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Research Article

Evaluation of *In vivo* Analgesic and Anti-inflammatory Activity of *Oroxylum indicum*, Baicalein, Chrysin with Phytochemical Analysis and Molecular Docking Study

Bhairav Kumar Pathak[1], Kamlesh M. Palandurkar[2], Meenakshi Singh[3], Anshuman Trigunayat[4], Amit Singh[5], Reena Giri[6], Kiran Rajendra Giri[7,*]

ABSTRACT

Background: *Oroxylum indicum* (OIE) is a native medicinal plant that has been widely employed in Ayurvedic medicine for thousands of years. Though studies have been published citing the analgesic and anti-inflammatory activity of *Oroxylum indicum* and chrysin and Baicalein, there has been no comparative study comparing their activittes and confirming them with molecular docking results. Molecular docking study of two phytochemicals Chrysin (PubChem CID 5281607) and Baicalein (PubChem CID 5281605) into the active sites of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). Evaluation and validation of Anti-inflammatory and Analgesic effects of a methanolic extract of the stem bark of *Oroxylum indicum* and its two constituents Chrysin and Baicalein in Charles foster rats with analysis of the phytoconstituent of *Oroxylum indicum* through HRMS analysis. **Methodology:** UHPLC-HRMS/MS analyses were performed on a Dionex Ultimate 3000 RS Series UHPLC system combined with a Q Exactive Plus High-Resolution Accurate Mass Spectrometry System. Hot plate and Tal flick model are used for screening of analgesic activity. TNF-alpha and IL-6 inflammatory markers were examined. Carrageenan model is used for anti-inflammatory analysis. **Result:** Interesting results has been obtained in the docking studies of Chrysin and Baicalein with COX-1 (PDB ID: 1EQG). The hydrogen bond interaction established between the Chrysin and Baicalein with the important amino acid, includes Arg 120, Tyr 355, Ser 530, Met 522 (Figure 1). The binding free energy of the Chrysin and Baicalein with target COX-1 was found to be -7.88 and -7.26 Kcal/mol. **Conclusion:** There is marked reduction in the TNF Alpha expression in the OIE group which is followed by Baicalein and Chrysine. The Baicalein group shows the most marked cumulative increase in reaction time for tail flick among all the groups of the intervention group followed by Chrysine and OIE.
Key words: *Oroxylum indicum,* Baicalein, Chrysine, Molecular docking, TNF alpha.

Bhairav Kumar Pathak[1], Kamlesh M. Palandurkar[2], Meenakshi Singh[3], Anshuman Trigunayat[4], Amit Singh[5], Reena Giri[6], Kiran Rajendra Giri[7,*]

[1]*Junior Resident, Department of Pharmacology, IMS, BHU, INDIA.*

[2]*Associate Professor, Department of Biochemistry, IMS, BHU, INDIA.*

[3]*Assistant Professor, Medicinal Chemistry Department, Faculty of Ayurveda, IMS, BHU, INDIA.*

[4]*Professor, Department of Pharmacology, IMS, BHU, INDIA.*

[5]*Professor, Department of Pharmacology, IMS, BHU, INDIA.*

[6]*Professor, Department of Pharmacology, GMC, Akola, INDIA.*

[7]*Associate Professor, Department of Pharmacology, IMS, BHU, INDIA.*

Correspondence

Kiran Rajendra Giri

Associate Professor, Department of Pharmacology, IMS, BHU, INDIA.

E-mail: drkirangiri@gmail.com

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INTRODUCTION

Inflammation is a retort of vascularized tissues to any kind of infection, stress or injury to tissues that transports cells and molecules of host defense system from the circulation to the sites where they are needed, in order to combat the offending stimuli. Pain is one of the most important adaptive and protective mechanisms. It is a very complex phenomenon and which cannot be simply characterized as a response to injury.[1]

Medicinal plants have been used virtually in all cultures as a source of medicine. Ayurvedic medicine has immense importance in India.

Oroxylum indicum is a native medicinal plant which has been widely employed in Ayurvedic medicine for thousands of years. It is generally used as medicinal herb. It accounts for active ingredient in widely known Ayurvedic formulations like Chyawanprash and dasamula.[2]

Oroxylum indicum leaves are known to contain various types of phytochemicals such as flavones and their glycosides like Baicalein (5, 67-trihydroxy flavone) and its 6 and 7-glucuronides, chrysin (5,7-dihydroxy flavone), scutellarein and its 7-glucuronides, anthraquinone and aloe-emodin, chrysin-7-O-glucuronide, chrysin-diglucoside and irridoids.[3-5]

Flavonoids have a noteworthy function in keeping human health and protecting it from diseases. Additionally, it plays an important role in averting and fighting allergies in addition to its anti-inflammation property. The anti-inflammatory property Chrysin has been used for a long time in Chinese traditional medicine and also in the cosmetic industry. Recently there has been increasing interest to explore in depth and to identify the mechanism responsible and for use of flavonoids as anti-inflammatory and analgesic agent. Baicalein is a bioactive flavonoid derived from several herbal plants which is widely used in traditional Chinese and Indian medicine.[6,7]

Evidence has shown that Baicalein has many pharmacological effects including anti-inflammatory, analgesic, antiallergic, antioxidant, antiviral, antiapoptotic, antitumor, and neuroprotective effects as well as a modulatory effect on the immune system.[8,9]

Though studies have been published citing the analgesic and anti-inflammatory activity of *Oroxylum indicum* and chrysin and Baicalein, there has been no comparative study comparing their activities and confirming them with molecular docking results. This study is an effort towards finding out more potential bio constituent of *Oroxylum indicum* showing analgesic and anti-inflammatory properties.

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OBJECTIVES

A molecular docking study of two phytochemicals, Chrysin (PubChem CID 5281607) and Baicalein (PubChem CID 5281605) into the active sites of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2)

Evaluate and validate the anti-inflammatory effect of a methanolic extract of stem bark of *Oroxylum indicum* and its two constituents Chrysin and Baicalein in Charles Foster rats

Evaluate and validate the Analgesic effect of Methanolic Extract of stem bark of *Oroxylum indicum* and its two constituents Chrysin and Baicalein in Charles foster rats

Analysis of Phytoconstituent of *Oroxylum Indicum* through HRMS analysis

MATERIALS AND METHODS

Molecular docking study

The molecular docking study of two phytochemicals Chrysin (PubChem CID 5281607) and Baicalein (PubChem CID 5281605) into active sites of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) was performed. The structures of ligands were retrieved from the PubChem database, and the energy minimization was carried out using OpenBabel-2.4. The crystal structures of COX-1 (PDB ID: 1EQG) with resolution 2.61 Å, co-crystallized with ligand IBP (Ibuprofen) and of COX-2 (PDB ID: 5IKT) with resolution 2.45 Å, co-crystalized with ligand TLF (2-[(3-chloro-2-methylphenyl) amino] benzoic acid) were taken from Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB).[10]

Protein preparation was accomplished using UCSF Chimera alpha version 1.17. Ligands and co-crystallized water molecules were removed from the protein molecules and polar hydrogens were added. The target protein ligand docking was performed using Autodock 4.2.6 (http://autodock.scripps.edu/) software utilizing Lamarckian Genetic Algorithm (LGA). In docking simulation, centres of the grids were located at the active site of respective proteins. The grid coordinates (X, Y, Z) for COX-1 and COX-2 were (27.38, 38.28, 200.14) and (164.27, 183.32, 195.10), respectively.[11] The best possible docking interaction was investigated and 2D and 3D figures were obtained by Biovia Discovery Studio Client 2021 software.

