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EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF SEED EXTRACT OF *CLITORIA TERNATEA* AND *OCIMUM SANCTUM* ON CARRAGEENAN INDUCED PAW EDEMA IN RATS

Yashsvee Verma[1], Anchal Verma[1*]

[1]Shri Rawatpura Sarkar Institute of Pharmacy, Kumhari, Durg (Chhattisgarh) India.

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***Corresponding Author**
Anchal Verma
Shri Rawatpura Sarkar Institute of Pharmacy, Kumhari, Durg (Chhattisgarh) India.

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ABSTRACT

Herbal medicines have long been used in traditional systems of healthcare for the treatment of inflammatory disorders due to their safety and efficacy. This study evaluates the anti-inflammatory activity of ethanolic seed extracts of *Clitoria ternatea* and *Ocimum sanctum* using the carrageenan-induced paw edema in rats. Both plants were extracted in ethanol. Extracts were administered orally at 200 mg/kg using 2% gum acacia as a suspending agent. Animals were divided into seven groups control, standard, individual extracts, and combination treatments of CTSE and OSSE in 1:1, 1:2, and 2:1 ratios. All treated groups showed significant reduction in paw edema ($p < 0.01$), with the 2:1 CTSE: OSSE combination exhibiting the highest anti-inflammatory activity, exceeding that of Diclofenac. GC-MS analysis revealed the presence of several phytoconstituents in the extracts, suggesting their role in mediating the observed effects. These findings indicate a synergistic interaction between CT and OS seed extracts.

KEYWORDS: *Clitoria ternatea*, *Ocimum sanctum*, GC-MS, Anti-inflammatory activity, Carrageenan, Diclofenac, Plethysmometer.

INTRODUCTION

Inflammation is a fundamental biological response to disruptions in tissue homeostasis.[1] It is a vital defence mechanism of the immune system, crucial for survival during infections or physical injuries. However, when inflammation becomes chronic, it can negatively affect

overall health.[2] Over recent decades, there has been a noticeable rise in the prevalence of inflammatory diseases and conditions, often linked to on-going inflammatory stimuli or genetic predispositions.[3] In 2019, immune-mediated inflammatory diseases (IMIDs) affected an estimated 67.6 million people worldwide. Common IMIDs include asthma, inflammatory bowel disease (IBD), multiple sclerosis (MS), rheumatoid arthritis (RA), psoriasis, and atopic dermatitis (AD).[4] Inflammation is typically identified by four hallmark symptoms: redness, swelling, heat, and pain. Because of its broad effects, inflammation is thought to impact nearly all aspects of normal physiology and disease processes.[5] Despite significant scientific efforts, the full scope of inflammation's impact on human health is still not completely understood. Key inflammatory mediators like interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) are central to initiating and regulating inflammatory responses.[6] These cytokines interact with receptors such as toll-like receptors (TLRs), IL-1R, IL-6R, and TNFR, triggering intracellular signaling pathways including the mitogen-activated protein kinase (MAPK), nuclear factor kappa-B (NF-κB), and Janus kinase/signal transducer and activator of transcription (JAK/STAT) cascades.[7]

Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to alleviate inflammation, pain, and fever.[8,9] However, long-term use of NSAIDs can lead to serious side effects like stomach ulcers, kidney damage, respiratory issues, and cardiovascular complications due to their widespread inhibition of cyclooxygenase (COX) enzymes.[10] As a result, there has been an increased effort to discover new anti-inflammatory and pain-relieving medications with fewer adverse effects to replace conventional NSAIDs.[11]

Plants and herbs have been an important contributor to the quality of human life for thousands of years.[12] Some of them are well known medicinal herbs. Herbal therapies extracted from medicinal plants are widely used, with approximately 75-80 % of the worldwide population, particularly in the process of development and underdeveloped World nations, relying on them for primary health care.[13] Health authorities have shown growing interest in herbal medicines, largely because they are often the primary healthcare option in less developed regions and are gaining popularity as alternative treatments in more developed countries.[14] Medicinal plants are known to be rich in secondary metabolites and essential oils, both of which have significant therapeutic value.[15] These plants are considered beneficial in treating various health conditions due to their perceived safety, affordability, effectiveness, and widespread availability.[16] This preference is largely due to their cultural

acceptability, compatibility, and the belief that natural products are safer and less toxic compared to synthetic drugs.[17,18] Many commercially available herbal products, whether marketed as food supplements or ayurvedic medicines, often contain a combination of ingredients.[19] These ingredients work synergistically, enhancing the therapeutic effects of each other, making the formulations more potent than individual components.[20] The use of such multi-ingredient herbal preparations continues to grow in popularity, particularly for their perceived safety and efficacy.[21] Today the large numbers of drugs in use are derived from plants, like *Papaver somniferum, Withania somnifera, Clitoria ternatea* & *Ocimum sanctum* etc. which is used in several traditional medicine systems to cure various diseases.[22]

