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RESEARCH ARTICLE

Analgesic and Anti-Inflammatory Activity of Ethanolic Extract of *Ipomoea Hederifolia* Linn

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SUMMARY

Pain and inflammation represent significant global health burdens, with plant-derived medicines contributing substantially to modern therapeutics. Ipomoea hederifolia L. has been traditionally employed for inflammation management, but scientific validation of its anti-inflammatory properties remains limited. This study systematically evaluates the analgesic and anti-inflammatory potential of I. hederifolia through comprehensive phytochemical characterization and preclinical pharmacological assessments. The ethanolic extract was prepared via continuous hot extraction and subjected to detailed phytochemical quantification (tannins, phenolics, alkaloids, flavonoids) and GC-MS analysis. Pharmacological evaluation included antioxidant capacity and protein denaturation inhibition in vitro, acute toxicity testing per OECD guidelines 423, acetic acid-induced writhing (peripheral analgesia), hot plate test (central analgesia), and carrageenan-induced paw edema (anti-inflammatory activity). Test groups received 200/400 mg/kg extract, compared against diclofenac (10/50 mg/kg) and tramadol (40 mg/kg) controls. Phytochemical analysis revealed substantial bioactive content (tannins 8.3%, flavonoids 8.02%). GC-MS identified 23 therapeutically relevant compounds correlating with observed bioactivities. The extract demonstrated potent DPPH radical scavenging activity (IC50 54.2 µg/mL) and 78.52% protein denaturation inhibition at 31.25 µg/mL. Significant analgesia occurred through both peripheral (57.3% writhing inhibition) and central (latency increase >150%) mechanisms, with dose-dependent anti-inflammatory effects (68.4% edema reduction). Remarkably, no toxicity was observed at 2000 mg/kg, indicating an exceptional safety profile for therapeutic development. This study provides conclusive pharmacological validation of I. hederifolia's traditional use, demonstrating its multifunctional therapeutic potential through robust antioxidant, analgesic, and anti-inflammatory activities. Further research should focus on compound isolation to fully realize its therapeutic value in pain and inflammation management.

Keywords: *Antioxidant, analgesic activity, anti-inflammatory activity, Ipomoea hederifolia.*

Ipomoea Hederifolia Linn'in Etanolik Ekstraktının Analjezik ve Anti-inflamatuar Aktivitesi

ÖZ

Ağrı ve inflamasyon, küresel sağlık açısından önemli bir yük oluşturmaktadır ve bitki kaynaklı ilaçlar modern tedavilere önemli katkı sağlamaktadır. Ipomoea hederifolia L. geleneksel olarak inflamasyonun tedavisinde kullanılmaktadır, ancak anti-inflamatuar özelliklerinin bilimsel olarak doğrulanması sınırlı kalmaktadır. Bu çalışma, kapsamlı fitokimyasal karakterizasyon ve preklinik farmakolojik değerlendirmeler yoluyla I. hederifolia'nın analjezik ve anti-inflamatuar potansiyelini sistematik olarak değerlendirmektedir. Etanolik ekstrakt, sürekli sıcak ekstraksiyon yoluyla hazırlanmış ve ayrıntılı fitokimyasal kantifikasyona (tanenler, fenolikler, alkaloidler, flavonoidler) ve GC-MS analizine tabi tutulmuştur. Farmakolojik değerlendirme, in vitro antioksidan kapasite ve protein denatürasyonu inhibisyonu, OECD kılavuzları 423'e göre akut toksisite testi, asetik asit kaynaklı kıvrılma (periferik analjezi), sıcak plaka testi (santral analjezi) ve karragenan kaynaklı pençe ödemi (antiinflamatuar aktivite) içermektedir. Test gruplarına 200/400 mg/kg ekstrakt verilirken, kontrol gruplarına diklofenak (10/50 mg/kg) ve tramadol (40 mg/kg) verildi. Fitokimyasal analiz, önemli miktarda biyoaktif içerik (tanenler %8,3, flavonoidler %8,02) olduğunu ortaya çıkarmıştır. GC-MS, gözlemlenen biyoaktivitelerle ilişkili 23 terapötik olarak önemli bileşik tespit etti. Ekstrakt, güçlü DPPH radikal temizleme aktivitesi (IC50 54,2 µg/mL) ve 31,25 µg/mL'de %78,52 protein denatürasyonu inhibisyonu gösterdi. Doz bağımlı antiinflamatuar etkilerle (ödemde %68,4 azalma) hem periferik (%57,3 kıvranma inhibisyonu) hem de merkezi (latens artışı >%150) mekanizmalar yoluyla önemli analjezi meydana geldi. Dikkat çekici bir şekilde, 2000 mg/kg'da herhangi bir toksisite gözlemlenmemiştir, bu da terapötik geliştirme için olağanüstü bir güvenlik profili olduğunu göstermektedir. Bu çalışma, I. hederifolia'nın geleneksel kullanımının kesin farmakolojik doğrulamasını sağlar ve güçlü antioksidan, analjezik ve antiinflamatuar aktiviteler yoluyla çok işlevli terapötik potansiyelini gösterir. İleri araştırmalar, ağrı ve inflamasyon yönetiminde terapötik değerini tam olarak gerçekleştirmek için bileşik izolasyonuna odaklanmalıdır.

Anahtar Kelimeler: *Antioksidan, analjezik aktivite, anti-inflamatuar aktivite, Ipomoea hederifolia.*

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INTRODUCTION

Pain and inflammation are widespread health concerns that, if unmanaged, may progress into chronic conditions like arthritis (Punchard, Whelan, & Adcock, 2004). Pain results from inflammatory mediators released after tissue injury or due to nerve compression from edema (Kidd & Urban, 2001). Similarly, inflammation involves biochemical changes such as cell membrane disruption, triggering arachidonic acid release. This activates cyclooxygenase (COX), producing thromboxanes and prostaglandins, key drivers of inflammation (Ijeoma, Aderonke, Ogbonna, Augustina, & Ifeyinwa, 2011).

