GC-MS spectra of the fixed oil of *T. stocksianum* have revealed that the test sample is composed of a significant quantity (21.16%) of Linolenic acid (**Table 2**) which could be responsible for the anti-inflammatory effect of the Fixed oil.

Docking studies

Fixed oil components with Cyclooxygenase enzyme (COX-2)

To support the anti-inflammation mechanism by components of fixed oils, docking studies were performed and binding interactions were analyzed for the components which were obtained in a higher ratio i.e. Palmitic acid in the case of saturated oil and linoleic acid in the case of unsaturated oil. Both chemical moieties were docked inside the binding pocket of the cyclooxygenase enzyme (COX-2). The results of 2D interactions are elaborated on in **Figure 4**.

The binding pocket amino acids include ARG D: 42 and ASN D: 43 that accounts for Conventional hydrogen bonds with the carbonyl group of linoleic acid while the double bonds and carbon chain gave π -alkyl interactions with TRY D: 130, CYC D: 36, PRO D: 153, CYS D: 47 and SYS D: 41. Similarly unsaturated fatty acid Palmitic acid interacts through unfavorable donor-donor linkage with ASN C: 537 and conventional hydrogen bond with GLY C: 533. In the case of carbon hydrogen bond, it was formed with PROC: 538. π -alkyl attractions were also observed in this case with PHE D: 142 and LEU D: 145. To further elaborate the linkages, 3D bindings were seen as given in **Figure 5**.

Both higher concentration compounds of fixed oil were analyzed in attaching pocket of human lipoxygenase enzyme to justify the mechanism of anti-inflammation. When the docking studies were analyzed, conventional hydrogen bonds were observed with TRP A: 144 with the carboxylic acid moiety while π -alkyl bindings of unsaturated double bonds were detected with LEU A: 153, TYR A: 515 and ILE B: 330. The binding pocket of the enzyme further consists of GLY B: 332, GLU B: 334, ASN B: 335, ASN B: 328, PRO B: 331, GLU A: 146, and MET A: 145. Carbon hydrogen bond and Vander Waals bonding were seen with ARG A: 384 and ARG A: 143. All results were depicted in **Figure 6** as a 2D image and **Figure 7** as a 3D image. In the case of Palmitic acid, the hydroxyl group on one side gave the best interaction of conventional hydrogen bond with GLU A: 287. Vander Waal forces were witnessed with GLN A: 329, ASP A: 290, ASP A: 442, ARG B: 520 and ASP A: 285. π -alkyl interactions were also the part of show with bindings between the carbon chain and LEU A: 244, PHE A: 286, ILE A: 365, VAL A: 361, ALA A: 439, LEU A: 288 and LYS B: 441.

Furthermore, to understand the interactions with added features, Ligplot viewing was performed for compounds inside the amino acids residual pockets. **Figure 8** Revealed linoleic acid inside the compact active site of enzyme with residual linkages.

Conclusion

Based on the above observed results, it could be summarized that fixed oil of *T. stocksianum* contained the anti-inflammatory potential via both inhibitions of lipoxygenase and cyclooxygenase alleyways of arachidonic acid. However, noteworthy activity was pragmatic through inhibition of prostaglandins. These observations strappingly sustained the traditional natural plant usage in the management of different inflammatory situations. Thus, it's understandable through binding affinity parameters that a large number of molecules persists the ability to inhibit COX-2 synergistically, thus preventing inflammation.

Declarations

Ethics approval and consent to participate

The Committee of animal ethics of the University of Malakand KPK accepted the protocols in strict compliance with internationally accepted principles for laboratory animal use and care after conforming to ARRIVE guidelines for the experimental animal studies through letter No; DREC/122 for Control and Supervision of Experiments on Animals standard.

Consent for publication

Not applicable

Availability of data and materials

The data is available on request from corresponding authors on reasonable request.

Competing interests

All other authors declare that they have no competing interests.

Funding

This study was unfunded

Authors' Contributions

FH, AZ and SWAS performed the overall experimental work. RZ completed the computational study, while SMHS and MIK drafted the manuscript. SMMS and MSJ supervise the overall experimental work and refined the manuscript for publication. All authors have checked the manuscript and approved the final version for publication.

Acknowledgments

The authors are thankful to the Pakistan Council of Scientific and Industrial Research (PCSIR) laboratories, Peshawar, Pakistan for making this research possible through GC-MS and Plethysmometer.

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Tables

Table 1: Chemical composition and relative percent concentrations of saturated Fixed oil of *T. stocksianum*.

