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Original Article

Ayurveda Section

Assessment of Comparative COX-1 and COX-2 Inhibition Efficacy of *Ehretia Laevis* Roxb. (*Khandu Chakka/Ajan Vruksha*) Leaves versus Diclofenac Sodium: An In-vitro Study

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ABSTRACT

Introduction: Pain is a significant problem globally. *Ehretia laevis* Roxb has pain-relief and anti-inflammatory properties. Cyclooxygenase (COX) is responsible for the production of prostaglandins, which control pain and inflammation. Nonsteroidal anti-inflammatory drugs (NSAIDs) reduce pain and inflammation by acting on COX.

Aim: To assess the comparative COX-1 and COX-2 inhibition efficacy of *Ehretia laevis* Roxb leaves versus diclofenac sodium.

Materials and Methods: This study is an analytical, experimental in-vitro study. Which was performed at Sciore Research Private Limited and affiliated with Bajaj College of Science, Wardha, and Mahatma Gandhi Ayurved College, Hospital, and Research Centre at Datta Meghe Institute of Higher Education and Research, Wardha, Maharashtra, India from May 2023 to June 2023. The study was performed according to the manufacturer's protocol from Cayman Chemical (item No: 701070) and the standard operating procedures of the test facility. For in-vitro processing, *Ehretia laevis* Roxb powder was mixed with a 10% Dimethyl Sulfoxide (DMSO) solution to obtain a concentration of 1 mg/mL and filtered. All assays were performed in triplicate. GraphPad Prism (Version 8.4.2) was used to calculate the IC50 values by plotting log (inhibitor) vs. normalised response/variable slope.

Results: Diclofenac sodium was a significantly more potent inhibitor of both COX-1 and COX-2 enzymes compared to *Ehretia laevis* Roxb powder. To achieve 50% inhibition of COX-1, diclofenac sodium required only 59.49 nanograms per milliliter (ng/mL), whereas *Ehretia laevis* Roxb powder needed a significantly higher dose of 301.6 micrograms per milliliter (µg/mL). This indicates that diclofenac sodium is roughly five times more potent for COX-1 inhibition. Similarly, for COX-2 inhibition, diclofenac sodium had an IC50 value of 14.23 ng/mL, while *Ehretia laevis* Roxb powder had an IC50 value of 245.0 µg/mL, indicating that diclofenac sodium is approximately 17 times more potent for COX-2 inhibition. Overall, the data suggested that diclofenac sodium has a stronger and more selective inhibitory effect on both COX-1 and COX-2 enzymes compared to *Ehretia laevis* Roxb powder.

Conclusion: The IC50 values for diclofenac sodium for COX-1 inhibition is 59.49 ng/mL and for COX-2 inhibition is 14.23 ng/mL. The IC50 value for *Ehretia laevis* Roxb for COX-1 inhibition is 301.6 µg/mL and for COX-2 inhibition is 245.0 µg/mL. Various extracts of *Ehretia laevis* Roxb should be tested further for their COX inhibition activities.

Keywords: Cyclooxygenase, Gastrointestinal health, Inflammation, Non-steroidal anti-inflammatory drugs, Pain

INTRODUCTION

Ehretia laevis Roxb. has analgesic and anti-inflammatory properties [1]. It contains chemical compounds that exhibit analgesic, antinociceptive, and anti-inflammatory activities [2]. While *Ehretia laevis* Roxb. shows analgesic and anti-inflammatory effects, its activity has not been compared to that of NSAIDs. The analgesic effect is ultimately based on the inhibition of COX-1 and COX-2. To avoid the side effects of modern medicine, many herbal preparations are used for pain relief and inflammation management. However, there is limited reliable data on the pain-relieving and anti-inflammatory properties of herbal preparations to understand their exact mechanism of action. *Ehretia laevis* Roxb. demonstrates analgesic and anti-inflammatory properties when used both topically and internally, but few studies have investigated these properties comprehensively.