Pain is a major global health issue, affecting an estimated 1 in 5 people worldwide, with an additional 1 in 10 adults diagnosed with chronic pain. Approximately 10% of the global population, around 60 million people, suffer from pain. In individual countries, the prevalence of pain ranges from 20% to 25% (Goldberg & McGee, 2011). Despite the availability of numerous medications for pain and inflammation, they remain among the most challenging and debilitating health issues, affecting 80% of adults globally. They are considered major clinical, social, and economic concerns in most communities worldwide (Yimer, Birru, Adugna, Geta, & Emiru, 2020). Prolonged, untreated pain is a pervasive disorder that can cause both physical damage and psychological issues (Krummenacher et al., 2014; Treede et al., 2019; Woolf, 2011). Uncontrolled inflammation not only hinders daily activities such as work, school, or social events but also accelerates the development of severe inflammatory diseases like rheumatoid arthritis, inflammatory bowel disease, atherosclerosis, and chronic periodontitis (Serhan & Levy, 2018). If left untreated, these debilitating illnesses are the primary causes of disability and can be fatal (Yimer et al., 2020).

Synthetic drugs are widely used for the treatment of pain and inflammation (Kidd & Urban, 2001; Punchard et al., 2004). Nonsteroidal anti-inflammatory drugs (NSAIDs) and opioid analgesics are commonly used for the treatment. However, these medications can cause numerous adverse effects (Grace, 1961; Punchard et al., 2004). These drugs cause various adverse effects such as nausea, vomiting, headache, dizziness, abdominal pain, indigestion, diarrhoea, and sickness (Sostres, Gargallo, Arroyo, & Lanas, 2010). They also lead to serious adverse effects, including hepatotoxicity (Sriuttha, Sirichanchuen, & Permsuwan, 2018), nephrotoxicity (Whelton, 1999), and gastrointestinal issues such as ulcers (Harirforoosh, Asghar, & Jamali, 2014). Therefore, herbal drugs can be a potential source to replace them. According to literature, alkaloids, flavonoids, steroids, and tannins exhibit analgesic and anti-inflammatory activity (Fan, Ali, & Basri, 2014; Ijeoma et al., 2011). Ipomoea species of plants have traditionally been employed to treat a wide range of ailments, including diabetes, constipation, hypertension, dysentery, arthritis, exhaustion, meningitis, rheumatism, kidney disease, and inflammation. Phytochemical analyses of the Ipomoea genus reveal that these plants possess antimicrobial, spasmogenic, hypotensive, anticancer, psychotomimetic, analgesic, and anti-inflammatory properties (Joel & Uwabunkeonye, 2020). Analgesic activity to some of the Ipomea species, such as Ipomoea imperati (Vahl.) Griseb. (Paula-Zurron et al., 2010), Ipomoea involucrate (Ijeoma et al., 2011), Ipomoea pes-caprae (Vieira et al., 2013), and Ipomoea pes-tigridis (Ramesh, 2010) are reported in the literature.

Ipomoea hederifolia L. (*I. hederifolia*), commonly known as "Jitirana," is a medicinal herb belonging to the family Convolvulaceae. It is also referred to as scarlet morning glory or scarlet creeper (Kurian, Banurekha, Venkateswarlu, & Kumar, 2023). While native to tropical and subtropical America, it is widely found in countries such as Brazil, Bangladesh, and India (Santos, Nurit-Silva, Arruda, & Leite, 2023). This plant features climbing, striated stems ranging from green to vinaceous, bright red corolla flowers, and simple, alternate, membranaceous, cordiform, or three-lobed leaves with semi-circular petioles around

7 cm long, resembling Ivy (Runner plant) (Santos et al., 2023). Phytochemical analysis reveals that *I. hederifolia* contains various constituents, including quinine, terpenoids, glycosides, alkaloids, steroids, flavonoids, saponins, and carbohydrates (Hossain, Uddin, Baral, Ferdus, & Bhowmik, 2022). The main chemical constituents include ergoline alkaloids, indolizidine alkaloids, nortropanic alkaloids, phenolic compounds, coumarins, norisoprenoids, diterpenes, isocoumarins, benzenoids, flavonoids, anthocyanosides, glycolipids, lignans, and triterpenes.

In traditional medicine systems, *I. hederifolia* is utilized for its hypotensive, anti-inflammatory, psychotomimetic, anticancer, laxative, diuretic, cathartic, and expectorant properties (Kurian et al., 2023; Meira, Silva, David, & David, 2012). Infusions and decoctions made from various parts of the plant, including the leaves, stems, roots, and fruits, are commonly used. They are employed as purgatives and treatments for tumors, dermatitis, rheumatism, nervous system issues, stomachaches, and intestinal parasites. The roots serve as a sternutatory, while the seeds can act as expectorants, diuretics, cathartics, and anti-inflammatory agents for conditions like constipation, edema, and parasitosis (Santos et al., 2023).

I. hederifolia has been traditionally used for its purported antinociceptive and anti-inflammatory effects, and preliminary phytochemical studies suggest the presence of bioactive compounds that may support these properties. However, no systematic scientific studies have yet validated its analgesic and anti-inflammatory potential. Therefore, this study aimed to evaluate the ethanolic extract of *I. hederifolia* for these activities using rodent models.

MATERIALS AND METHODS

Drugs and chemicals

Carrageenan, acetic acid, diclofenac, distilled water, tramadol, 1,1-diphenyl-2-picrylhydrazyl (DPPH), and egg albumin were obtained from the local vendors.

Collection, identification, and preparation of plant material

The *I. hederifolia* plant was collected in October 2023 from the roadside near the village of Khambale, Dist. Sangli 415311, Maharashtra, India. The plant was identified as *Ipomoea hederifolia* L. and confirmed by Dr. Shankar M. Shendage, Associate Professor, Department of Botany, Balwant College, Vita (Sangli), India. The voucher specimen (Specimen No. SS 002) has been deposited in the laboratory of Balwant College, Vita, for future reference.

Preparation of plant extract

After collecting the *I. hederifolia* plant, the material was washed under tap water, and the flowers were removed. The remaining plant parts were shade-dried for two weeks. The shade-dried material was then ground to obtain a coarse powder. For the extraction process, 40 grams of the powdered material was placed in a bag of cotton cloth, and the bag was kept in a glass thimble. Sufficient ethanol was added to the reservoir, and the hot continuous extraction process was initiated using a Soxhlet extractor at 70°C (K. Mali, Sutar, Devade, & Dias, 2019). This extraction continued until the ethanol in the thimble became colorless, which took about 6 hours. The ethanolic extract was then dried using a rotary evaporator, resulting in a brownish gummy residue. From 13 batches of 40 grams of crude *I. hederifolia* powder, a total of 38.1 grams of residue was obtained, yielding a percentage of 7.33%. The *I. hederifolia* extract (IHE) was stored in a desiccator for further use (Kaushik, Kumar, Kaushik, & Rana, 2012). The dry extract was dissolved in (0.5%) carboxymethyl cellulose (CMC) and administered orally to the animals as per body weight (AlRashdi et al., 2012).