Collection, identification, and extract preparation

The stem bark of *Oroxylum indicum* plant was purchased from an Ayurvedic Store in the local market of Varanasi and the two phytoconstituents Chrysin and Baicalein were purchased from Sigma Aldrich. The authentication of stem bark was done from the Dravyaguna Department, Banaras Hindu University, Varanasi.

Methanolic extract was prepared using the maceration method. For this, about 250 g of the crushed stem bark (solute) was taken in a stoppered flask and filled with 3 litres of ethanol (solvent). This flask was then kept at room temperature for about a period of 7 days with frequent agitation.[12] After the following days the solvent was filtered out and was heated in a round bottom flask at a temperature of 55 Celsius, until the moisture content from the extract was evaporated. This leftover of the extract was then mixed in carboxy methyl cellulose for administering. Chrysin and Baicalein were also mixed in carboxy methyl cellulose to prepare a deliverable form of phytoconstituent.

Dose determination

A pilot study was performed for dose determination in Charles Foster rats.

Based on the study of acute toxicity of *Oroxylum indicum* in animals, the lethal dose was at dose of 3000mg/Kg.[13] Three doses were calculated for each extract, and out of those the dosage that showed significant results was taken for the study in each group. For the Methanolic extract of *Oroxylum indicum* the dose selected for the rats was 300mg/kg; was administered orally to the rats, using a feeding tube for a period of 3 days prior to induction of pain and inflammation. The extract was in the form of a suspension in 0.5% Carboxy methyl cellulose. Chrysin dose selected was 40 mg/Kg; for Baicalein dose selected was 40 mg/Kg; for Morphine dose selected was 5 mg/Kg; for Indomethacin dose selected was 10 mg/Kg.

Selection of animals

The study was conducted as per CPCSEA guidelines. Adult rats of Charles Foster species of either sex weighing100gm to 150gm were purchased from the animal house of Institute of Medical Sciences, Banaras Hindu University, Varanasi. The rats were kept in polypropylene cages in the animal house of department of pharmacology, with 12/12 hours light and dark cycle at27±2 Celsius and were provided with standard environmental conditions including temperature, humidity, aeration and food.

Inclusion criteria

Normal animals of either sex weighing between 100 g to 150 g were included in the study.

Exclusion criteria

Diseased animals, pregnant rats, aged rats, or animals weighing less than 100g were excluded from the study.

Method of inflammation induction

Paw edema was induced by injecting 0.1 ml of 1% w/v carrageenan suspended in 1% CMC into the sub-plantar tissues of the left hind paw of each rat. The paw thickness was measured before injecting the carrageenan and after 60, 120, 180, 240 min. using plethysmometer. The anti-inflammatory activity was calculated as percentage inhibition of oedema in the animals treated with extract under test in comparison to the carrageenan control group.[14]

The percentage (%) inhibition of edema is calculated using the formula

The percentage (%) inhibition of edema is calculated using the formula

Where T t is the thickness of the paw of rats given test extract at the corresponding time and T o is the paw thickness of rats in the control group at the same time.

Method of pain induction

Hotplate: After maintaining the constant temperature rat is placed on the hot plate and observed for either paw licking or jumping reaction. The reaction time is recorded by a stop-watch. Repeated readings are taken at 20, 60, and 90 minutes after the drug administration. Cut off time for rat is 20-30 sec and for mice it is 15 seconds to 20 seconds.[15]

Tail-flick: Firstly, the animal is positioned into restrainer and leaving the tail exposed outside the restrainer and make the markings on the tail. Clean the tail with the help of cotton soaked in water or ethanol. Thereafter, leave the tail for drying, and then let the mouse or rat settle down in the restrainer. When the rat or mouse gets settled, keep the restrainer on the instrument called the tail flick analgesiometer. 1/3rd tail proximally should be left due to the thick and keratinized skin, and then keep the tail on the place made for it above hot wire and measure the height of the tail from the wire of the analgesiometer. The time of tail flick is measured and recorded. The cut off time is set at 15-20 sec in case of mouse whereas in the case of rat the cut off time is 20-30 sec to avoid any further injury to the tail.[16]

Blood collection

The rats were anaesthetized using pentobarbital. The blood was then obtained from the animals *via* the retroorbital route, using a thin capillary tube. 5mlof blood from each rat in each group was kept in small test tubes, properly labelled. The blood in the tubes was allowed to stand for a while. These tubes were then centrifuged at 2000rpm in a centrifuge machine for the serum to separate from the components of blood. The serum was then used to test various parameters using appropriate kits.

High-resolution mass spectrometry analyses

Qualitative analysis of phytochemicals was in given sample for High-Resolution Accurate Mass Spectrometry were acquired using Q Exactive plus from Thermo Fischer Scientific with Hypersil Gold (100 × 2.1 mm, 3.0 µm) column was used. Eluent phase consist of: Solvent A: 100% Water + 0.1% Formic Acid, Solvent B: 100% Acetonitrile + 0.1% Formic Acid, Solvent C: 100% Methanol + 0.1% Formic Acid with 0.3 mL/min of flow rate. Linear gradient elution parameters were set as follows:

Initial 0–5% B was held for 2 min and 30% B in 7 min, later gradient was linearly increased from 60% B in 15 min, and to 90% B in 23 minutes returned to 5% in 28 min and held for re-equilibration until 4 min. The injection volume was 5 µL.

The UPLC elution was exposed to Q-Exactive plus high-resolution mass spectrometer with electrospray ionization (Thermo Scientific). Full mass scan data was acquired at resolving power of 70,000. Meanwhile, high-resolution extracted ion chromatography was adopted to extract the candidates from the high-quality, accurate raw mass data both in negative and positive ion modes. Then, data mining was processed based on common biotransformation reactions as well as the reported metabolites in the literature.

RESULTS AND OBSERVATIONS

The interaction of ligand and target has been revealed with the molecular docking studies. The binding energy and the type of interaction such as Hydrogen bonding, Van der Waals, Electrostatic and Non-covalent interactions, play major roles in revealing the interaction between target and ligand molecules. Interesting results has been obtained in the docking studies of Chrysin and Baicalein with COX-1 (PDB ID: 1EQG). The hydrogen bond interaction established between the Chrysin and Baicalein with the important amino acid, includes Arg 120, Tyr 355, Ser 530, Met 522 (Figure 1). The binding free energy of the Chrysin and Baicalein with target COX-1 was found to be -7.88 and -7.26 Kcal/mol. The multiple hydrogen bonding observed between the hydroxyl group of benzene ring and amino acids Arg 120, Tyr 355, Met 522. The side chain carboxyl group present, also involved in the hydrogen bonding with some important amino acid such as, Ser 530.