Inflammation and pain are regarded as two of the most prevalent and significant issues affecting people in their daily lives. They are considered key indicators of many illnesses. Consequently, numerous medications and methods for pain management have been discussed since ancient times. Non-Steroidal anti-inflammatory drugs (NSAIDs) function by preventing the production of prostaglandins, a class of chemicals involved in pain and inflammation. In contrast, opioids function differently; they mask the sensation of pain rather than combating it. Paracetamol inhibits COX, affecting the central and peripheral nervous systems in distinct ways.

COX enzymes produce prostaglandins, which regulate pain and inflammation. By acting on COX, NSAIDs effectively relieve these conditions. Two main types of COX enzymes exist: COX-1 and COX-2. COX-1 is present in various human tissues, including the gut, where it helps to protect the stomach from fluids and regulates intestinal function. It also plays a role in renal and platelet functions. In contrast, COX-2 is frequently found in inflammatory and painful areas.

Since COX-1 regulates the kidneys, platelets, and digestive tract, inhibiting it can lead to several negative effects. Therefore, it is often recommended to use COX-2 inhibitors to alleviate pain and inflammation. Celecoxib is a selective COX-2 NSAID, while ibuprofen, naproxen, ketorolac, and indomethacin are non-selective. Diclofenac sodium sodium and meloxicam are unclassified anti-COX medications.

Cell membranes consist of a phospholipid bilayer, and cell damage from infection or injury can cause them to rupture. When this happens, phospholipases are activated, converting some phospholipids into prostaglandins, which results in fever, inflammation, and pain. Arachidonic acid, present in cell membranes, is released from phospholipid membranes by phospholipase A2. This acid can enter the body through either the lipoxygenase pathway or the COX pathways

(COX-1 and COX-2), leading to the production of prostaglandin G-2 and H2. Prostaglandins, thromboxane, and prostacyclins can cause fever, inflammation, and pain, while thromboxane leads to platelet aggregation. Leukotrienes, derived from the lipoxygenase pathway, induce smooth muscle contraction and bronchospasm [3-4].

Study objective: The objectives of the study were to:

- Assess the inhibitory activity of *Ehretia laevis* Roxb. leaves on COX-1
- Assess the inhibitory activity of diclofenac sodium on COX-1
- Compare the inhibitory activities of *Ehretia laevis* Roxb. leaves and diclofenac sodium on COX-1
- Assess the inhibitory activity of *Ehretia laevis* Roxb. leaves on COX-2
- Assess the inhibitory activity of diclofenac sodium on COX-2
- Compare the inhibitory activities of *Ehretia laevis* Roxb. leaves and diclofenac sodium on COX-2

MATERIALS AND METHODS

This study is an analytical, experimental in-vitro study conducted at Sciore Research Private Limited, Plot No. 40, above Jai Bhavani Mata, Engitech, Sector 10, MIDC, Bhosari, Pune, Maharashtra 411026 from May 2023 to June 2023. The study was performed according to the manufacturer's protocol and standard operating procedures provided by Cayman Chemical (Item No: 701070) [5-8].

Study Procedure

Sample preparation: For in-vitro processing, *Ehretia laevis* Roxb. powder was mixed with a 10% DMSO solution to obtain a concentration of 1 mg/mL and filtered. Different test substances were prepared for the study as described below.

Experimental procedures:

COX-1 Inhibition Activity (Cayman Chemical; Item No: 701070)

To prepare the tubes, 160 µL of 1X reaction buffer, 10 µL of heme, and 10 µL of inactive COX-1 (provided in the kit) were added. COX-1 100% initial activity tubes were prepared by adding 160 µL of 1X reaction buffer, 10 µL of heme, and 10 µL of COX-1 (provided in the kit). A 10 µL sample of the inhibitor (test item/standard) was added to the COX-1 inhibitor tubes, while 10 µL of the inhibitor vehicle was added to the 100% initial activity and background tubes. The tubes were incubated at 37°C for 10 minutes. The reaction was initiated by adding 10 µL of arachidonic acid to all reaction tubes, which were then quickly mixed and incubated for exactly 30 seconds at 37°C.