Clitoria ternatea, commonly known as butterfly pea, Asian pigeon wings, blue bell, or Darwin's pea.[23-25] belongs to the Fabaceae family and is found in two main varieties—blue and white flowered.[26,29] It is rich in a wide array of phytochemicals, including flavonoids, tannins, anthocyanins, terpenoids, alkaloids, saponins, phenolic acids, essential fatty acids, and proteins.[30] Notable bioactive constituents include delphinidin-3,3',5'-triglucoside, kaempferol, rutin, beta-sitosterol, p-coumaric acid, linoleic acid, sinapic acid, and various tocopherols.[31] The plant exhibits numerous pharmacological properties such as antioxidant, anti-inflammatory,[32] antimicrobial, neuroprotective, nootropic, antidiabetic, anticancer, and immunomodulatory activities.[33-36] It has been shown to enhance acetylcholine levels and acetyl cholinesterase activity, indicating its potential in improving cognitive function.[37] Compounds such as flavonol glycosides, anthocyanin's, and ternatins contribute to its ability to suppress inflammation by inhibiting inflammatory enzymes (iNOS, COX, LOX)[38] and modulating cytokine expression (e.g., reducing TNF-α, IL-6; increasing IL-10)[39] These properties position *Clitoria ternatea* as a valuable botanical with significant therapeutic potential.[40-42]

Ocimum sanctum, commonly known as Tulsi or Holy Basil,[43-46] is a sacred plant in Ayurveda and Indian culture, often referred to as "The Queen of Herbs"[47-48] and "The Elixir of Life."[49] Belonging to the Lamiaceae family, it exists primarily in two forms—Krishna Tulsi (black) and Ram Tulsi (green)—both valued for their similar therapeutic properties.[50-52] Tulsi is rich in a variety of bioactive compounds, including flavonoids, alkaloids, terpenoids, phenolics,[53-55] and essential fatty acids such as linoleic and linolenic acid. Key constituents like eugenol, ursolic acid, oleanolic acid, rosmarinic acid, and β-sitosterol contribute to its wide-ranging pharmacological effects.[56-58] These include anti-inflammatory,

antioxidant, antidiabetic, anticancer, antiviral, antimicrobial, and neuroprotective activities.[59] *Ocimum sanctum* anti-inflammatory potential is largely attributed to its inhibition of COX-1 and COX-2 enzymes[60-63], highlighting its role in managing chronic inflammation and related disorders.[64] Its revered status in traditional medicine is supported by a growing body of scientific evidence confirming its therapeutic efficacy.[65-65]

In consonance with this, it was envisioned that combining the seed extracts of *Clitoria ternatea* and *Ocimum sanctum*, would be beneficial in potentiating anti-inflammatory effect of both the extracts. The objective of the present investigation was therefore to alcoholic extraction of *Clitoria ternatea* & *Ocimum sanctum* seeds and Evaluation of anti-inflammatory effect of the combined extracts on paw edema in rats.

MATERIALS AND METHODS

Chemicals & Instruments

Diclofenac and Carrageenan were purchased from Dhamtech pharma and consultants, Mumbai, Ethanol and Dimethyl Sulphoxide (DMSO) were purchased from local market of Raipur, Plethysmometer (Orchid scientific), GC-MS (Shimadzu GC -2030).

Collection of Plant Material

Dried seeds of *Clitoria ternatea* and *Ocimum sanctum* were sourced from a Regional market in Raipur. They underwent identification and authentication by Shri Narayan Prasad Awasthi Government Ayurved College, Raipur (C.G.), based on a comparison of their morphological characteristics with those described in established literature.