The surprising result was obtained on molecular docking of Chrysin with both the receptor targets, Chrysin gives improved binding interactions in COX-1 and COX-2 with binding affinity -7.88 kcal/mol and -5.82 Kcal/mol than Baicalein with binding affinity -7.26 kcal/mol and -5.63 Kcal/mol, respectively. Among both receptor targets, Chrysin shows greater binding in COX-1 than COX-2 with inhibition constant value of 1.72 µM and 54.33 µM, respectively. Similar result was obtained for ligand Baicalein. They bound more predominantly to COX-1 with inhibition constant 4.78 µM rather than on COX-2 with 74.2 µM. The docking score of Chrysin and Baicalein on COX-1 is comparatively higher than the score obtained for the COX-2 receptor model suggestive of the fact that these ligands have suitable binding and interactions with COX-1 as compared with the COX-2. However, due to the higher number of hydrogen bonding in Baicalein and partial docking score variations among Chrysin and Baicalein with both receptors targets it was difficult to explain better suitability of the binding interactions of an individual ligand.

The binding affinities obtained from molecular docking and the values of important physicochemical parameters important for drug development have been enlisted in Table 1a and 1b. The binding interactions of the compounds with COX-1 and COX-2 are shown in Figure 1 and Figure 2, respectively. Both ligands Chrysin and Baicalein formed hydrogen bond with the Ser530 residues of COX-1 and COX-2.

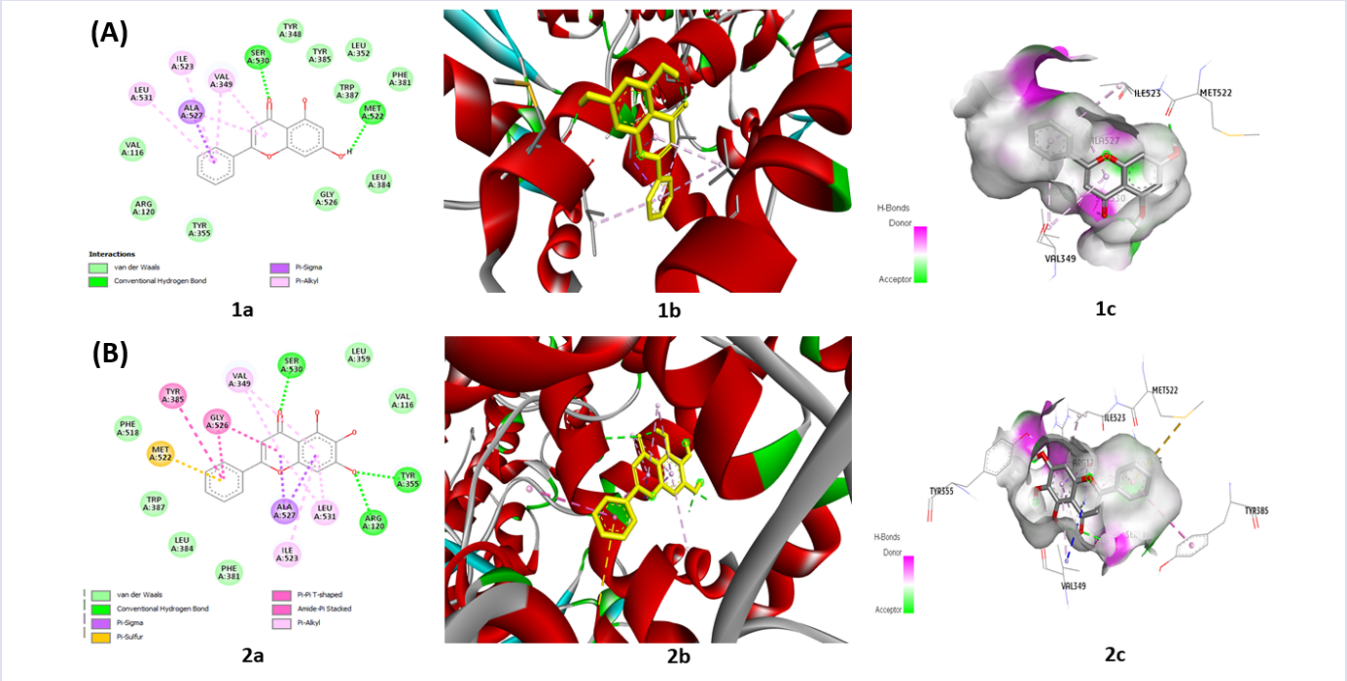


Figure 1: (A) Docking of Chrysin with COX-1 protein. Figures 1a,1b and 1c represent 2D structure, 3D structure and H-bond interacting surface, respectively. (B) Docking of Baicalein with COX-1 protein. Figures 2a, 2b and 2c represent 2D structure, 3D structure and H-bond interacting surface, respectively.

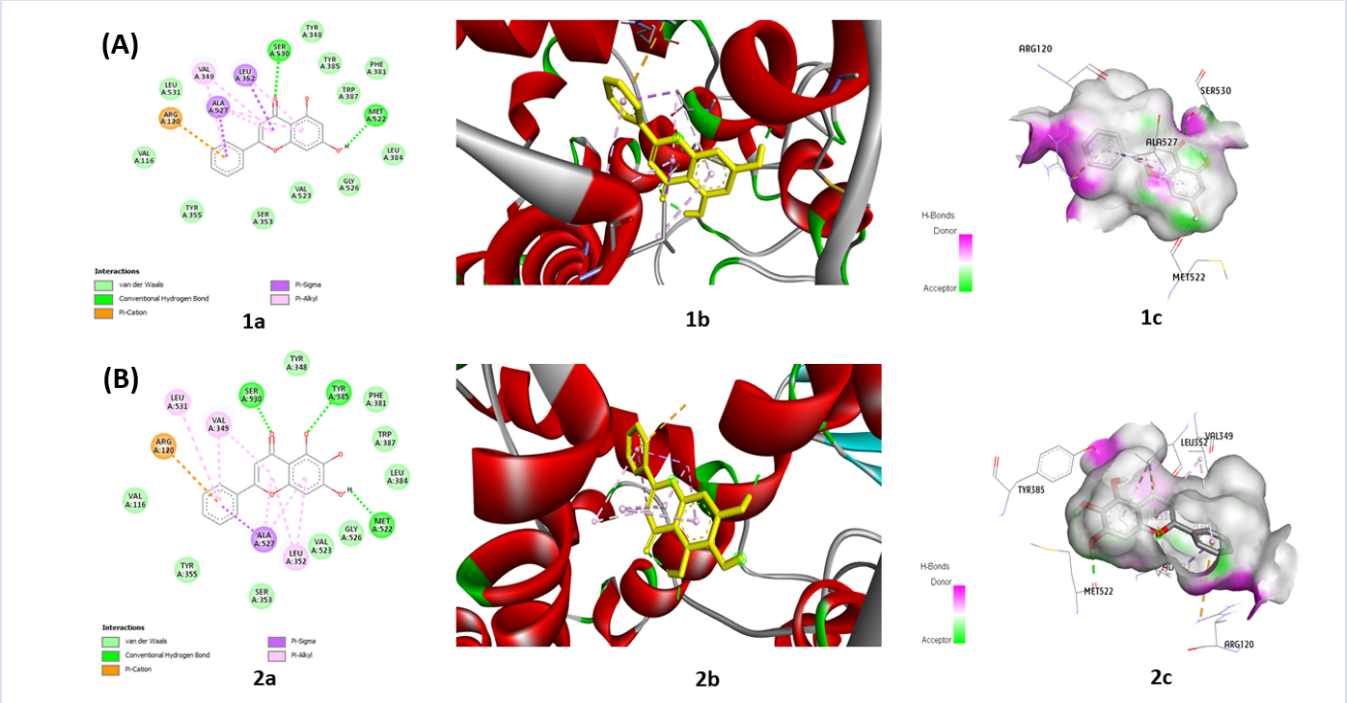


Figure 2: (A) Docking of Chrysin with COX-2 protein. Figures 1a,1b and 1c represent 2D structure, 3D structure and H-bond interacting surface, respectively. (B) Docking of Baicalein with COX-2 protein. Figures 2a, 2b and 2c represent 2D structure, 3D structure and H-bond interacting surface, respectively.