To stop enzyme catalysis, 30 µL of saturated Stannous Chloride solution was added to each reaction tube. The tubes were vortexed and incubated for five minutes at room temperature. The prostaglandins were quantified using the ELISA method as described in the kit manufacturer's protocol. The %B/B0 (% Sample Bound/Maximum Bound) was calculated to evaluate the binding of the inhibitor to the enzyme. The average nonspecific binding (NSB) absorbance was subtracted from the sample absorbance, divided by the corrected B0, and then multiplied by 100 to obtain %B/B0.

COX-2 Inhibition Activity (Cayman Chemical; Item No: 701080)

Background tubes were prepared by adding 160 µL of 1X reaction buffer, 10 µL of heme, and 10 µL of inactive COX-2 (provided in the kit). COX-2 100% initial activity tubes were prepared by adding 160 µL of 1X reaction buffer, 10 µL of heme, and 10 µL of COX-2 (provided in the kit). A 10 µL sample of the inhibitor (test item/standard) was added to the COX-2 inhibitor tubes, while 10 µL of the inhibitor vehicle was added to the 100% initial activity and background tubes. The tubes were incubated at 37°C for 10 minutes. The reaction was initiated by adding 10 µL of arachidonic acid to all reaction tubes, which were then quickly mixed and incubated for exactly 30 seconds at 37°C. To stop enzyme catalysis, 30 µL of saturated Stannous Chloride solution was added to each reaction tube. The tubes were vortexed and incubated for five minutes at room temperature. The prostaglandins were quantified using the ELISA method as described in the COX-1 and COX-2 (human) inhibitor screening assay kit (Cayman Chemical, Item No: 701070 and Item No: 701080) [9]. The %B/B0 (% Sample Bound/Maximum Bound) was calculated to evaluate the binding of the inhibitor to the enzyme. The average NSB absorbance was subtracted from the sample absorbance, divided by the corrected B0, and then multiplied by 100 to obtain %B/B0 [5-8].

STATISTICAL ANALYSIS

All assays were performed in triplicate. GraphPad Prism (Version 8.4.2) was used to calculate the IC50 values by plotting log (inhibitor) versus normalized response/variable slope. Descriptive statistics were employed to present the data.

RESULTS

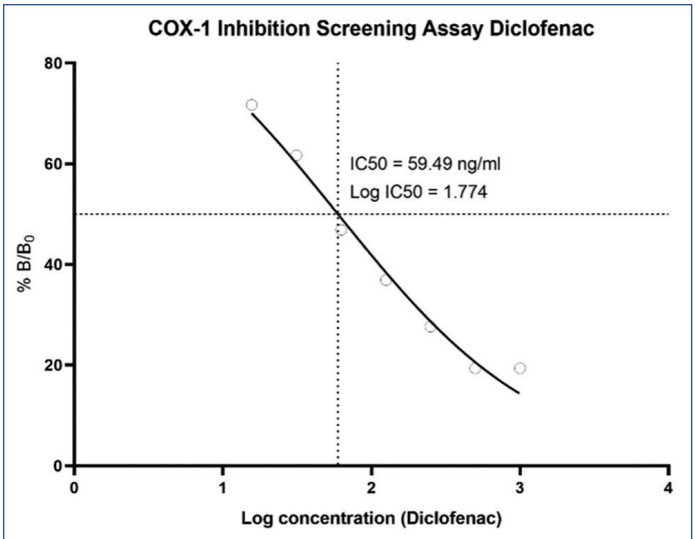
COX-1 inhibition activity: Across all tested doses, diclofenac sodium exhibits a consistently lower level of COX-1 inhibition compared to *Ehretia laevis* Roxb. powder. Diclofenac sodium displays a more gradual decrease in inhibition with increasing doses. The inhibition percentages remain around 47% at lower doses (15.6 ng/mL and 31.3 ng/mL) and then gradually decline to around 19% at higher doses (500 ng/mL and 1000 ng/mL) as per [Table/Fig-1,2].