S.No	Name	Retention Time	Conc. (%)
1.	C6:0; Hexanoic acid, methyl ester	3.025	0.02
2.	C8:0; Caprylic acid, methyl ester	4.914	0.02
3.	C10:0; Capric acid, methyl ester	6.749	0.02
4.	C11:0; Undecanoic acid, methyl ester	7.607	0.01
5.	C12:0; Lauric acid, methyl ester	8.491	0.29
6.	C14:0; Myristic acid, methyl ester	10.907	0.38
7.	C15:0; Pentadecanoic acid, methyl ester	12.560	0.05
8.	C16:0; Palmitic acid, methyl ester	14.552	2.77
9.	C17:0; Margaric acid, methyl ester	16.848	0.11
10.	C18:0; Stearic acid, methyl ester	19.524	0.73
11.	C20:0; Arachidic acid, methyl ester	27.074	0.26
12.	C22:0; Behenic acid, methyl ester	34.206	0.41
13.	C23:0; Tricosanoic acid, methyl ester	37.469	0.07
14.	C24:0; Tetracosanoic acid, methyl ester	40.554	0.43

Table 2:Chemical composition and relative percent concentrations of unsaturated Fixed oil of *stocksianum*

S.No	Name	Retention Time	Conc. (%)
1.	C16:1c; Palmitoleic acid, methyl ester	15.084	0.46
2.	C18:1; Oleic acid, methyl ester	20.065	3.80
3.	C18:1n9T; Elaidic acid, methyl ester	20.065	4.70
4.	C18:2c; Linoleic acid, methyl ester	21.675	23.84
5.	C18:2t;Octadecadienoic acid, methyl ester	21.676	22.60
6.	C18:3n6; g-Linoleic acid, methyl ester	22.343	0.13
7.	C18:3n3; Linolenic acid, methyl ester	24.218	21.16

Table 3: Group of animals and fixed oil quantities given for acute toxicity studies.

Groups	Animals	Fixed Oil (ml/kg)
1	5	1
2	5	2
3	5	3

Table 4: *Concentration dependent anti-inflammatory effect of Fixed oil (FO) of T. stocksianum in carrageenan induced paw edema model*

Treatments	Saline	FO			Aspirin
Dose	10ml/kg	1ml/kg	2ml/kg	3ml/kg	10mg/kg
NPV	0.07 ± 0 .05	0.086 ± 0.07	0.080 ± 0.07	0.066 ± 0.05	0.068 ± 0.04
1h	0.215 ± 0.08	0.198 ± 0.12	0.165 ± 0.15	0.145 ± 0.06*	0.135 ± 0.18*
2h	0.218 ± 0.10	0.185 ± 0.16	0.15 ± 0.07*	0.115 ± 0.05**	0.10 ± 0.13**
3h	0.221 ± 0.07	0.170 ± 0.14	0.135 ± 0.09*	0.071 ± 0.08**	0.070 ± 0.07**
4h	0.218 ± 0.09	0.172 ± 0.15	0.138 ± 0.17*	0.073 ± 0.07**	0.081 ± 0.10**
5h	0.213 ± 0.06	0.173 ± 0.13	0.14 ± 0.13*	0.076 ± 0.06**	0.096 ± 0.08

NPV; Normal paw volume in ml. Values are reported as mean ± SEM, n = 06. Data was analyzed by ANOVA followed by post hoc Dunnett’s test. Asterisks show significant values from control. **P* ≤ 0.05, ***P* ≤ 0.01.

Table 5: Anti-inflammatory potential of Fixed oil (FO) of *T. stocksianum* against Arachidonic acid, Prostaglandin E₂ and Leukotriene induced edema in mice at 3rd h.

Samples/Standards	Dose	Phlogistic agent	Paw volume at 3 rd h	% Inhibition
FO	3 ml/kg	Arachidonic acid	0.081 ± 0.09**	68.36
Caffeic acid	100 mg/kg		0.085 ± 0.10**	66.80
Aspirin	100 mg/kg		0.235 ± 0.13 ^{n.s}	08.2
FO	3 ml/kg	Prostaglandin E ₂	0.080 ± 0.05**	68.75
		Leukotriene	0.098 ± 0.14**	58.75

Values are presented as mean ± SEM, n = 06. Data was analyzed by ANOVA followed by post hoc Dunnett's test in Arachidonic acid induced edema animal model vial data was analyzed by student's *t* test in Prostaglandin and Leukotriene induced paw edema. Asterisks show significant **P* ≤ 0.05, ***P* ≤ 0.01., and n.s; indicates non-significant percent inhibition compared to respective Phlogistic agent.

Table 6: Anti-inflammatory Potential of Fixed Oil of *T. stocksianum*, in Arachidonic acid induced paw edema in mice.