Preparation of Extracts

The dried seeds of *Clitoria ternatea* and *Ocimum sanctum* were ground into a coarse powder using a grinder. Each seeds powder (300 g) was then separately placed in a Soxhlet apparatus for extraction using ethanol as the solvent (boiling range 60–80 ^{0}C) over a period of 48 hours. Following extraction, the ethanol was evaporated at 40 ^{0}C to yield a concentrated extract. A preliminary phytochemical screening was performed on the obtained residue to identify various bioactive compounds. The final extracts were filtered and stored in dark bottles at room temperature for future use.

Organoleptic Description of Extract

An organoleptic assessment was carried out on the extracts to evaluate their color, odor, consistency, and opalescence. This evaluation was crucial for determining the physical attributes of the extracts, as these properties can often reflect their chemical composition and overall quality.

Preliminary Phytochemical Screening

The seed extracts of *Clitoria ternatea* & *Ocimum sanctum* were subjected to qualitative phytochemical screening to identify various bioactive compounds. This analysis focused on detecting secondary metabolites known to contribute to the therapeutic properties of the plants. The compounds tested included alkaloids, carbohydrates, glycosides, flavonoids, phenols, tannins, amino acids, proteins, saponins, sterols, terpenoids, quinones, and oxalates. Their presence was confirmed using established qualitative methods. The findings provided insight into the chemical composition of the extracts, which is essential for understanding their pharmacological potential. Notably, secondary metabolites such as flavonoids, alkaloids, glycosides, and saponins are well known for their anti-inflammatory, antioxidant, and antimicrobial properties.

GC-MS Analysis

Gas Chromatography–Mass Spectrometry (GC-MS) analysis of the ethanolic seed extracts of *Clitorea ternatea* & *Ocimum sanctum.* A 1 µL sample was injected into the system using a micro syringe. In the resulting chromatogram, each peak corresponded to compounds that had passed through the gas chromatography column and was detected as it exited. The x-axis represented the retention time, while the y-axis showed the signal intensity, indicating the quantity of each compound. As the compounds emerged from the column, they entered the mass spectrometer, where electron ionization was employed. This involved bombarding the molecules with electrons, fragmenting them into smaller, charged particles known as ions. Each ion had a unique mass-to-charge (m/z) ratio, which was used to generate a mass spectrum—a graphical representation that serves as a distinct fingerprint for each compound. Prior to analysis, critical parameters were configured, including the oven temperature, carrier gas flow rate, and ionization energy. The oven temperature was set at 100 ^{0}C, with helium as the carrier gas flowing at a rate of 1 mL/min. The electron ionization source operated at approximately 70 electron volts (eV). Separation was carried out using an Elite-1 column made of 100 % dimethylpolysiloxane. Compounds identification was accomplished by

comparing the retention times and mass spectra to reference data in the instrument's integrated library.

In vivo Study

Experimental Animals

A total of 35 Wistar rats (150–180 g), of both sexes, were procured from M/S Chakraborty Enterprise (CCSEA Registration No: 1443/PO/Bt/S/11/CPCSEA). The animals were housed and maintained under standard environmental conditions at the animal facility of Shri Rawatpura Sarkar Institute of Pharmacy, Kumhari, Durg (Registration No: 1188/PO/Re/S/08/CPCSEA). The experimental protocol (Proposal No: SRIP/IAEC/2023-24/B/13) was approved by the Institutional Animal Ethics Committee (IAEC).The rats were kept in a controlled environment with room temperature: 22 ± 2 ^{0}C, Relative humidity: 50 ± 5 %, Light/dark cycle. The animals were housed in colony cages (five animals per cage) and had free access to standard feed and drinking water.

Evaluation of *In vivo* anti-inflammatory potential

Carrageenan-Induced Paw Edema

The carrageenan-induced paw edema model in rats was employed to assess the anti-inflammatory effects of the plant seed extracts. Inflammation was induced by injecting 0.1 mL of 1 % carrageenan into the sub-plantar surface of the rat's hind paw. Albino rats (150–250 g) of both sexes were fasted overnight, with free access to water. The rats were divided into seven groups (n = 5). Doses of 200 mg/kg of *Clitorea ternatea* seeds extract (CTSE)[66], 5 g/kg of *Ocimum sanctum* seeds extract (OSSE)[67] and 10 mg/kg of Diclofenac[68], were chosen for further experimentation.