High inhibition at lower doses: *Ehretia laevis* Roxb. powder shows a strong inhibitory effect on COX-1 at lower doses (15600 ng/mL and 31300 ng/mL), with inhibition percentages exceeding 83%. There is a clear trend of decreasing inhibition with increasing doses. At the highest dose (1000000 ng/mL), the inhibition drops to 27.6%, which is a significant decrease compared to the lower doses, as per [Table/Fig-3,4].

COX-2 inhibition activity: Diclofenac sodium exhibits a consistently lower level of COX-2 inhibition compared to *Ehretia laevis* Roxb. powder across all tested doses. The inhibition percentages for diclofenac sodium gradually decrease with increasing doses, starting around 51% at the lowest dose (15.6 ng/mL) and dropping to around 15% at the highest dose (1000.0 ng/mL), as per [Table/Fig-5,6].

Dose (ng/mL)	Log Dose	Absorbance			Average	Corrected			%B/B0		
NSB	-	0.003	0.002	0.004	0.003	-	-	-	-	-	-
B0	-	0.962	0.958	0.960	0.960	-	-	-	-	-	-
15.6	1.19382	0.691	0.696	0.687	0.691	0.688	0.693	0.684	71.669	72.192	71.276
31.3	1.49485	0.590	0.599	0.597	0.595	0.587	0.596	0.594	61.146	62.083	61.875
62.5	1.79588	0.454	0.455	0.450	0.453	0.451	0.452	0.447	46.933	47.064	46.541
125.0	2.09691	0.358	0.359	0.354	0.357	0.355	0.356	0.351	36.987	37.118	36.594
250.0	2.39794	0.270	0.269	0.266	0.268	0.267	0.266	0.263	27.826	27.695	27.433
500.0	2.69897	0.192	0.186	0.188	0.189	0.189	0.183	0.185	19.711	19.057	19.319
1000.0	3	0.192	0.186	0.188	0.189	0.189	0.183	0.185	19.711	19.057	19.319

[Table/Fig-1]: COX-1 inhibition: Diclofenac sodium.



[Table/Fig-2]: COX-1 Inhibition: Diclofenac sodium.

steeper decrease in inhibition with increasing doses, as per [Table/Fig-1-8]. Even though *Ehretia laevis* Roxb. powder might have a higher inhibitory effect at some doses, the required amount could be impractical for therapeutic use. The interaction of *Ehretia laevis* Roxb. powder with COX enzymes could reveal ways to improve its potency and reduce the required dose for both COX-1 and COX-2 inhibition. Future research could investigate whether alternative extraction methods or formulations could enhance the potency of *Ehretia laevis* Roxb. powder, potentially reducing the required dosage.

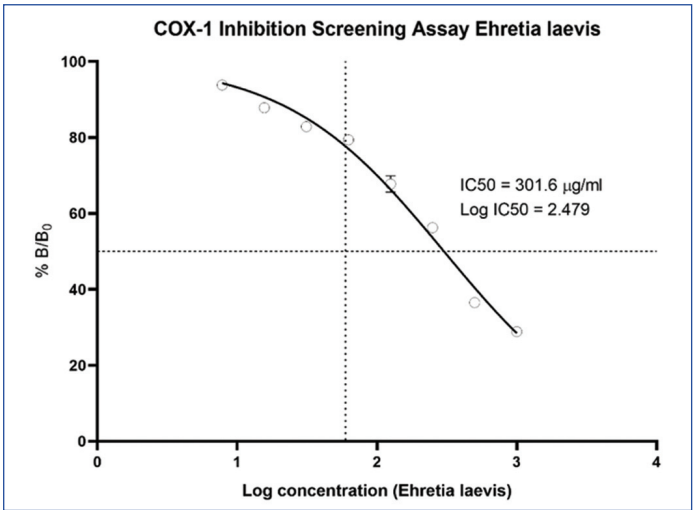
The trend of inhibition with increasing dose: Both *Ehretia laevis* Roxb. powder and Diclofenac sodium exhibited a dose-dependent inhibition, meaning that the inhibition increased with increasing doses. However, the rate of increase differed between the two substances. Diclofenac sodium showed stronger inhibition at all doses.