Preliminary phytochemical screening of the extract

Standard phytochemical screening tests were carried out to detect the presence of secondary metabolites to relate the analgesic and anti-inflammatory activity of IHE with the presence or absence of active

constituents. Thus, the test for carbohydrate, tannin, saponin, flavonoid, alkaloid, quinine, terpenoid, glycoside, anthraquinone glycoside, triterpenoid, phenol, steroid, cardiac glycoside, and cyanogenic glycoside (Vanlalhruaia & Lalbiaknunga, 2020) was performed using reference procedures (Hossain et al., 2022).

Determination of alkaloids

A sample extract of I. *hederifolia* was prepared by dissolving 100 mg of plant extract in 10 mL of 2N hydrochloric acid and filtering. 1 mL of filtrate was transferred to a separating funnel and washed three times with 10 mL of chloroform. After washing, the extract was separated, and the pH was maintained at 7 by using 0.1 N sodium hydroxide. Solution of atropine sulphate (100 µg/mL) was prepared in distilled water. After preparation of the sample extract, 1 mL of the extract was mixed with 5 mL of pH 4.7 phosphate buffer. Subsequently, 5 mL of bromocresol green (BCG) was added, and the mixture was shaken with 1 mL, then 2 mL, 3 mL, and 4 mL of chloroform in a separating funnel. The chloroform layer extracts were collected in a 10 mL volumetric flask. If the volume is less than 10 mL, it was adjusted by adding chloroform. The same procedure was repeated for the standard solution of atropine sulphate at concentrations of 20 to 100 µg/mL to establish a calibration curve. The assay was compared with atropine equivalents. Finally, the absorbance of the chloroform layer containing the complex was measured at 470 nm against a blank prepared similarly but without the extract (Madhu, Sailaja, Satyadev, & Satyanarayana, 2016). This absorbance measurement allows for the determination of the concentration of the alkaloids in the sample extract.

Determination of flavonoids

A sample extract of *I. hederifolia* was prepared by dissolving 100 mg of plant extract in 100 mL of distilled water (1 mg/mL), and a solution of quercetin (100 µg/mL) was prepared in methanol. After preparation of the sample extract, 1 mL of the extract was mixed with 4 mL of distilled water in a 10 mL volumetric flask. Then, 0.3 mL of 5% sodium nitrite was added, followed by the addition of 0.3 mL of 10% aluminum chloride after 5 minutes. After another 5 minutes, 2 mL of 1M sodium hydroxide was added. The volume was then made up to 10 mL using distilled water. For the reference standard quercetin, solutions ranging from 10 to 100 µg/mL were prepared in the same manner as described above, without the sample extract. The absorbance of the resulting solutions was measured at 510 nm against a blank prepared similarly but without the sample extract. The assay is then expressed in terms of quercetin equivalent concentration (Chandran & Kavitha Chandran, 2016).

Determination of phenolics

A sample extract of *I. hederifolia* was prepared by dissolving 100 mg of plant extract in 100 ml of distilled water (i.e., 1 mg/mL), and a solution of gallic acid (100 µg/mL) was prepared in distilled water. Then 1 mL of the sample extract was mixed with 1 mL of Folin-Ciocalteu reagent (FC reagent). After 5 minutes, 10 mL of 7% sodium carbonate solution was added to the mixture. Distilled water was then added and mixed thoroughly to make up the volume to 25 mL in a volumetric flask. The mixture was kept in the dark for 90 minutes at 23 °C. After incubation, the absorbance of the sample extract and standard was measured at 760 nm against a blank. For the reference standard, gallic acid solutions ranging from 30 to 210 µg/mL were prepared in the same manner as described above. The total phenolic compound content is expressed as gallic acid equivalent concentration. (Chandran & Kavitha Chandran, 2016).

Determinations of tannins

A sample extract of *I. hederifolia* was prepared by dissolving 100 mg of plant extract in 100 mL of distilled water (i.e., 1 mg/mL), and a solution of tannic acid (100 µg/ml) was prepared in distilled water. In a 10 mL volumetric flask, 1 mL of sample extract was mixed with 7.5 mL of distilled water. Subsequently, 0.5 mL of Folin-Ciocalteu reagent was introduced, followed by the addition of 1 mL of 35% sodium car-

bonate solution. The flask was then filled to the 10 mL mark with distilled water and thoroughly shaken before being left at room temperature for 30 minutes. Meanwhile, a series of reference standard solutions containing tannic acid at concentrations of 20 to 100 µg/mL was prepared. Using a UV/Visible spectrophotometer, the absorbance of both the test and standard solutions was measured against a blank at 700 nm. The tannin content was quantified in terms of tannic acid equivalent concentration (Lahare, Yadav, Bisen, & Dashahre, 2021).

Gas chromatography-mass spectroscopy (GCMS) analysis

The ethanolic IHE was subjected to analysis using GC–MS (Shimadzu TQ 8050 Plus with HS 20, Japan), equipped with an automation tool AOC-20i+s, which was used for rinsing with pre-solvent 2 times, rinsing with post-solvent 2 times. Its injection port dwell time was 0.3 seconds with 5 times pumping. The washing volume was 8 µL. In the presence of purge flow of 3 mL/min, the pressure, column oven temperature, and injection temperature were maintained at 54.4 kPa, 50°C, and 260°C, holding time 2 minutes respectively. The column flow rate was maintained at 1.01 mL/min. Throughout the experiment run time of 4 min to 50 min, the injection mode was direct at a linear velocity of 36.5 cm/sec, and the injection temperature was 250 °C. The column oven temperature was 50°C. To have control of the system and acquire the data, we engaged the Shimadzu GC–MS solution software provided by the supplier. By comparing their mass spectra to data from the library NIST14.lib (National Institute of Standards and Technology), the compounds present in ethanolic IHE were identified (Willie, Uyoh, & Aikpokpodion, 2020).