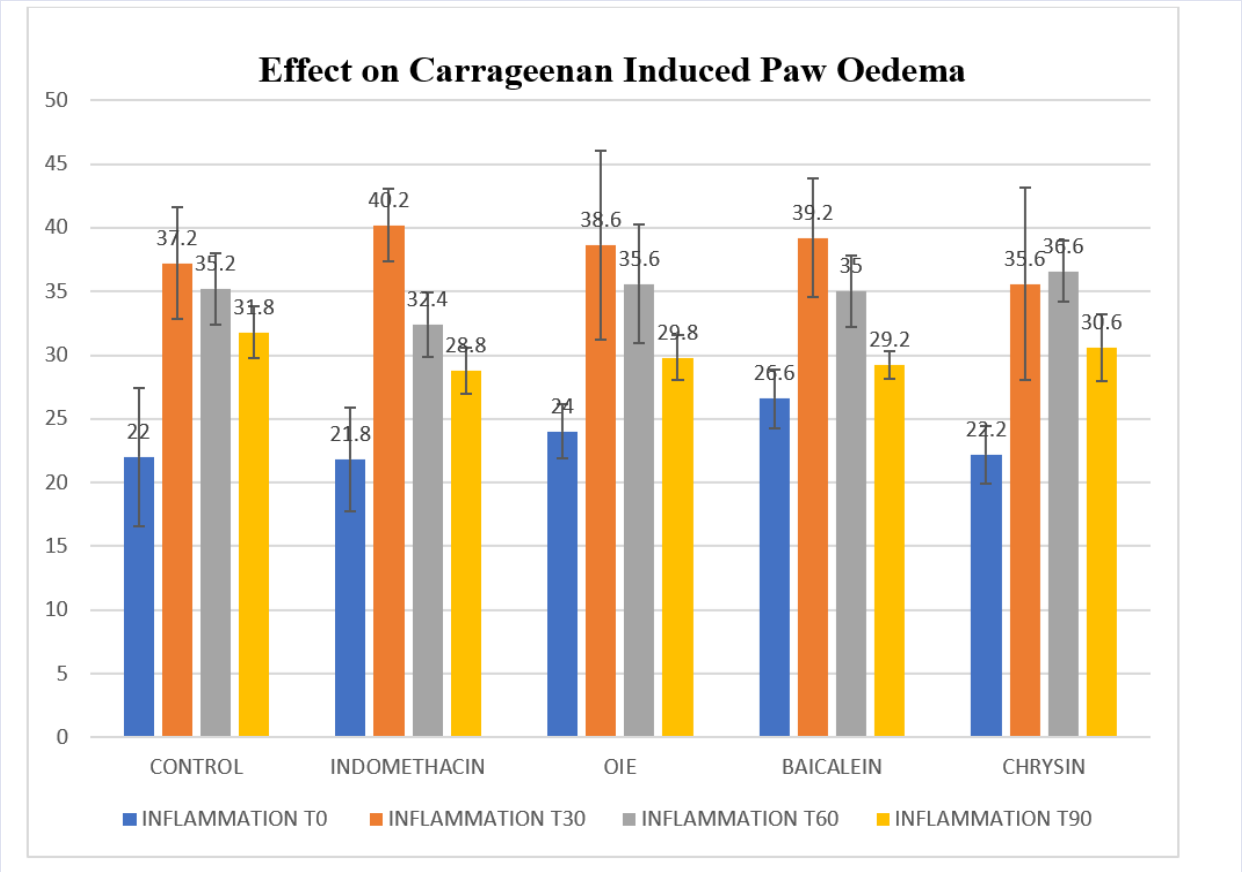


Figure 3: Estimation of paw oedema reduction
(Results are mean±SD of 5 rats in each group and compared to control group. Statistical analysis was done by one-way analysis of variance followed by Dunnet test for multiple comparisons)

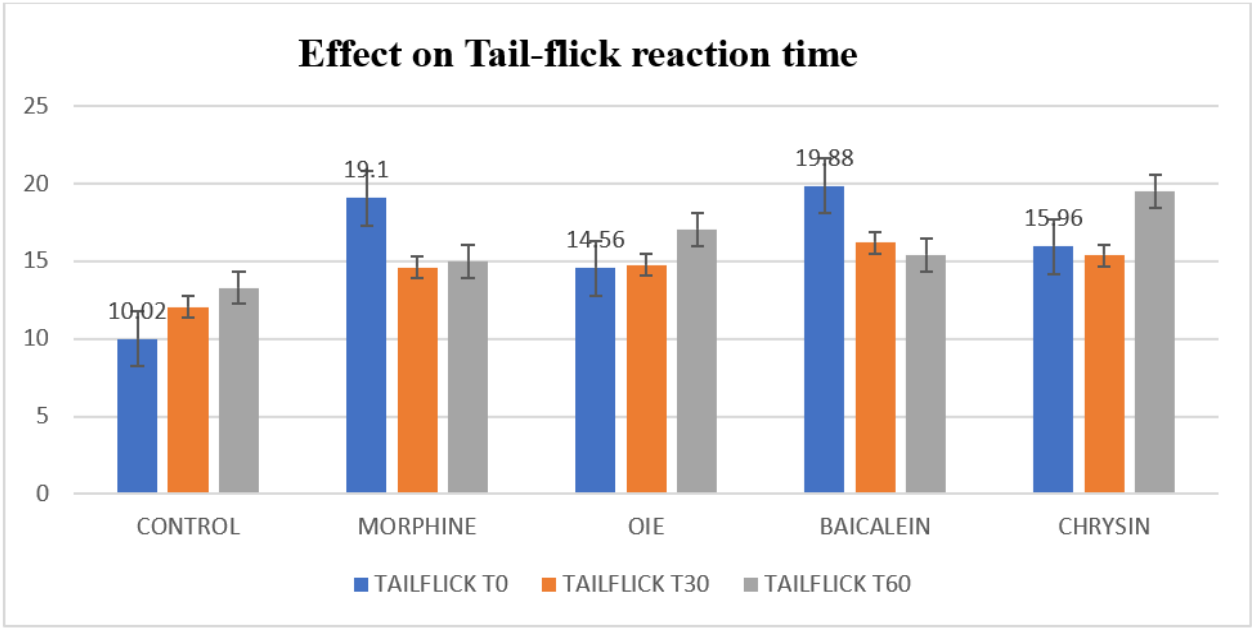


Figure 4: Estimation of Tail-flick reaction time
(Results are mean±SD of 5 rats in each group compared to the control group. Statistical analysis was done by one-way analysis of variance followed by the Dunnet test for multiple comparisons)