Highest inhibition achieved: Diclofenac sodium achieved a higher maximum inhibition (84.05%) compared to *Ehretia laevis* Roxb. powder (72.40%).

Overall, Diclofenac sodium demonstrated a stronger COX inhibition

Dose (µg/mL)	Log dose	Absorbance			Average	Corrected			%B/B0		
NSB	-	0.003	0.002	0.004	0.003	-	-	-	-	-	-
B0	-	0.962	0.958	0.960	0.960	-	-	-	-	-	-
7.8	0.89279	0.911	0.899	0.901	0.904	0.908	0.896	0.898	94.583	93.333	93.542
15.6	1.19382	0.843	0.847	0.850	0.847	0.840	0.844	0.847	87.500	87.897	88.229
31.3	1.49485	0.801	0.795	0.799	0.798	0.798	0.792	0.796	83.125	82.500	82.917
62.5	1.79588	0.754	0.764	0.778	0.765	0.751	0.761	0.775	78.229	79.271	80.729
125.0	2.09691	0.677	0.647	0.637	0.654	0.674	0.644	0.634	70.208	67.083	66.042
250.0	2.39794	0.545	0.544	0.540	0.543	0.542	0.541	0.537	56.458	56.354	55.938
500.0	2.69897	0.342	0.359	0.360	0.354	0.339	0.356	0.357	35.313	37.083	37.188
1000.0	3	0.268	0.288	0.284	0.280	0.265	0.285	0.281	27.604	29.688	29.271

[Table/Fig-3]: COX-1 inhibition: Ehretia Laevis Roxb. powder.



[Table/Fig-4]: COX-1 inhibition: Ehretia Laevis Roxb. powder.

Ehretia laevis Roxb. powder shows a strong inhibitory effect on COX-2 at lower doses (7800 ng/mL and 15600 ng/mL) with average inhibition percentages exceeding 79%. However, the inhibition significantly decreases with increasing doses, reaching around 29% at the highest dose (1000000 ng/mL), as per [Table/Fig-7,8].

IC50 values: The IC50 values for diclofenac sodium are presented in ng/mL, while for *Ehretia laevis* Roxb., they are presented in µg/mL. We converted diclofenac sodium to µg/mL, as shown in [Table/Fig-9,10].

- Diclofenac sodium COX-1: 59.49 ng/mL=0.05949 µg/mL
- Diclofenac sodium COX-2: 14.23 ng/mL=0.01423 µg/mL

Diclofenac sodium shows a more consistent inhibition effect across the tested dose range, while *Ehretia laevis* Roxb. powder exhibits a effect compared to *Ehretia laevis* Roxb. powder at all tested doses.

DISCUSSION

Diclofenac sodium is a much more potent inhibitor of both COX-1 and COX-2 enzymes compared to *Ehretia laevis* Roxb. powder. To achieve 50% inhibition of COX-1, diclofenac sodium requires only 59.49 nanograms per milliliter (ng/mL), whereas *Ehretia laevis* Roxb. powder needs a significantly higher dose of 301.6 micrograms per milliliter (µg/mL). This translates to diclofenac sodium being roughly five times more potent for COX-1 inhibition. Similarly, for COX-2 inhibition, diclofenac sodium has an IC50 value of 14.23 ng/mL, while *Ehretia laevis* Roxb. powder has an IC50 value of 245.0 µg/mL, as per. This indicates that diclofenac sodium is approximately 17 times more potent for COX-2 inhibition. Overall, the data suggest that diclofenac sodium has a stronger and more selective inhibitory effect on both COX-1 and COX-2 enzymes compared to *Ehretia laevis* Roxb. powder.