DPPH radical scavenging activity

The antioxidant activity of the ethanolic extract of *I. hederifolia* was assessed using the scavenging activity of DPPH free radicals. Initially, a 0.1 mM DPPH solution was prepared by dissolving 10 mg of DPPH in 250 mL of methanol. Subsequently, 2 mL of the extract and standard ascorbic acid, each at various concentrations, were transferred to separate 10 mL volumetric flasks. To each volumetric flask, 3 mL of the DPPH solution was added. All samples of extract and ascorbic acid at different concentrations were covered with aluminium foil and incubated in a dark area for half an hour. After the 30-minute incubation period, the absorbance of each sample was measured using a UV-visible spectrophotometer at 517 nm. The DPPH solution without any extract or standard served as the control. The antioxidant activity of the extract was determined by calculating the reduction in DPPH absorbance compared to the control, using a suitable formula (Hossain et al., 2022).

$$Percentage\ of\ free\ radical\ DPPH\ scavenging = \frac{Ac - As}{Ac} \times 100 \qquad (01)$$

Where Ac is the absorbance of the control and As is the absorbance of the sample.

***In vitro* anti-inflammatory assay**

The assay was carried out with minor modifications in accordance with the procedures described by Mali et al. (Mali, Kokate, Ghorpade, Dias, & Mahajan, 2025). For the protein denaturation assay, egg albumin was prepared by diluting 1 mL of albumin in 10 mL of distilled water. Plant extracts and solutions of diclofenac were prepared at concentrations of 31.25 µg/mL to 1000 µg/mL in distilled water. Each reaction vessel was assembled with 0.2 mL of egg albumin, 1.4 mL of phosphate-buffered saline, and 1 mL of the test extract. For the negative control, distilled water was utilized. After 15 minutes of incubation at 37 °C, the mixtures were heated for 5 minutes at 70 °C. The absorbance was measured at 660 nm after cooling. The percentage inhibition of protein denaturation was calculated using the following formula:

$$\%Denaturation\ inbition = \frac{1 - D}{C} \times 100 \qquad (02)$$

Where D is the absorbance reading of the test sample, and C is the absorbance reading without the test sample (negative control).

Acute oral toxicity study

The extract of *I. hederifolia* was evaluated for its *in vivo* toxicological properties following the OECD guideline 423, which involves a single-dose 14-day acute oral toxicity study by using six healthy female Albino Wistar rats. Then the animals were kept fasting overnight, providing water only. After that, a 2000 mg/kg body weight aqueous solution with suspending agent (0.5%) CMC of IHE was administered orally by oral gavage. The dose volume of 10 mL/kg body weight was selected. Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hrs, with special attention given during the first 4 hrs. and daily thereafter, for a total of 14 days. The acute toxicity range of the test substance is determined based on the mortality status of the animals. This information assists in categorizing the toxicity level of the tested drug (OECD, 2002).

Experimental animals

Adult Wistar albino rats, weighing between 180 - 280 g, and Swiss albino mice, weighing between 20 - 35 g, of either gender were procured from National Institute of Biosciences 69, At Post Dhangawadi, Off Pune-Bangalore Highway, Tal Bhor, Dist Pune 412205 (1091/GO/BT/S/07/CPCSEA) and utilized for the study. The animals were housed in cages in rooms with regulated temperature, air-cooling, and a 12-hour light-dark cycle. They were provided with free access to water and a standard laboratory diet. A one-week acclimatization period to the laboratory conditions was allowed for the animals, and they were fasted overnight before the commencement of the experiments. The study protocol was approved by the Institutional Animal Ethics Committee of Biocyte Institute of Research and Development, Sangli, Maharashtra, India 416416 (IAEC/BiRD/Sangli/2024-25-03), and all experiments were conducted in accordance with the guidelines laid down by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Animal grouping and dosing

Wistar albino rats and Swiss albino mice of either sex were randomly divided into four groups, each consisting of 6 rats and 6 mice, respectively. Group I served as the normal control and received distilled water only. Group II served as the positive control and was treated with standard drug tramadol (40 mg/kg, *p.o.*) for the hot plate test, diclofenac (50 mg/kg, *p.o.*) for the writhing test in mice, and diclofenac (10 mg/kg, *p.o.*) for the carrageenan-induced paw edema in rats. Groups III and IV were designated as test groups and received the IHE at doses of 200 and 400 mg/kg (*p.o.*), respectively. The doses were selected based on an acute toxicity study. All treatments were administered orally, and the maximum volume administered was 10 mL/kg.

Hot plate method

The Wistar albino rats were divided into four groups, each carrying six animals. Animals were fasted overnight prior to study, and each of them acclimatized to Eddy's hot plate for 5 min. After this, the hot plate temperature was maintained at 55°C, and then the rat was placed on it. The reaction time was recorded as the time taken for the rat to exhibit a response to the thermal pain, such as paw licking or jumping. Reaction times were recorded before (0 min) and at 30, 60, 90, and 120 min after the administration of the test extracts and standard drug (Tramadol). A maximum reaction time of 45 seconds was set to prevent any injury to the rats (Greene, 1972; Kumar, Singh, Pranav, & Ray, 2011). If the recorded reaction time exceeded 45 seconds, the animal was immediately removed from the hot plate, and it was considered to have reached maximum analgesia (Raveendran et al., 2019).

The maximum possible analgesia (MPA) was calculated as follows:

$$MPA = \frac{Reaction\ time\ for\ treatment - Reaction\ time\ for\ control}{(45\,\mathrm{sec} - Reaction\ time\ for\ control)} \times 100 \quad (03)$$

Acetic acid-induced writhing test

The writhing model is a chemical nociceptive test involving the intraperitoneal injection of irritating compounds into animals to induce peritonitis. Swiss albino mice were divided into four groups, each carrying six mice, and fasted overnight prior to the study. In this study, intraperitoneal injection of 10 mg/kg of a 0.6% acetic acid solution was administered 30 minutes after drug administration. Each mouse was placed into a glass beaker, and a five-minute observation period was allowed. The number of writhes, characterized by stretching of the abdomen and at least one hind limb simultaneously, was recorded for 10 minutes of observation. The writhing percentage was calculated and compared to the standard (Kaushik et al., 2012).

$$\%\ inhibition\ of\ writhing = \frac{Mean\ number(control) - Mean\ number(test)}{Mean\ number(control)} \times 100 \quad (04)$$

Carrageenan-induced paw edema method

Rats were divided into four groups, each containing six rats. Inflammation was induced in the left hind paw using carrageenan (1% carrageenan suspended in 0.9% NaCl) injected in subplantar region. The test drug, normal saline, and standard drug were administered to the rats before one hour of giving carrageenan injection. Group I served as the normal control and received the vehicle, group II served as the positive control and received the standard drug, and group III-IV received the test extract. Paw volume was measured using the volume displacement technique with a plethysmometer apparatus. Paw volume was measured immediately after carrageenan injection and after 30, 60, 90, 120, and 150 minutes. The percentage inhibition of inflammation was calculated using the following ratio (Ijeoma et al., 2011)**:**

$$Percentages\ inhibition\ of\ inflammation = \frac{[(Vt - Vo)control - (Vt - Vo)]test}{(Vt - Vo)control} \times 100 \quad (05)$$

Where Vt is the average volume for each group after treatment, and Vo is the average volume for each group before any treatment.