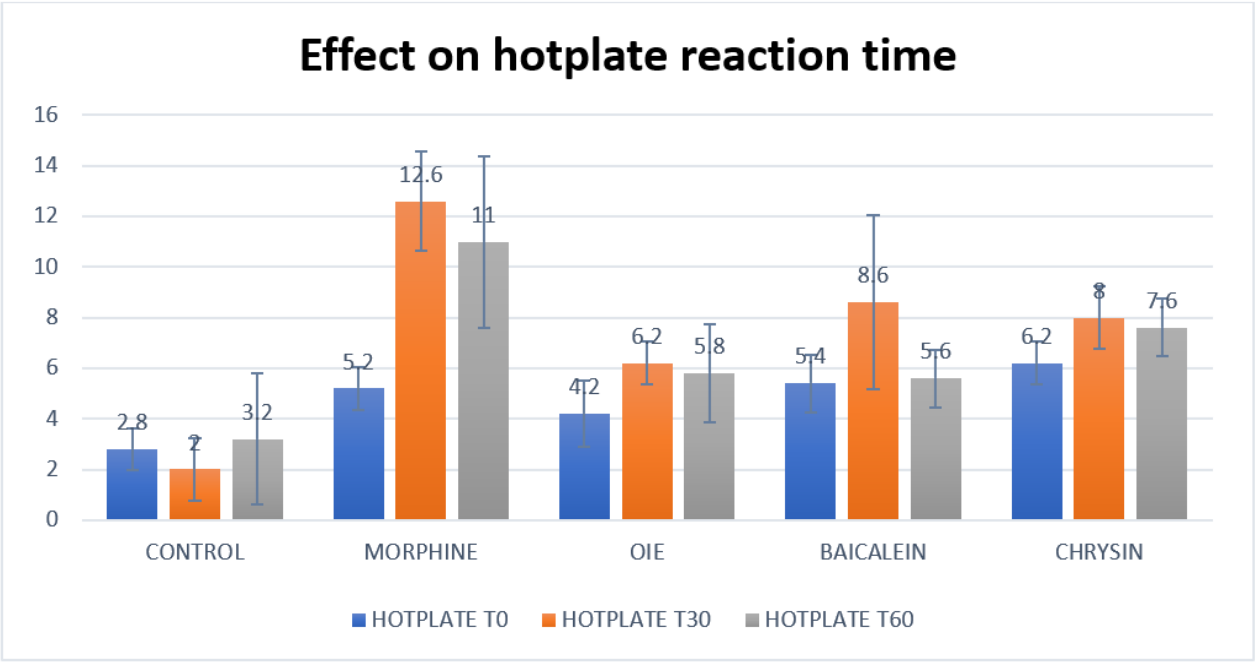
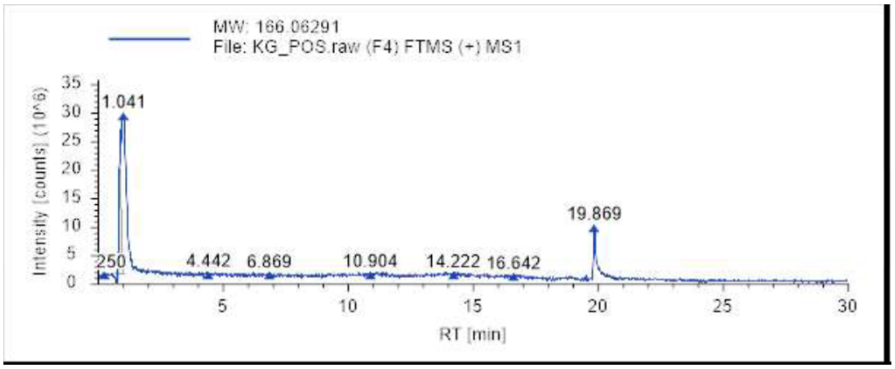
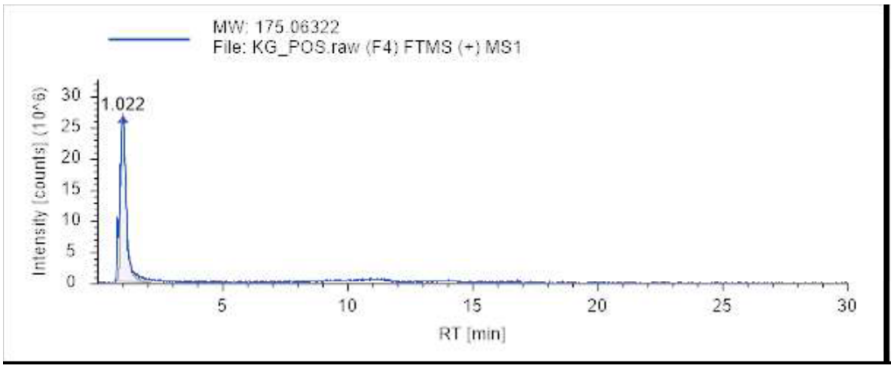


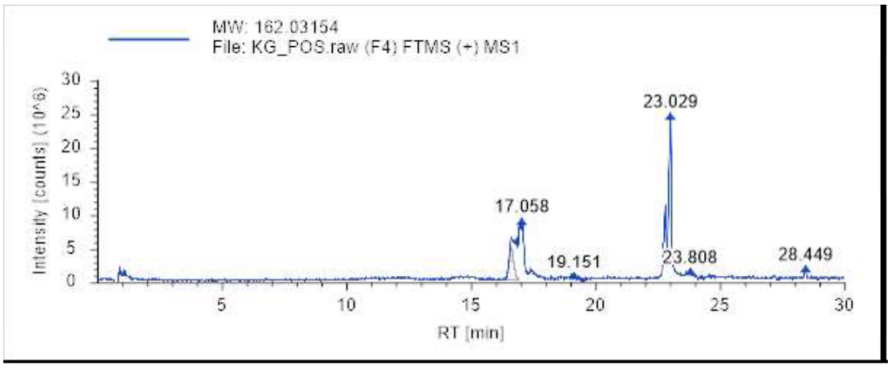
Figure 5: Estimation of hotplate reaction time
(Results are mean±SD of 5 rats in each group compared to control group. Statistical analysis was done by one-way analysis of variance followed by Dunnet test for multiple comparisons)