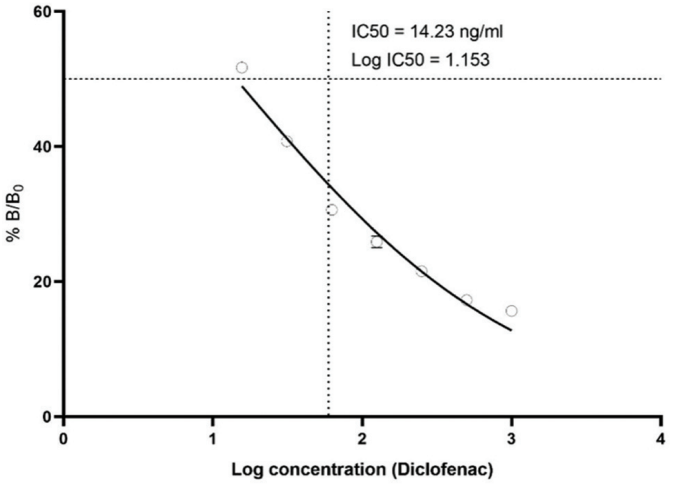
Selectivity Ratio (COX-1/COX-2 IC50): As per [Table/Fig-11]:

- Diclofenac sodium: 4.18 (0.05949 / 0.01423) → COX-2 selective
- *Ehretia laevis*: 1.23 (301.6 / 245.0) → Less selective

The positive control significantly decreased COX-1 activity, and none of the plant extract quantities examined were able to inhibit the enzyme with IC50 values lower than the positive control. Due to the known beneficial effects of COX-1 activity, extracts with lower inhibitory strength against COX-1 are advised. However, it is noteworthy that *Ehretia laevis* Roxb. showed significant activity with an IC50 value of 301.6 µg/mL. COX-1 enzymatic activity stimulates the production of beneficial prostaglandins that are responsible for the maintenance and protection of the intestinal mucosa (Dennis

Dose (ng/mL)	Log dose	Absorbance			Average	Corrected			%B/B0		
NSB	-	0.003	0.004	0.004	0.004	-	-	-	-	-	-
B0	-	0.881	0.866	0.878	0.875	-	-	-	-	-	-
15.6	1.19382	0.454	0.455	0.450	0.453	0.453	0.454	0.449	51.790	51.933	51.359
31.3	1.49485	0.358	0.359	0.354	0.357	0.358	0.359	0.354	40.877	41.021	40.446
62.5	1.79588	0.270	0.269	0.266	0.268	0.270	0.268	0.266	30.826	30.682	30.395
125.0	2.09691	0.220	0.235	0.226	0.227	0.220	0.235	0.226	25.097	26.811	25.783
250.0	2.39794	0.192	0.186	0.188	0.189	0.192	0.186	0.188	21.923	21.205	21.493
500.0	2.69897	0.150	0.148	0.156	0.151	0.150	0.148	0.156	17.097	16.869	17.783
1000.0	3	0.140	0.141	0.131	0.137	0.140	0.141	0.131	15.954	16.069	14.926

[Table/Fig-5]: COX-2 inhibitions: Diclofenac sodium.



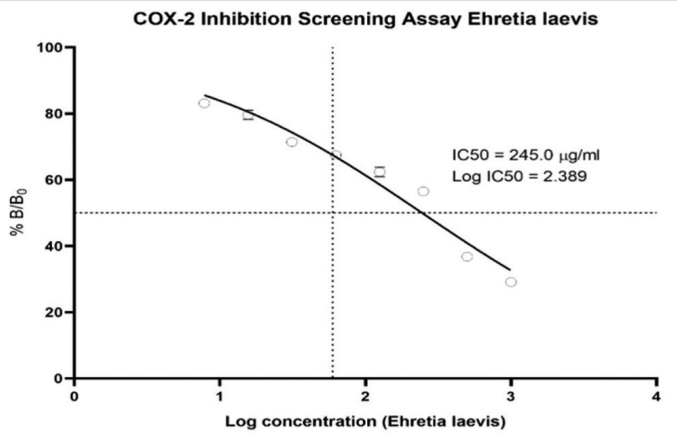
[Table/Fig-6]: COX-2 inhibitions: Diclofenac sodium.