Statistical analysis

Results are expressed as mean ± Standard Error of Mean (SEM). Data was analysed using GraphPad Prism software version 10.1.0. Comparison between different groups was done by One-Way Analysis of Variance (ANOVA) followed by Tukey's test. A *p*-value less than 0.05 was considered statistically significant.

RESULTS

Preliminary phytochemical screening

Preliminary phytochemical screening of the extract (Table 1.) revealed the absence of anthraquinone glycosides and cyanogenic glycosides. However, the analysis confirmed the presence of multiple bioactive compounds, including carbohydrates, tannins, saponins, flavonoids, alkaloids, quinine, terpenoids, glycosides, triterpenoids, phenols, steroids, and cardiac glycosides. These findings suggest that IHE contains phytoconstituents potentially responsible for analgesic and anti-inflammatory effects.

Table 1. Results of preliminary phytochemical screening

Sr. No.	Phytoconstituent	Test name	Observation	Result
1	Carbohydrate	Benedict test	Reddish brown colour	+ ve
		Molisch test	Reddish or purple colour	+ ve
2	Tannin	Ferric chloride test	Green or black colour	+ ve
3	Saponin	Foam test	White foam	+ ve
4	Flavonoid	Shinoda test	Magneta or red colour	+ ve
5	Alkaloid	Mayer's test	Yellow colour	+ ve
		Wagner's test	Brown or reddish colour	+ ve
		Dragendroff test	Orange-red colour	+ ve
6	Quinine	Test for quinine	Red hue obtained	+ ve
7	Terpenoids	Salkowski test	Reddish brown colour	+ ve
8	Glycoside	Bromine water test	Yellow colour	+ ve
9	Anthraquinone Glycoside	Bontrager's test	Pink, Violet, or red colour	- ve
10	Triterpenoid	Liebermann Burchard test	Deep red colour	+ ve
11	Phenol	Ferric chloride test	Green colour	+ ve
12	Steroid	Salkowski test	Brown colour	+ ve
13	Cardiac glycoside	Test for cardiac glycoside	Blood red colour	+ ve
14	Cyanogenic glycoside	Picrate-impregnated paper test	Picrate-impregnated paper colour changes	- ve

+ ve: positive; -ve: negative

Quantitative estimation of phytoconstituents

Quantitative analysis of phytoconstituents in IHE is presented in Table 2. Tannins, phenolic compounds, alkaloids, and flavonoids were quantified and expressed as gram percentage (gm%) relative to standard reference compounds. The analysis revealed a higher concentration of tannins compared to phenolic compounds in the extract.

Table 2. Total secondary metabolite content in gm% equivalent to standards

Phytoconstituent	According to the calibration curve of the standard	Result expressed for the extract in	Secondary metabolites (gm%)
Tannins	Tannic acid	gm% equivalent to tannic acid	8.332
Phenolic compounds	Gallic acid	gm% equivalent to gallic acid	4.928
Alkaloids	Atropine sulphate	gm% equivalent to atropine sulphate	7.186
Flavonoids	Quercetin	gm% equivalent to quercetin	8.021

Gas chromatography mass spectrometry analysis

GC-MS analysis of *I. hederifolia* extract (Figure 1.) identified 73 compounds through NIST14 library matching. Among these, 23 bioactive compounds with established pharmacological properties are presented in Table 3. The majority of these identified compounds demonstrate significant antioxidant, analgesic, and anti-inflammatory activities.

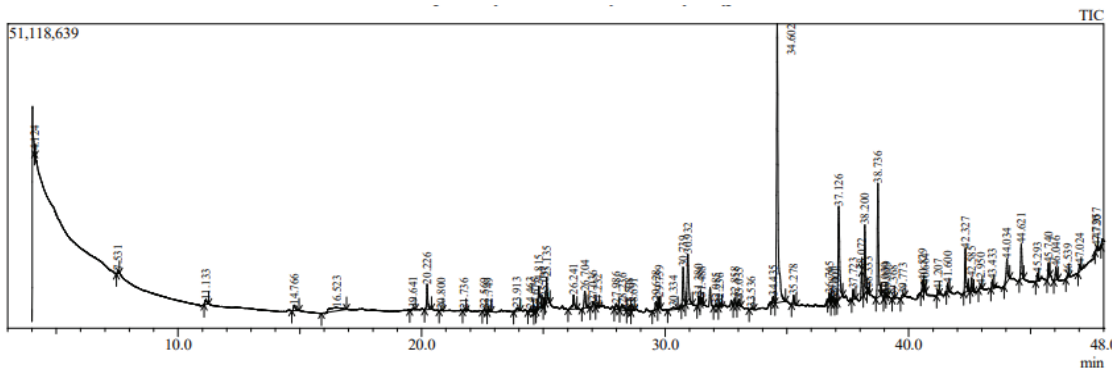

Figure 1. GCMS Chromatogram of the ethanolic extract of *I. hederifolia*

Table 3. Bioactive compounds present in the ethanolic extract of *I. hederifolia*