Group 1: Control group - Saline solution (5 ml/kg, p.o.) + Carrageenan (0.1ml of 1% in normal saline)

Group 2: Standard group - Diclofenac (10 mg/kg, p.o.) + Carrageenan (0.1 ml of 1% w/v in saline solution)

Group 3: Test group (D1) - (CTSE) (200 mg/kg, p.o.) + Carrageenan (0.1 ml of 1% w/v in saline solution)

Group 4: Test group (D2) - (OSSE) (200 mg/kg, p.o.) + Carrageenan (0.1 ml of 1% w/v in saline solution)

Group 5: Test group (D3) – Combined seeds extract (CTSE & OSSE) 1:1 (200 mg/kg, p.o.) + Carrageenan (0.1 ml of 1% w/v in saline solution)

Group 6: Test group (D4) - Combined seeds extract (CTSE & OSSE) 1:2 (200 mg/kg, p.o.) + Carrageenan (0.1 ml of 1% w/v in saline solution)
Group 7: Test group (D5) - Combined seeds extract (CTSE & OSSE) 2:1 (200 mg/kg, p.o.) + Carrageenan (0.1 ml of 1% w/v in saline solution)

A mark was made on the right hind paw just below the tibio-tarsal junction so that every time the paw could be dipped in the column of the plethysmometer up to the mark to ensure a constant paw volume. The Individual & Combined seeds extracts at a dose of 200 mg/kg was administered orally to the treated groups, and Diclofenac at a dose of 10 mg/kg was administered orally to the standard group. After 30 minutes edema was induced in the left hind paw by injecting 0.1 mL of carrageenan in saline (1% w/v) into the plantar tissue of all the animals. The paw volume was measured at 0 min, 60 min (1 h), 120 min (2 h), 180 min (3 h), and 240 min (4 h). After the administration of carrageenan to each group, the difference between the initial and subsequent readings gave the actual edema volume. The ability of the anti-inflammatory drug to suppress paw inflammation was expressed as the percentage of inhibition of paw edema, and this percentage was calculated according to the following equation[63]:

Percentage of inhibition (%) = $(X - Y) / X \times 100$
Where, X = Mean increase in paw volume of rats in the control group,
Y = Mean increase in paw volume of rats in the drug-treated group."

Statistical Analysis

The data were evaluated using one-way ANOVA, followed by Dunnett's multiple comparison tests, with analysis performed using Graph Pad Prism version 5.0. Results are presented as Mean ± SEM, and differences with p-values less than 0.05 were regarded as statistically significant.

RESULTS AND DISCUSSION

Organoleptic Description of Extract

The organoleptic evaluation of *Clitoria ternatea* & *Ocimum sanctum* seed extract involves assessing its sensory characteristics including color, odor, taste, texture, and appearance. These properties provide preliminary qualitative insights into the nature and quality of the extract (Table I).

Table I: Organoleptic description of ethanolic seed extracts of *Clitoria ternatea* & *Ocimum sanctum*.

Parameter	*Clitoria ternatea* ethanolic seed extract	*Ocimum sanctum* ethanolic seed extract
Appearance	Thick liquid or semi-solid, homogenous	Thick, viscous extract or semi-solid
Color	Light yellow to golden yellow	Pale yellow to yellowish-brown
Odor	Mild, earthy, slightly woody or nutty	Strong aromatic, spicy, clove-like, camphoraceous
Taste	Mildly bitter, earthy, slightly astringent	Pungent, slightly sweet, bitter-spicy
Texture	Smooth, slightly viscous or sticky if concentrated	Viscous, smooth, slightly oily or resinous

Phytochemical Screening

The extractive yield in ethanol was found to be 34.5 % w/w for *Clitoria ternatea* seeds and the extractive yield in ethanol was found to be 36.7 % w/w for *Ocimum sanctum* seeds.

Phytochemical screening of medicinal plants is essential for discovering potential sources of therapeutic agents and compounds with industrial relevance. In this study, the individual ethanolic seed extracts of *Clitoria ternatea* and *Ocimum sanctum* were analysed to identify their phytochemical components. The results revealed the presence of several bioactive compounds, including proteins, carbohydrates, glycosides, resins, alkaloids, steroids, tannins, flavonoids, saponins, and phenols. These findings suggest that the seeds of both *Clitoria ternatea* and *Ocimum sanctum* are rich in a variety of phytochemicals, which may contribute to their medicinal efficacy and potential for industrial use. A detailed summary of the phytochemical screening is presented in Table II.