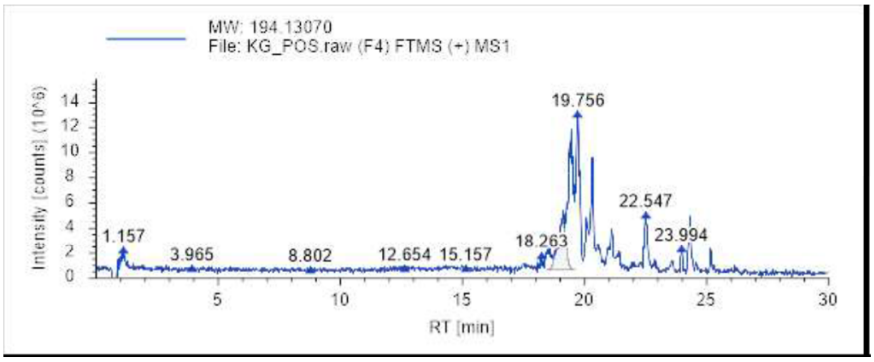
Apocynin



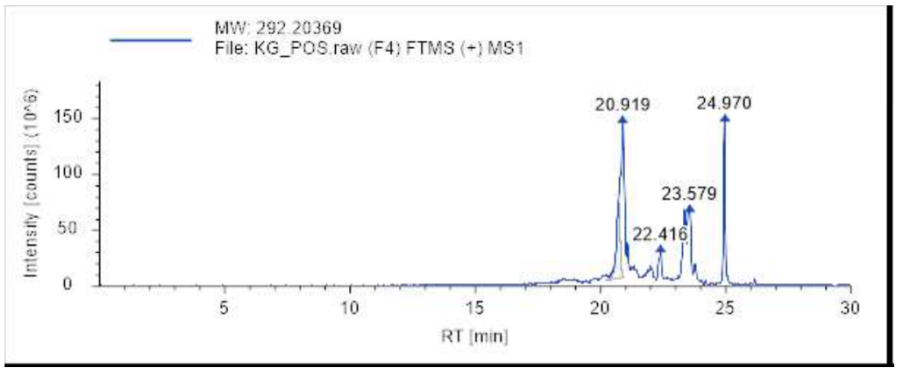
Indole-3-acetic acid



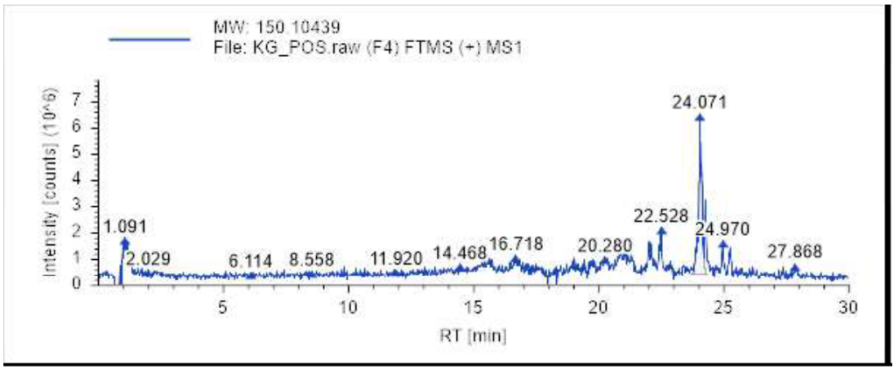
7-Hydroxycoumarine



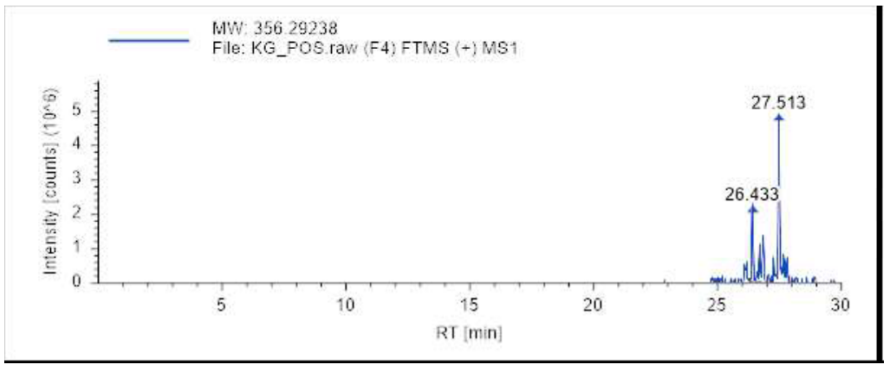
Sedanolide



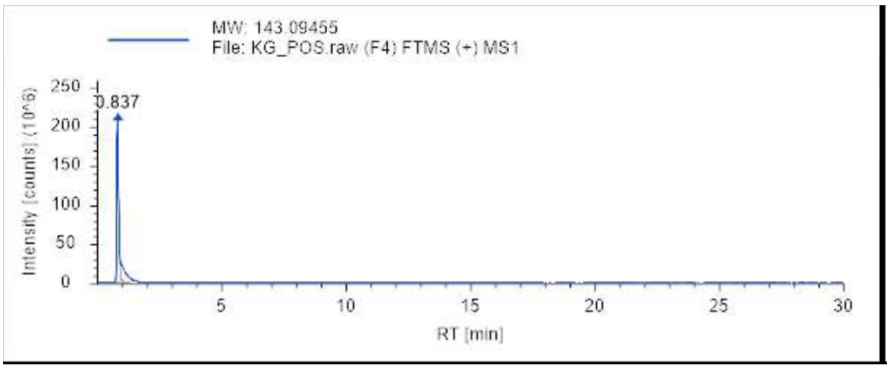
12-Oxo phytodienoic acid



Carvone



Monoolein



DL-Stachydrine

Figure 6

Table 1a: Binding energy (Kcal/mol) and Inhibition constant (μM) of the docked ligands with COX-1 and COX-2 receptor.

Compounds	Binding affinity (Kcal/mol)		Inhibition constant (μM)	
	COX-1	COX-2	COX-1	COX-2
Chrysin	-7.88	-5.82	1.72	54.33
Baicalein	-7.26	-5.63	4.78	74.2

Table 1b: Hydrogen bonding analysis with respect to COX-1 and COX-2 receptor model.

Receptor model					
COX-1 receptor			COX-2 receptor		
Amino acid residues	Number of Hydrogen bonds		**Amino acid residues**	Number of Hydrogen bonds	
	Chrysin	**Baicalein**		**Chrysin**	**Baicalein**
SER 530	1	1	**SER 530**	1	1
TYR 355	0	1	**TYR 385**	0	1
ARG 120	0	1	**MET 522**	1	1
MET 522	1	0			

Table 2: Comparison of serum TNF-α.

CONTROL RATS (0.5% CMC)	OIE	BAICALEIN	CHRYSINE	INDOMETHACIN
28.38±4.28	18.55±1.81	23.36±0.82	25.41±1.03	17.1±0.98

Table 3: Estimation of Serum IL6.

CONTROL RATS (0.5% CMC)	OIE	BAICALEIN	CHRYSINE	INDOMETHACIN
408.83±2.31	354±5.51	319.6±4.84	321.8±2.78	315.8±0.98

(Results are mean±SD of 5 rats in each group compared to the control group. Statistical analysis was done by one way analysis of variance followed by the Dunnet test for multiple comparisons)

Ser530 residue is very crucial for initiation of prostaglandin synthesis and is acetylated by aspirin exerting analgesic effect.[2]Chrysin, another important flavonoid, showed a comparable binding affinity of -7.88 kcal/mol with COX-1 active site forming hydrogen bonding with Ser530 and Met522 residues of COX-1. Both Chrysin and Baicalein showed preferential binding with COX-1 than COX-2 with a binding affinity of -7.88 kcal/mol and -7.26 kcal/mol forming hydrogen bonding with Ser530 residue. Efficient binding of the flavonoids Baicalein and Chrysin can partly explain why alcohalic fraction showed potent analgesic activity. However, further compound specific *in vivo*, *in vitro* and *in silico* studies can establish the exact mechanism of analgesic activity more clearly.

At T = 0, the mean baseline reduction in paw volume in Baicalein group shows the maximum effect followed by OIE, Chrysin. Reduction in the volume is more marked as compared to Indomethacin and Control. At T = 30, the mean baseline reduction in paw volume in the Indomethacin group shows marginally higher effect than Baicalein followed by OIE and Chrysin. The reduction in the volume is more marked as compared to Control. At T = 60, the mean baseline reduction in paw volume in the Chrysin group shows maximum effect, followed by Baicalein, OIE. Reduction in the volume is more marked as compared to Indomethacin and Control. At T = 90, the mean baseline reduction in paw volume in all the groups shows a majorly similar effect in Baicalein, OIE, Chrysin.