RT	Compound	Class	Area %	Activity	Reference
11.133	Propane, 1,1,3-triethoxy-	Retinaldehyde	0.53	Antioxidant activity, Anti-inflammatory, Upper respiratory infections.	(Willie et al., 2020)
14.766	Dodecane	Alkane	0.65	Antibacterial activity	(Padma et al., 2019)
20.226	Tetradecane	Alkane	1.81	Antimicrobial Activity, Wound healing activity, Anti-viral and Antitumor activities	(Velmurugan & Anand, 2017)
22.749	Pentadecane	Alkane	0.19	Anti-inflammatory, Analgesic, and Antipyretic	(Okechukwu, 2020)
23.913	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4	Benzofurans	0.42	Antimicrobial	(Mujeeb, Bajpai, & Pathak, 2014)
24.463	9,19-Cyclolanostan-3-ol, acetate, (3. beta.)-	Triterpenoid	0.29	Anti-mosquito larvicidal activity	(Ali, Mohammed, & Imad, 2016)
25.135	Hexadecane	Alkane	1.82	Antibacterial, Antioxidant activities	(Velmurugan & Anand, 2017)
28.236	1,1,4,7-Tetramethyldeca-hydro-1H-cyclopropa [e] azulene-4,7-diol	Azulene	0.82	Antioxidant, Antimicrobial	(Willie et al., 2020)
28.661	Thunbergol	Diterpene alcohol	0.08	Antidiabetic, Anticancer, Analgesic, anti-inflammatory, and Antimicrobial.	(Shah et al., 2023)
29.628	Tetradecanoic acid, ethyl ester	Fatty acid ester	0.62	Antioxidant, Anticancer, Nematicide, Hypocholesterolemic, Lubricant.	(Krishnamoorthy & Subramaniam, 2014)
30.739	Neophytadiene	Sesquiterpene	3.05	Analgesic, Anti-inflammatory, Antimicrobial, Antioxidant, Antifungal	(Pratama, Tunjung, Sutikno, & Daryono, 2019; Willie et al., 2020)
31.380	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Terpene alcohol	0.49	Anti-inflammatory, Antioxidant, and Analgesic	(Musa et al., 2022; Rajeswari, Murugan, & Mohan, 2012)

RT	Compound	Class	Area %	Activity	Reference
33.035	Hexadecanoic acid, methyl ester	Fatty acid ester	0.45	Anti-inflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge, Antihistaminic, Antieczemic, Antiacne, Alpha reductase inhibitor, Antiandrogenic, Antiarthritic, Anti coronary, Antifibrotic.	(Krishnamoorthy & Subramaniam, 2014; Usha et al., 2021)
33.536	Isophytol	Terpenoid alcohol	0.15	Anti-inflammatory and Antioxidant	(Musa et al., 2022)
34.602	Hexadecanoic acid, ethyl ester	Fatty acid ester	25.18	Antioxidant activities and Anti-inflammatory	(Musa et al., 2022; Sheela & Uthayakumari, 2013)
35.278	E, E, Z-1,3,12-Nonadeca-triene-5,14-diol	Fatty alcohol	0.76	Antimicrobial activity	(Mohammed, Ghaidaa, & Imad, 2016)
36.871	9-Octadecenoic acid, methyl ester, (E)-	Fatty acid ester	0.47	Anti-inflammatory, Antiandrogenic cancer preventive, Dermatitigenic hypocholesterolemic, 5-alpha reductase inhibitor, Anemiagenic, Insectifuge	(Krishnamoorthy & Subramaniam, 2014)
37.126	Phytol	Diterpene	8.76	Anticancer, Antioxidant, Anti-inflammatory, Diuretic, Antitumor, Chemopreventive, Antimicrobial, Use in vaccine formulations.	(Krishnamoorthy & Subramaniam, 2014; Musa et al., 2022; Sheela & Uthayakumari, 2013; Velmurugan & Anand, 2017; Willie et al., 2020)
38.333	(E)-9-Octadecenoic acid ethyl ester	Fatty acid ester	0.21	Steroids, fertility.	(Idu, Aihiokhai, Imoni, Akokigho, & Olali, 2021)
38.736	Octadecanoic acid, ethyl ester	Saturated fatty acid ethyl ester	8.73	Antioxidant, Anti-inflammatory, Antifungal, Antitumor, Antibacterial	(Ganesh & Mohankumar, 2017; Idu et al., 2021)
41.600	4,8,12,16-Tetramethylhepta-decan-4-olide	Diterpenoid	0.57	Anticancer	(Pratama et al., 2019)
42.327	Methyl 19-methyl-eicosa-noate	Fatty acid ester	3.48	Antifungal and antigenotoxic	(Ganesh & Mohankumar, 2017)
49.900	Squalene	Triterpene	1.37	Antioxidant, Antistatic, Antibacterial, Anticancer, Antitumor.	(Sheela & Uthayakumari, 2013; Willie et al., 2020)

DPPH radical scavenging activity

The antioxidant potential of *I. hederifolia* was evaluated using the DPPH free radical scavenging assay. As shown in Figure 2, the extract demonstrated concentration-dependent antioxidant activity, with percentage inhibition increasing proportionally with extract concentration. The maximum inhibition of 83.87% was observed at 250 µg/mL, while the minimum inhibition (36.95%) occurred at 1.9 µg/mL. The IC_{50} value of the extract (54.2 µg/mL) was significantly higher than that of ascorbic acid (2.28 µg/mL), indicating comparatively lower antioxidant potency relative to the reference standard.

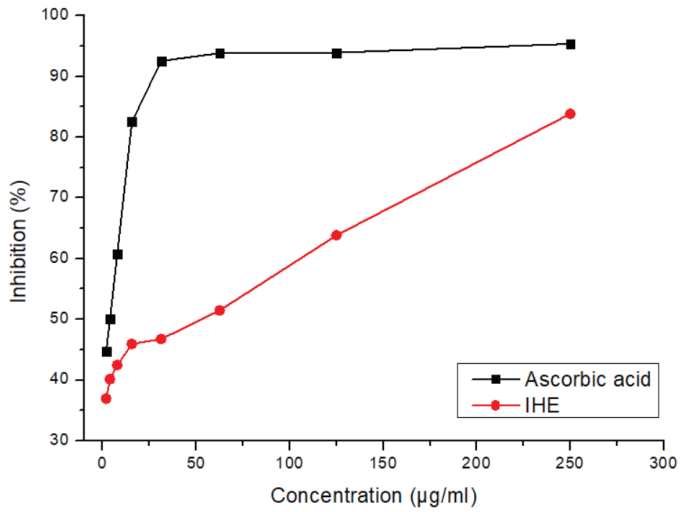

Figure 2. DPPH radical scavenging activity of ethanolic extract of *I. hederifolia* and ascorbic acid

***In vitro* anti-inflammatory assay**

The *in vitro* anti-inflammatory activity of IHE was assessed using the protein denaturation assay. As illustrated in Figure 3, both IHE and the reference standard (diclofenac sodium) exhibited concentration-dependent inhibition of protein denaturation. At the highest tested concentration (1000 µg/mL), IHE demonstrated 89.21% inhibition compared to 95.19% for diclofenac. Similarly, at the lowest concentration (31.25 µg/mL), the inhibition percentages were 78.52% for IHE and 81.30% for the standard drug, indicating comparable dose-response profiles between the test extract and positive control.