The COX-2 inhibitory activity IC50 value of 245 µg/mL was also intriguing. The role of phenolic compounds in plant extracts' anti-inflammatory properties was highlighted in COX-1 and COX-2 inhibition experiments. These findings are not statistically significant when compared to diclofenac sodium; nonetheless, the study is noteworthy for discovering natural inhibitors with a lower risk of developing gastrointestinal health consequences compared to diclofenac sodium. As a result, selectively inhibiting COX-2 activity is advantageous in controlling skin inflammation in pathological circumstances. The incorporation of COX-2 selective extracts into product formulations could improve skin beauty by reducing scarring, dark patches, and uneven skin associated with chronic inflammation.

Gallic acid from Terminalia b. has an IC50 of 74 nM against COX-2 and an IC50 of 1500 nM against COX-1 [10]. The ethanolic extract of leaves of Canarium patentinervium Miq inhibited the activity of COX-1 and COX-2 with IC50 values equal to 0.60±0.01 µg/mL and 1.07±0.01 µg/mL, respectively [11]. Abroma augusta and Desmodium gangeticum were tested for COX activity. The aqueous extract (100

Dose (µg/mL)	Log dose	Absorbance			Average	Corrected			%B/B0		
NSB	-	0.003	0.004	0.004	0.004	-	-	-	-	-	-
B0	-	0.881	0.866	0.878	0.875	-	-	-	-	-	-
7.8	0.89279	0.734	0.719	0.731	0.728	0.733	0.718	0.730	83.789	82.086	83.469
15.6	1.19382	0.691	0.691	0.712	0.698	0.690	0.690	0.711	78.870	78.883	81.274
31.3	1.49485	0.620	0.635	0.621	0.625	0.620	0.635	0.621	70.811	72.526	70.926
62.5	1.79588	0.599	0.584	0.590	0.591	0.599	0.584	0.590	68.411	66.697	67.383
125.0	2.09691	0.545	0.560	0.535	0.547	0.545	0.560	0.535	62.240	63.954	61.097
250.0	2.39794	0.499	0.492	0.494	0.495	0.499	0.491	0.493	56.996	56.155	56.397
500.0	2.69897	0.313	0.325	0.329	0.322	0.313	0.324	0.329	35.749	37.043	37.583
1000.0	3	0.245	0.260	0.260	0.255	0.245	0.260	0.259	28.004	29.708	29.639

[Table/Fig-7]: COX-2 inhibition: Ehretia Laevis Roxb. powder.



[Table/Fig-8]: COX-2 inhibition: Ehretia Laevis Roxb. powder.

and Norris, 2015). Because of the prolonged ulceration associated with COX-1 inhibition, extracts from these plants are unlikely to serve as natural inhibitors.

Test item	IC50 values	
	COX-1 inhibition	COX-2 inhibition
Diclofenac sodium	59.49ng/mL	14.23ng/mL
Ehretia Laevis Roxb. Powder	301.6 µg/mL	245.0 µg/mL

[Table/Fig-9]: IC50 values.

Test Item	COX-1 IC50 (µg/mL)	COX-2 IC50 (µg/mL)
Diclofenac sodium	0.05949	0.01423
Ehretia Laevis Roxb. Powder	301.6	245.0

[Table/Fig-10]: IC50 Values in same units.

mg/mL) of Desmodium gangeticum had a COX-2 IC50 value of 39.5 µg/mL and a COX-1 IC50 value of 49.5 µg/mL. The petroleum ether extract (250 mg/mL) of the roots of Abroma augusta showed COX-1 (IC50=36.5 µg/mL) and COX-2 (IC50=59 µg/mL) inhibition [12].