There is marked reduction in the TNF Alpha expression in the OIE group which is followed by Baicalein and Chrysine. Less TNF Alpha expression implies the anti-inflammatory property of OIE significantly.

At T = 0, the mean baseline increases in reaction time for flicking the tail is in Baicalein group, followed by Morphine, Chrysin, OIE. Increase in reaction time was maximum in the Baicalein group. At T = 30, the mean baseline increases in reaction time for flicking the tail is in Baicalein group, followed by Chrysin, OIE, Morphine. Increase in reaction time was maximum in the Baicalein group. At T = 60, the mean baseline increases in reaction time for flicking the tail is in Chrysine group followed by the OIE, Baicalein, Morphine. The Increase in reaction time was maximum in the Chrysine group. The Baicalein group shows the most marked cumulative increase in reaction time for tail flick among all the groups of interventions.

At T = 0, the mean baseline increase in reaction time is in Chrysine group followed by Baicalein, Morphine, OIE. Increase in reaction time was maximum in the Chrysine group.

At T = 30, the mean baseline increase in reaction time is in Morphine group followed by Baicalein, Chrysin, OIE. Increase in reaction time was maximum in the Morphine group.

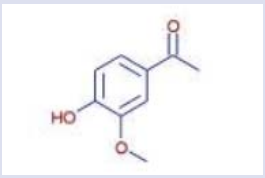
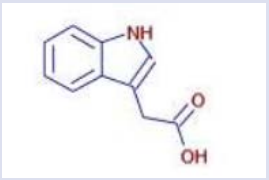
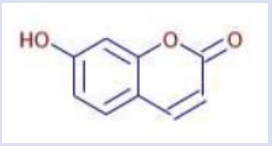
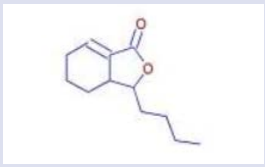
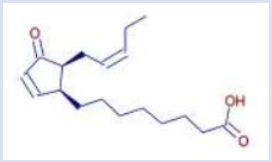
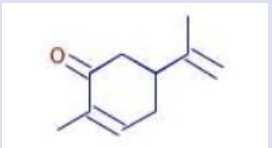
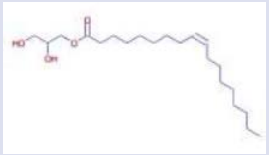
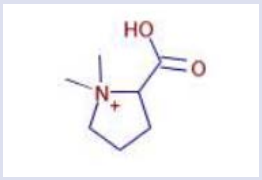
At T = 60, the mean baseline increase in reaction time is in Morphine group followed by Chrysin, OIE, Baicalein. The Increase in reaction time was maximum in the Morphine group.

The Baicalein group shows the most marked cumulative increase in reaction time for tailflick among all the groups of intervention group followed by Chrysine and OIE.

DISCUSSION

The carrageenan-induced paw edema is a distinct model of acute phase inflammation in which a variety of inflammatory mediators are involved in its development and it has wildly been used to evaluate the anti-inflammatory effect of natural plant products and their phytoconstituents. In the present study, we demonstrated that OIE, Chrysine, Baicalein showcased anti-inflammatory effects in carrageenan-induced rat paw edema in a dose dependent manner. Our results also established earlier findings that OIE, Chrysine, Baicalein exhibit a perceptible anti-inflammatory effect in experimental models.[17]

Table 4: Phytoconstituent of *Oroxylum Indicum* showing analgesic and antinflammtory property.

Name	Formula	RT [min]	Area (Max.)	References
Apocynin	C9 H10 O3 	0.897	122917678.9	Ref.26,27
Indole-3-acetic acid	C10 H9 N O2 	1.022	413691427.6	Ref.28, 29
7-Hydroxycoumarine	C9 H6 O3 	16.63	59274229.95	Ref.30
Sedanolide	C12 H18 O2 	19.138	90799509.02	Ref.31,32
12-Oxo phytodienoic acid	C18 H28 O3 	20.745	730448155	Ref.33,34
Carvone	C10 H14 O 	24.091	56671807.57	Ref.35,36,37
Monoolein	C21 H40 O4 	26.438	9873727.407	Ref.38
DL-Stachydrine	C7 H13 N O2 	0.829	1188219065	Ref.39,40,41

On the other hand, it is well characterized that neutrophil infiltration plays a key role in the inflammation induced by carrageenan in hind paw.[18] Our results indicated that all the three i.e. OIE, Chrysine, Baicalein elicited a marked reduction in the infiltration of acute phase reactants into the carrageenan-treated paws.

The results of the present study are comparable with the previous study of Ahmad *et al*. and Gepdiremen *et al*. We propose in the present work, that the presence of flavonoids and tannins in the OIE extract has helped in reduction of inflammation.[19,20]

A growing line of evidence has demonstrated that flavonoids, phenolic acids, and triterpenoid possessed antinociceptive and anti-inflammatory effects in animal models.[21] Studies have also reported that flavonoids such as rutin, quercetin, luteolin produced significant antinociceptive and anti-inflammatory activities.[22] Hence, it is proposed that the anti-inflammatory activities of OIE, Chrysine, Baicalein may be related to their phenolic content.

Here in this study, we estimated the serum TNF- α of Charles foster rat who were administered OIE, Chrysine, Baicalein. Previous studies have demonstrated that the level of TNF-α is closely associated with the pathophysiology of inflammation and pain. In the present study, decrease in serum TNF-α is associated with reduction in pain and inflammation. TNF-α is the most-studied cytokine and it is an important pro-inflammatory cytokine with a major role in the regulation of cellular processes.[23]

Previous studies have shown that TNF-α induces a pro-inflammatory response in the tissue with elevated TNF-α levels. So, in the current study, the significant (p<0.05) decrease in the gene expression of TNF-α in the Charles foster rats can be attributed to decrease in inflammation and an increase in the reaction time for hotplate and tail-flick.

In this study, OIE at 300mg/kg; Chrysine at 40mg/Kg; and Baicalein at 40mg/kg doses showed significant increase in the threshold of pain at all time intervals in both the hot plate model and tail flick model of acute pain as compared to carboxy methylcellulose group. We propose based on the results of studies, that the extract of OIE is as efficient as that of the plant extract of Lactucascariola and Artemisia absinthium.[24,25]

One of the compounds in *Oroxylum Indicum* at Rt: 0.897 was apocynin. Based on research conducted *in vitro* and *in vivo* through a variety of molecular mechanisms, apocynin exerts anti-inflammatory actions that can reduce or even prevent inflammatory diseases. In a study by Boshtam M, Kouhpayeh S apocynin inhibits NADPH oxidase (NOX) in phagocytic cells while inducing the generation of reactive oxygen species (ROS) in non-phagocytic cells.[26] Apocynin treatment considerably reduced inflammatory foci and erosions, according to a histopathologic investigation. Colonic expression of iNOS, COX-2, TNF-, and MCP-1 was markedly reduced in the group treated with apocynin. In mice treated with apocynin, the anti-inflammatory mediators Nrf2 and HO-1 were markedly activated. Against chemically induced colonic inflammation, apocynin demonstrated notable anti-inflammatory activity.[27]

Indole-3-acetic acid (IAA). In a study by Ji Y, Yin W, IAA dramatically reduced the amount of LPS-induced IL-1, IL-6, and monocyte chemoattractant protein-1 (MCP-1) expression as well as the production of reactive oxygen species (ROS) and nitric oxide (NO). Nuclear factor kappa B (NF-B) p65 nuclear translocation caused by LPS was inhibited by IAA therapy. Additionally, under both basal and LPS-stimulated conditions, IAA-treated cells showed a dose-dependent up-regulation of heme oxygenase-1 (HO-1) activity.[28,29] IAA detected in *Oroxylum Indicum* at Rt.1.022 which we propose to be responsible for anti-inflammatory activity of OIE.