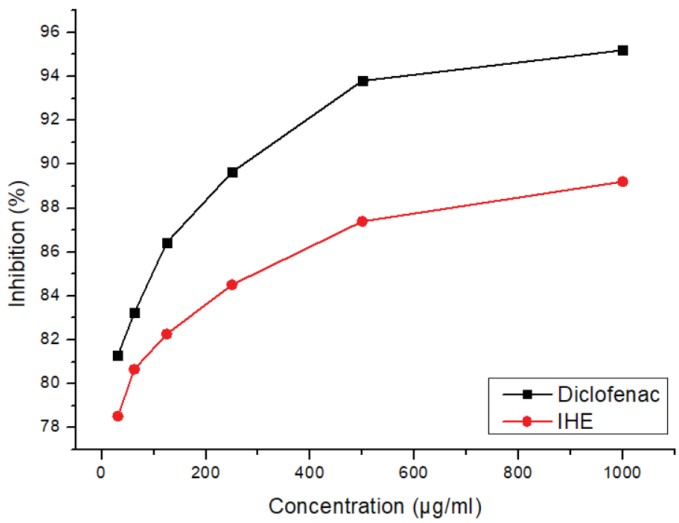

Figure 3. Comparison of protein denaturation inhibition by diclofenac and ethanolic extract of *I. hederifolia*

Acute oral toxicity study

The acute oral toxicity study revealed no adverse effects at the maximum tested dose (2000 mg/kg). All animals survived the observation period with normal food intake and body weight gain. Comprehensive clinical evaluations, including assessments of skin, fur, eyes, mucous membranes, respiratory function, and neurological status (with particular attention to tremors, convulsions, and coma), showed no abnormalities. Behavioral patterns and autonomic functions remained unaffected, with no signs of toxicity or mortality observed. Based on these results, doses of 200 mg/kg (10% of the maximum dose) and 400 mg/kg were selected for subsequent analgesic and anti-inflammatory evaluations.

Hot plate test

The results of the analgesic effect by using the hot plate method are shown in Table 4 and Figure 4. This method was performed to determine the central analgesic activity of IHE in rats. Low dose of IHE showed significant (p<0.05) central analgesic activity by delaying the reaction time at 30-minutes time interval of observation as compared with the normal control (Figure 4.). Similarly, all other groups showed significant (p<0.001) analgesic activity at 30, 60, 90, and 120-minutes time intervals when compared with the normal control group. At a 90-minutes time interval the low-dose extract showed significant (p<0.05) analgesic activity when compared with the standard drug, and the high-dose extract showed significant (p<0.001) analgesic activity. The intergroup comparison between low-dose and high-dose extract showed significant (p<0.05) analgesic activity at a 30-minutes time interval. The maximum possible analgesic activities were observed at 60 minutes for extracts 200 mg/kg and 400 mg/kg which were 39.45% and 52.35% respectively, as compared with the standard drug (tramadol 40 mg/kg), which produced 56.95% analgesic activity. The maximum analgesic activities observed at 90 minutes for extracts 200 mg/kg and 400 mg/kg were 31.87% and 75.54% respectively, as compared with the standard drug (tramadol 40 mg/kg) that produced 79.05% analgesic activity.

Table 4. Effect of ethanolic extract of *I. hederifolia* on hot plate latency time in rats

Group	**Normal Control**	**Standard**		**Low-dose extract**		**High dose extract**	
Drug	Distilled water	Tramadol		IHE		IHE	
Dose	10 mL/kg	40 mg/kg		200 mg/kg		400 mg/kg	
Time (Min)	**Paw licking and jumping response (Sec) ± SEM and % MPA**						
0	16.90±0.47	17.84±1.03	3.36%	24.69±3.88	27.74%	22.17±3.11	18.75%
30	17.02±0.36	26.05±1.65$^{\#c}$	32.25%	28.82±3.68$^{\#a*a}$	42.17%	26.04±2.74$^{\#c*a}$	32.24%
60	17.32±0.57	33.08±2.05$^{\#c}$	56.95%	28.24±3.25$^{\#c}$	39.45%	31.81±2.80$^{\#c}$	52.35%
90	17.76±0.61	39.29±1.21$^{\#c}$	79.05%	26.45±3.61$^{\#c\dagger a}$	31.87%	38.34±1.91$^{\#c\dagger c}$	75.54%
120	17.19±0.62	40.11±3.41$^{\#c}$	82.42%	18.64±1.33$^{\#c}$	05.19%	23.55±1.95$^{\#c\dagger c}$	22.84%

Values represent mean ± standard error of the mean; n=6, analysis was performed using One-Way ANOVA followed by Tukey's multiple comparison test, p value less than 0.05 was considered statistically significant. a: p<0.05, b: p<0.01, c: p<0.001, #: Data compared with control, †: Data compared with standard treatment, *: Data compared with low dose and high dose.