Treatments	Ard	Aspirin	C. Acid	FO
Dose	0.5% w/v	100mg/kg	100mg/kg	3 ml/kg
1h	0.215 ± 0.022	0.198 ± 0.004	0.166 ± 0.010*	0.143 ± 0.021
2h	0.23 ± 0.007	0.198 ± 0.008	0.115 ± 0.007**	0.115 ± 0.007**
3h	0.265 ± 0.006	0.235 ± 0.009	0.081 ± 0.006**	0.083 ± 0.004**
4h	0.251 ± 0.001	0.231 ± 0.003	0.078 ± 0.003**	0.09 ± 0.006**
5h	0.235 ± 0.004	0.203 ± 0.007	0.086 ± 0.007**	0.096 ± 0.007**

Ard; Arachidonic acid, C. Acid; caffeic acid, FO; Fixed Oil. Values are reported as mean ± SEM, n = 06. Data was analyzed by ANOVA followed by post hoc Dunnett's test. Asterisks show significant values from control. **P* ≤ 0.01, ***P* ≤ 0.001

Table 7: Anti-inflammatory effect of Fixed Oil of *T. stocksianum*, in prostaglandin and Leukotriene's induced paw edema test.

Treatments	PGE ₂	F. Oil	LT	F. Oil
Dose	0.01 g/ml	3 ml/kg		3 ml/kg
1h	0.231 ± 0.060	0.098 ± 0.015**	0.223 ± 0.08	0.156 ± 0.012**
2h	0.24 ± 0.057	0.093 ± 0.014**	0.233 ± 0.084	0.121 ± 0.011**
3h	0.256 ± 0.049	0.08 ± 0.005***	0.235 ± 0.084	0.098 ± 0.079***
4h	0.251 ± 0.040	0.086 ± 0.012***	0.236 ± 0.033	0.100 ± 0.068***
5h	0.246 ± 0.055	0.095 ± 0.010***	0.236 ± 0.021	0.106 ± 0.061**

F. Oil; Fixed oil, PGE₂; prostaglandin E₂. LT; Leukotriene. Values are reported as mean ± SEM, n = 06. Data was analyzed by student's *t* test. Asterisks show significant **P* ≤ 0.05, ***P* ≤ 0.01, ****P* ≤ 0.001 percent inhibition compared to respective Phlogistic agent.

Figures

Figure 1

Percent inhibition produced by Fixed oil (1, 2 and 3 ml/kg) of *T. stocksianum* in carrageenan induced paw edema model in mice. Each percent point represents the mean $\pm$ SEM for group of 06 mice. Data was analyzed by ANOVA followed by post hoc Dunnett's test. Asterisks show significant values from control. *P $\leq$ 0.05, **P $\leq$ 0.01.

Figure 2

Percent inhibition produced by Fixed oil (3 mg/kg) of *T. stocksianum* in Arachidonic acid induced paw edema model in mice. Each percent point represents the mean $\pm$ SEM for group of 06 mice. Data was analyzed by ANOVA followed by post hoc Dunnett's test. Asterisks show significant values from control. *P $\leq$ 0.05, **P $\leq$ 0.01.

Figure 3

FO; Fixed oil, PGE₂; prostaglandin E₂. LT; Leukotrienes. Values are reported as mean $\pm$ SEM, n = 06. Data was analyzed by student's t test. Asterisks show significant *P $\leq$ 0.05, **P $\leq$ 0.01, ***P $\leq$ 0.001 percent inhibition compared to respective Phlogistic agent

Figure 4

a) Two dimensional (2D) pattern showed the interactions of linoleic acid inside binding pocket with amino acids residues of COX-2. b) Showed the interaction of Palmitic acid inside binding pocket

Figure 5

a) displayed the Discovery studio visualizer 3-Dimensional image of linoleic acid inside binding pocket of COX-2 enzyme. b) Demonstrated the 3-Dimensional image of Palmitic acid inside binding pocket Fixed oil components with Lipxygenase enzyme (5-LOX)

Figure 6

a) Discovery studio visualizer 2-Dimensional view of linoleic acid inside binding pocket of 5-LOX enzyme.
 b) Demonstrated the 2-Dimensional image of Palmitic acid inside binding pocket

Figure 7

a) Discovery studio visualizer 3-Dimensional image of linoleic acid inside binding pocket of 5-LOXenzyme with yellow colour indicating the compound. b) Demonstrated the 3-Dimensional image of Palmitic acid inside binding pocket with solid yellow color showing the compound.

Figure 8

Image captured through Ligplot in order to get better understanding. All the amino acids of pocket were displayed on side with compound in center and TRP 144 amino acid on the right side. Bondings were shown in dotted red color lines.