Table II: Qualitative phytochemical screening of various secondary metabolites in the seed extracts of *Clitoria ternatea* and *Ocimum sanctum*.

Secondary Metabolites	*Clitoreaternatea* ethanolic Seed extract	*Ocimum sanctum* ethanolic Seed extract
Alkaloids	+	+
Carbohydrates	+	+
Glycosides	+	+
Flavonoids	+	+
Phenol	+	+
Tannins	+	+
Amino acids and proteins	+	-
Saponins	+	+
Terpenoids	+	+
Quinones	+	-

Oxalate	-	-
Resins	-	-
Reducing sugar	-	-
Steroids	-	+

GC-MS analysis

GC-MS analysis of the ethanolic seed extract of *Clitoria ternatea* identified twelve bioactive compounds, with both major and minor components making up 100 % of the total composition, as detailed in Table III and illustrated in Fig. 1. The phytochemical constituents of the *Clitoria ternatea* seed extract (CTSE) were characterized through this GC-MS study, and the corresponding chromatogram is shown in Fig. 1. Table III lists the IUPAC names of the detected compounds along with their retention times (RT), peak areas, percentage composition, and similarity indices. Among the twelve identified phytochemicals, seven exhibited anti-inflammatory and anti-arthritic activities.

Similarly, GC-MS analysis of the unsaponifiable fraction of *Ocimum sanctum* seed extract revealed seven compounds, which together accounted for 100 % of the total composition, as shown in Table IV and Fig. 2. The phytochemical profile of the *Ocimum sanctum* seed extract (OSSE) was established through this analysis, with the resulting chromatogram presented in Fig. 2. Table IV provides the IUPAC names, retention times, peak areas, percentage composition, and similarity indices of the identified compounds. All seven compounds were found to possess anti-inflammatory and anti-arthritic properties.

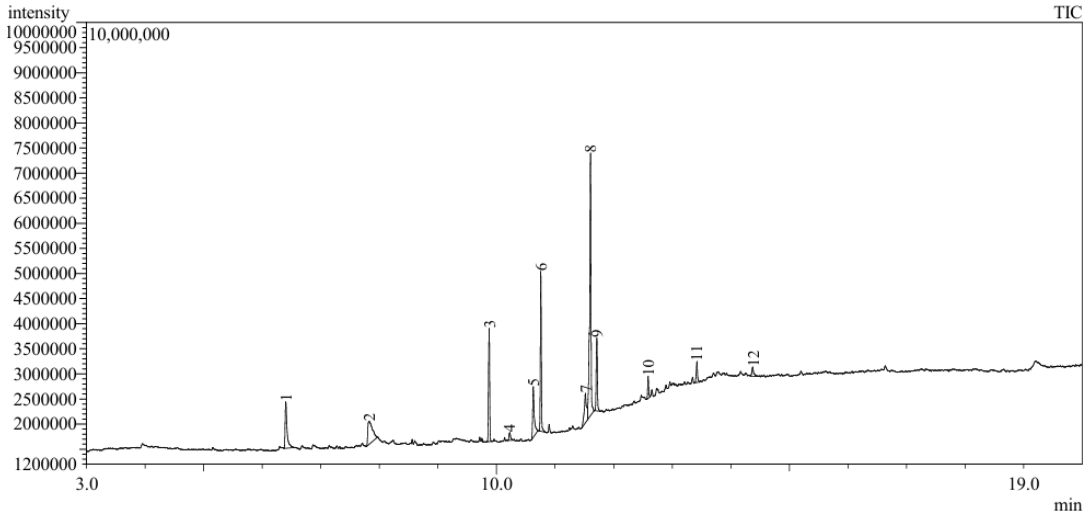

Fig. 1: Analysis of essential extract from *Clitoria ternatea* seeds by GC-MS.