The phenol compounds in *S. repanda* crude extract. 7-Hydroxycoumarine, phytocompounds alone with their favorable pharmacokinetics profile suggests good lead and efficiency of *S. repanda* with no toxicity risks.[30] An extract of *Apium graveolens* var. dulce leaves shows various antinflmmatoty property. Sedanolide is one of the compounds present in it which is also present in *Oroxylum indicum* detected at Rt: 19.138. In a study by Zhang YY 12-Oxo phytodienoic acid attenuates inflammation by inhibiting mPGES-1 and modulating macrophage polarization *via* the NF-κB and Nrf2/HO-1 pathway and could be a promising candidate for developing anti-inflammatory drugs. 12-Oxo phytodienoic acid was detected on OIE at Rt.20.745. Carvone shows its antinflammtory action by inhibiting of NO, PGE2 and COX-2 and iNOS expression, also shows decreased MDA levels and increased GSH content.[25] Monoolein, isolated from Ishige sinicola, inhibits lipopolysaccharide-induced inflammatory response by attenuating mitogen-activated protein kinase and NF-κB pathways.[38] Stachydrine and Sakuranetin against the inflammatory target proteins IL-6 and TNF-α by using molecular docking analysis, Stachydrine possessed high and specific inhibitory activity on tumor necrosis factor-α and interleukin-6.[39-41] Carvone, Monoolein, Stachydrine is detected in OIE extract at Rt. 24.091, 26.438, 0.829.Presence of various anti-inflammatory compounds and their inhibitory activity against NO, PGE2, IL6, TNF-alpha clearly explain mechanism by which *Oroxylum Indicum* shows antiinflammtory activity.

CONCLUSION

The present study was aimed evaluating and validating the ayurvedic literature for the anti-inflammatory and analgesic property of *Oroxylum indicum* and its two phytoconstituents Chrysine and Baicalein by using scientifically accepted and proven models of inflammation and pain in Charles foster rats. The results of the present work were evaluated by the mechanical model of Tail-flick and Hotplate for pain and carrageenan induced paw oedema and serum concentrations of TNF Alpha and IL6 for inflammation.

The pain model successfully showed an increase in reaction time thereby validating the analgesic activity of methanolic extract *Oroxylum indicum* stem bark and its two constituents Baicalein and Chrysine. The inflammation model also showed a significant drop in TNF Alpha, IL6 gene expression and reduction in paw volume which validates the anti-inflammatory activity of a methanolic extract *Oroxylum indicum* stem bark and its two constituents Baicalein and Chrysine. With binding affinities of -7.88 kcal/mol and -7.26 kcal/mol, respectively, and Ser530 residue hydrogen bonds, both Chrysin and Baicalein demonstrated preferential interaction with COX-1 over COX-2. *Oroxylum indicum's* anti-inflammatory properties can be explained by the presence of several substances with inhibitory effect against inflammatory mediators like nitric oxide (NO), prostaglandin E2 (PGE2), interleukin 6 (IL-6), and tumour necrosis factor alpha (TNF-alpha).

AUTHOR CONTRIBUTIONS

Conceptualization BP, KG, KP and methodology BP, KG, MS, KP, and formal analysis BP, KG, KP, and.; investigation BP, KG, KP; writing-original draft preparation BP, KG, KP, MS and RG; writing-review and editing, visualization AT, AS, KG, BP, RG, KP. All authors have read and agreed to the published version of the manuscript.

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DATA AVAILABILITY STATEMENT

HRMS data is shared as supplementary file.

All authors agree with MDPI Research Data Policies.

INSTITUTIONAL REVIEW BOARD STATEMENT

This project is approved by Institutional Animal Ethical committee. Letter No. 542/GO/ReBi/S/02/CPCSEA.

INFORMED CONSENT STATEMENT

Not applicable

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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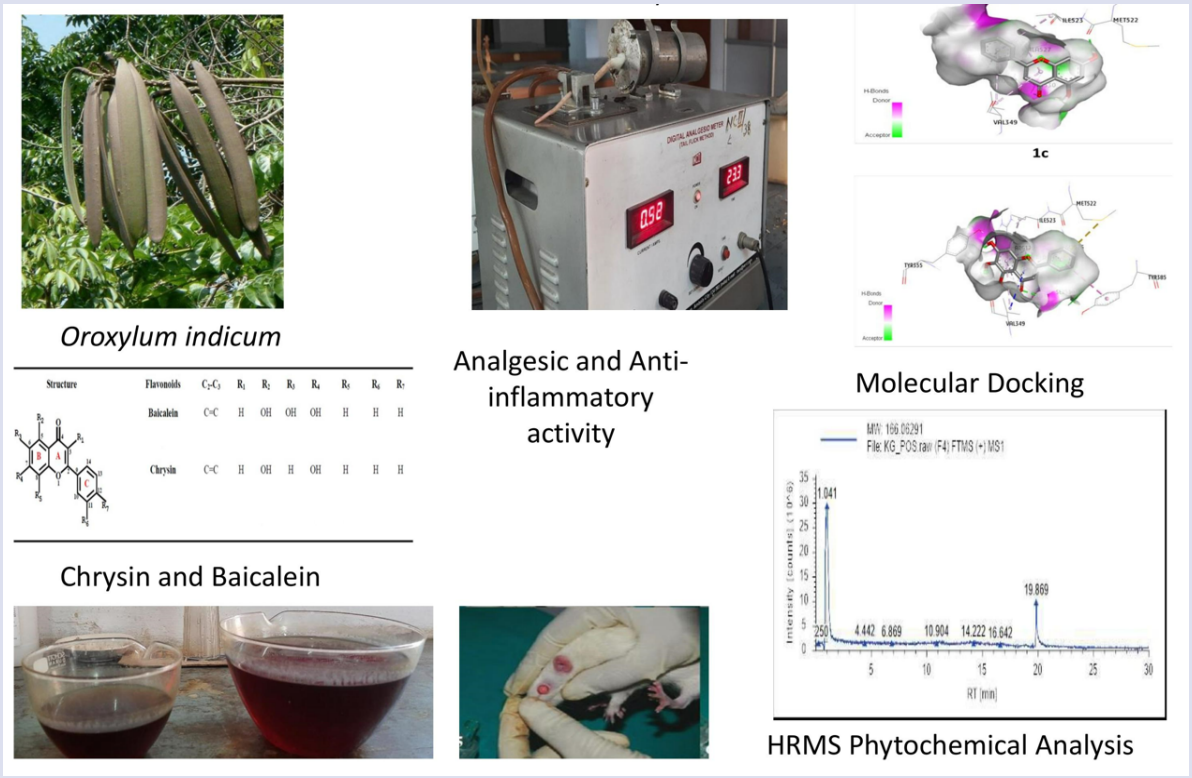
GRAPHICAL ABSTRACT



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