Velappan S. et al., (2014) explained that the methanolic extract of *Ehretia laevis* Roxb. reduced inflammation in an animal model [13]. Jyothirmai N. et al., (2016) discussed the anti-inflammatory activity

of different extracts of *Ehretia laevis* Roxb. in an animal model [14]. Joshi UP (2018) described the anthelmintic properties of *Ehretia laevis* Roxb. [15]. Rangnathrao TS et al., (2019) explained the anticancer and hepatoprotective activity of various extracts of *Ehretia laevis* Roxb. in an in-vitro study [16]. Sivasankari V. et al., (2013) reported a higher antioxidant property of methanol extract [17]. Rangnathrao TS et al., (2019) also discussed the antioxidant and hepatoprotective characteristics of *Ehretia laevis* Roxb. in an animal model [18]. Bande D. et al., (2018) described the blood coagulation properties of *Ehretia laevis* Roxb. [19]. Tichkule SV et al., (2019) examined the role of *Ehretia laevis* Roxb. in fracture healing [20]. Deshpande RR et al., (2014) reported the antimicrobial activity of *Ehretia laevis* Roxb. [21]. Panja S. (2020) discussed the antimicrobial, anticancer, and larvicidal characteristics of *Ehretia laevis* Roxb. [22]. Yende SR et al., (2021) explained the inhibition of TNF-α in arthritis [23]. Rushikesh T et al., (2018) reported the antimicrobial properties of *Ehretia laevis* Roxb. against Gram-positive and Gram-negative pathogens [24]. Thakre R et al., (2023) described the wound healing activity of different extracts of *Ehretia laevis* Roxb. in burn wounds in an animal model [25]. Thakre R et al., (2021) explored the wound healing activity of *Ehretia laevis* Roxb. in chronic venous ulcers [26]. Thakre R et al., (2021) also examined the histological changes in wound healing by *Ehretia laevis* Roxb. [27]. Thakre R et al., (2023) reported on the effectiveness of medicated threads made from *Ehretia laevis* Roxb. [28]. Thakre R et al., (2019) discussed the antimicrobial properties of polar and non-polar extracts of *Ehretia laevis* Roxb. [29]. Thakre R et al., (2021) also described the effectiveness of *Ehretia laevis* Roxb. in managing shoulder pain [30].

All of these studies indicate the clinical importance of the herbal plant *Ehretia laevis* Roxb. In vitro studies are crucial for generating empirical evidence for future pre-clinical and clinical studies. Inhibition studies on COX-1 and COX-2 should be planned for various extracts of *Ehretia laevis* Roxb. to identify the most effective extract. Although in-vitro studies might generate limited evidence, they pave the way for further pre-clinical and clinical investigations.

Limitation(s)

To ascertain variability and significance, statistical tests require several replicates. In the absence of replicates, the results may not be trustworthy. Cross-study comparisons are challenging, as IC50 values can be affected by variables such as assay type, buffer conditions, incubation period, and enzyme source. Due to variations in metabolism, protein binding, and tissue distribution, in-vitro IC50 values do not always correspond to in vivo effects.

CONCLUSION(S)

The IC50 values for diclofenac sodium for COX-1 inhibition are 59.49 ng/mL and for COX-2 inhibition, 14.23 ng/mL. The IC50 values for *Ehretia laevis* Roxb. for COX-1 inhibition are 301.6 µg/mL and for COX-2 inhibition, 245.0 µg/mL. Various extracts of *Ehretia laevis* Roxb. should be tested further for COX inhibition activities. Due to the prolonged ulceration associated with COX-1 inhibition, extracts from these plants are unlikely to serve as natural inhibitors. The COX-2 inhibitory activity IC50 value of 245 µg/mL is also intriguing. The role of phenolic compounds in the anti-inflammatory properties of plant extracts was highlighted in COX-1 and COX-2 inhibition experiments. Selectively inhibiting COX-2 activity is advantageous in controlling skin inflammation in pathological contexts. The integration of COX-2 selective extracts into product formulations may improve skin appearance by reducing scarring, dark patches, and uneven skin associated with chronic inflammation.

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