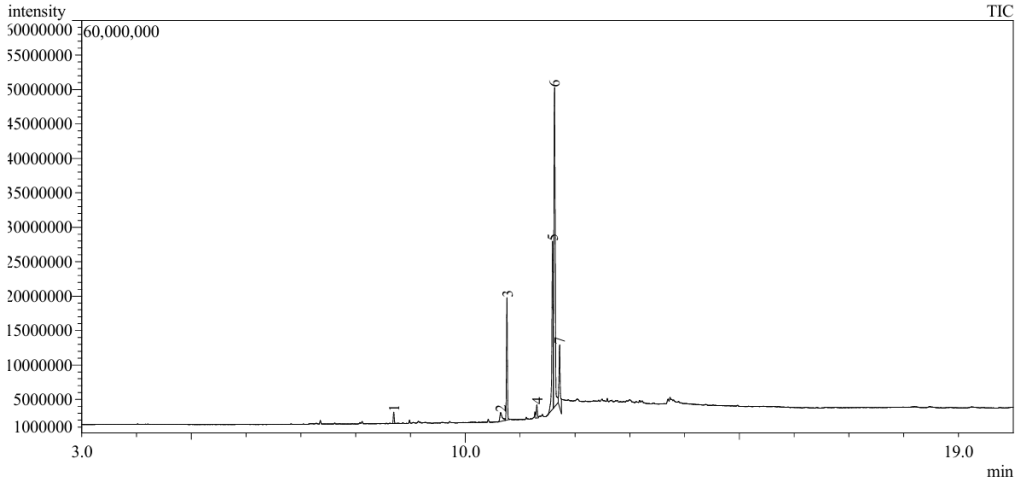

Fig. 2: Analysis of essential extract from *Ocimum sanctum* seeds by GC-MS.

Table III- List of compounds identified from GC-MS analysis of ethanolic seeds extract of *Clitoria Ternatea.*

Peak #	R. Time	Area	Area %	Similarity	Name
1	6.401	1993642	7.40	93	2-Decenal, (E)-
2	7.823	2461572	9.14	84	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-
3	9.873	2745701	10.20	93	Isopropyl myristate
4	10.224	280492	1.04	93	n-Heptadecanol-1
5	10.629	1960200	7.28	92	n-Hexadecanoic acid
6	10.757	3890008	14.44	94	Hexadecanoic acid, ethyl ester
7	11.515	1608055	5.97	92	cis-Vaccenic acid
8	11.604	8655205	32.14	95	(E)-9-Octadecenoic acid ethyl ester
9	11.711	1806650	6.71	94	Octadecanoic acid, ethyl ester
10	12.588	553178	2.05	92	Eicosanoic acid, ethyl ester
11	13.419	586485	2.18	01	Docosanoic acid, ethyl ester
12	14.373	390266	1.45	78	Ethyl tetracosanoate
		26931454	100.00		

Table IV: List of compounds identified from GC-MS analysis of ethanolic seeds extract of *Ocimum Sanctum.*

Peak #	R. Time	Area	Area %	Similarity	Name
1	8.694	2088016	1.32	96	Caryophyllene oxide
2	10.646	3769457	2.38	92	n-Hexadecanoic acid
3	10.760	21661388	13.68	94	Hexadecanoic acid, ethyl ester
4	11.304	2710379	1.71	93	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-
5	11.590	39288782	24.82	95	Linoleic acid ethyl ester
6	11.629	74857577	47.28	95	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-
7	11.719	13946091	8.81	94	Octadecanoic acid, ethyl ester
		158321690	100.00		

Antiinflammatory Activity

Carrageenan-induced rat paw edema assay

The study involved evaluating the individual and combined extracts of *Clitoria ternatea* and *Ocimum sanctum* (in 1:1, 1:2, and 2:1 ratios) for their in vivo anti-inflammatory activity, utilizing the carrageenan-induced rat paw edema method. Table **V** & Fig. **3** present the changes in paw thickness following different treatments, comparing the effect of seed extracts and a standard drug (Diclofenac) to the normal saline control at various time points. At a dose of 10 mg/kg, Diclofenac exhibited a 79.85 % reduction in paw edema after 4 h. In comparison, the combination of extracts showed a maximum edema inhibition of 76.84 % with the CTSE:OSSE (2:1) mixture at the 4 h mark. The observed anti-inflammatory effect was found to correlate with the ethanolic content of the extracts, with mixtures containing a higher proportion of CT showing enhanced inhibition of paw edema.

Table V: Effect of formulation of seeds extracts *Clitorea ternatea* & *Ocimum sanctum* on carrageenan-induced paw edema in rats (mean±SEM).

Group	Dose (Mg/Kg)	Mean edema volume (ml) and inhibition (%)							
		1hr		2hr		3hr		4hr	
		Mean ± SD	% inhibition	Mean ± SD	% inhibition	Mean ± SD	% inhibition	Mean ± SD	% Inhibition
Control	0.9% Nacl (5ml/kg)	2.26±0.10	4.24	2.18±0.07	6.57	2.1±0.08	10.99	2.06±0.04	10.88
Standard	20 mg/kg	1.9±0.08 ***	19.82	1.4±0.06 ***	43.53	0.9±0.06 ***	63.71	0.5±0.06 ***	79.85
D1	200 mg/kg	2.12±0.04 *	11.80	1.74±0.04 ***	24.63	1.28±0.04 ***	40.31	1.04±0.04 ***	48.46
D2	200 mg/kg	2.12±0.04 *	11.16	1.82±0.04 ***	21.39	1.5±0.06 ***	32.44	1.2±0.06 ***	42.70
D3	200 mg/kg	2.1±0.06 *	15.31	1.72±0.07 ***	30.62	1.32±0.07 ***	46.76	0.96±0.12 ***	61.30
D4	200 mg/kg	2.1±0.07 *	15.88	1.72±0.07 ***	31.77	1.28±0.09 ***	49.26	0.9±0.06 ***	64.31
D5	200 mg/kg	2±0.06 ***	20.01	1.52±0.07 ***	39.22	1.02±0.07 ***	59.23	0.58±0.09 ***	76.84

Statistical analysis was done by one-way analysis of variation (ANOVA) followed by Dunnett's test. ***P < 0.001, **P < 0.01, *P < 0.05. Standard and test were compared with the carrageenan group.

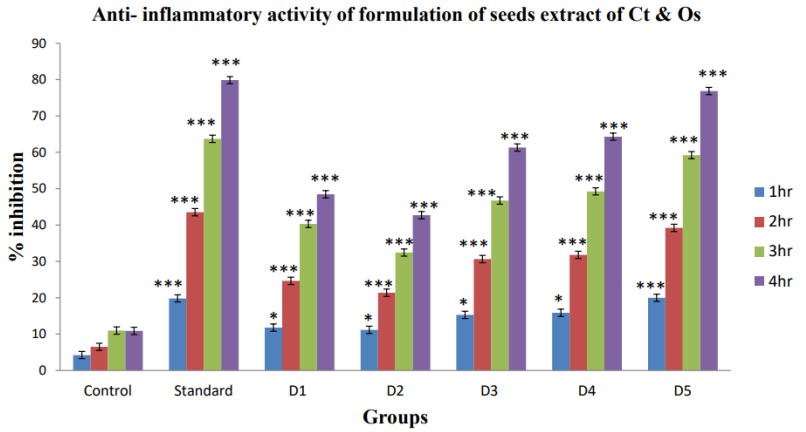

Fig. 3: Anti-inflammatory activity of formulation of seeds extracts *Clitoria ternatea* and *Ocimum sanctum*.

CONCLUSION

The present study demonstrated that ethanolic seed extracts of *Clitoria ternatea* (CT) and *Ocimum sanctum* (OS) exhibit significant anti-inflammatory activity in the carrageenan-induced paw edema model in rats. The highest percentage inhibition of paw edema was observed in the combination group D5 (CTSE: OSSE, 2:1) with 76.84% inhibition at 4 hours, indicating a potent and prolonged anti-inflammatory response compared to individual extracts. The enhanced efficacy of the combination suggests a synergistic interaction between the phytoconstituents of CT and OS. Phytochemical classes such as flavonoids, terpenoids, phenolics, tannins, glycosides, and triterpenoids specifically compounds like quercetin, kaempferol, rutin, delphinidin, taraxerol, and rosmarinic acid are likely contributors to the observed pharmacological activity. However, further studies involving bioactivity-guided fractionation, compound isolation, and mechanistic evaluations are warranted to identify the key bioactive constituents and elucidate their roles in anti-inflammatory pathways. These findings support the therapeutic potential of CT and OS seed extracts in managing inflammatory conditions.

AUTHOR'S CONTRIBUTION

All the experimental work and manuscript writing was carried out by YV under the supervision of AV. Conceptualization and data interpretation was done by AV.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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