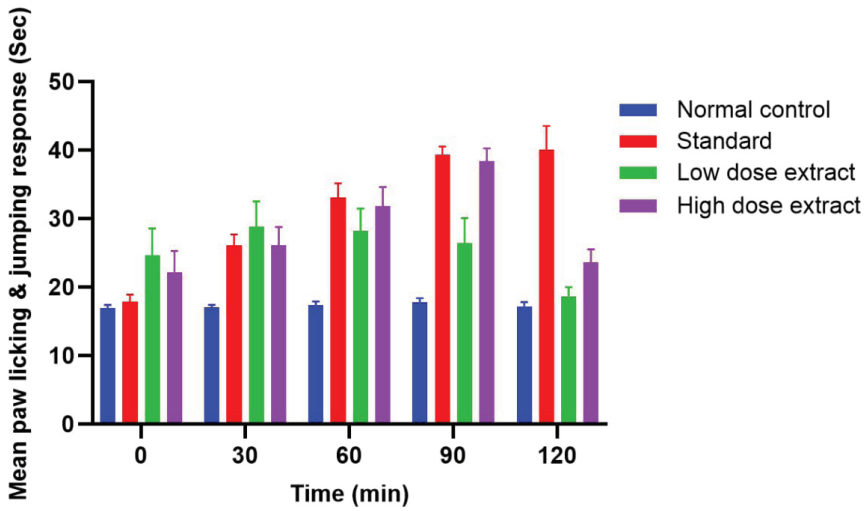

Figure 4. Comparison between groups vs mean response (sec) of the hot plate method

Acetic acid-induced writhing test

The results of the acetic acid-induced writhing test model in mice by using ethanolic IHE are shown in Table 5 and Figure 5. In this test, IHE at both test doses (i.e., 200 and 400 mg/kg) showed statistically significant (p<0.05) peripheral analgesic activity in a dose-dependent manner as compared to the normal control. High-dose (400 mg/kg) of the extract produced significant (p<0.001) protection against the number of writhings induced by acetic acid. The extent of reduction of writhing in the different doses of the extract was different, i.e., significantly lower in 200 mg/kg (p<0.01) and comparable with 400 mg/kg (p<0.001) as compared to the standard drug of 50 mg/kg (p<0.001) diclofenac sodium.

Inter-group comparison between the two extract doses does not show a statistically significant (p > 0.05) difference. The high dose of IHE (400 mg/kg) along with the standard drug (diclofenac sodium 50 mg/kg) showed comparable % protection of the numbers of writhing with percentage protection values of 50.23 % and 57.20 % respectively.

Table 5. Effect of the ethanolic extract of *I. hederifolia* on acetic acid-induced writhing in mice

Group	Drug	Dose	Mean number of writhing ± SEM	% Protection
Normal Control	Normal Saline	10 mL/kg	35.83±2.76	0
Standard	Diclofenac Sodium	50 mg/kg	15.33±1.11$^{\#c}$	57.20
Low dose IHE	IHE	200 mg/kg	24.66±1.52$^{\#b\dagger b}$	31.16
High dose IHE	IHE	400 mg/kg	17.83±1.25$^{\#c}$	50.23

Values represent mean ± standard error of the mean; n=6, analysis was performed using One-Way ANOVA followed by Tukey's multiple comparison test, p value less than 0.05 was considered statistically significant. a: $p<0.05$, b: $p<0.01$, c: $p<0.001$, #: Data compared with control, †: Data compared with standard treatment.

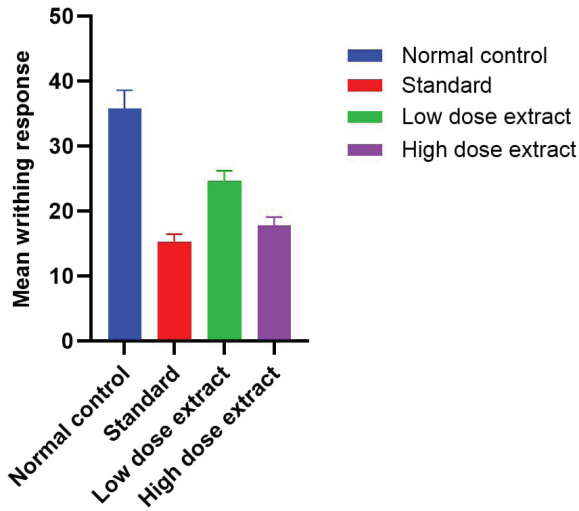

Figure 5. Comparison between groups and the mean number of writhing in the writhing test

The carrageenan-induced paw edema method

The results of the anti-inflammatory effect by using the carrageenan-induced paw edema method are shown in Table 6 and Figure 6. This method was performed to determine the acute inflammatory activity of IHE in rats. The mean paw volume was taken 60 minutes before each carrageenan induction. The results showed that low dose (200 mg/kg), high dose (400 mg/kg) of IHE and standard drug (Diclofenac 10 mg/kg) at 60, 90, 120,150 minutes showed significant (p<0.001) anti-inflammatory activity as compared with the control group. Low dose of extract at 120 minutes showed significant (p<0.05) anti-inflammatory activity as compared to the standard. Both low and high doses of extract at 150 minutes showed significant (p<0.001) anti-inflammatory activity. The inter-group comparison between low-dose and high-dose extract showed significant (p<0.05) anti-inflammatory activity. The maximum anti-inflammatory activity at 150 minutes of low-dose and high-dose extracts showed 52.77 % and 61.69 % respectively, as compared with the standard drug that produced 74.69 %.

Table 6. Effect of ethanolic IHE on carrageenan-induced paw edema method

Group	**Normal Control**	**Standard**		**Low-dose extract**		**High dose extract**	
Drug	Distilled water	Diclofenac		IHE		IHE	
Dose	10 mL/kg	10 mg/kg		200 mg/kg		400 mg/kg	
Time (min)	**Mean paw volume (CC) ± SEM and % Inhibition**						
-60	0.383±0.014	0.372±0.02		0.418±0.008		0.412±0.003	
30	0.740±0.009	0.728±0.009	0.28%	0.779±0.016	-1.12%	0.751±0.025	5.04%
60	0.815±0.012	0.701±0.021$^{\#c}$	23.84%	0.745±0.020$^{\#a*a}$	24.30%	0.690±0.007$^{\#c*a}$	35.64%
90	0.810±0.011	0.686±0.008$^{\#c}$	26.46%	0.682±0.02$^{\#c}$	38.17%	0.642±0.014$^{\#c}$	46.13%
120	0.801±0.005	0.608±0.010$^{\#c}$	43.54%	0.664±0.02$^{\#c\dagger a}$	41.15%	0.620±0.014$^{\#c}$	50.24%
150	0.798±0.015	0.477±0.015$^{\#c}$	74.69%	0.614±0.017$^{\#c\dagger c}$	52.77%	0.571±0.007$^{\#c\dagger c}$	61.69%

Values represent mean ± standard error of the mean; n=6, analysis was performed using One-Way ANOVA followed by Tukey's multiple comparison test, p value less than 0.05 was considered statistically significant. a: p<0.05, b: p<0.01, c: p<0.001, #: Data compared with control, †: Data compared with standard treatment, *: Data compared of low dose and high dose.

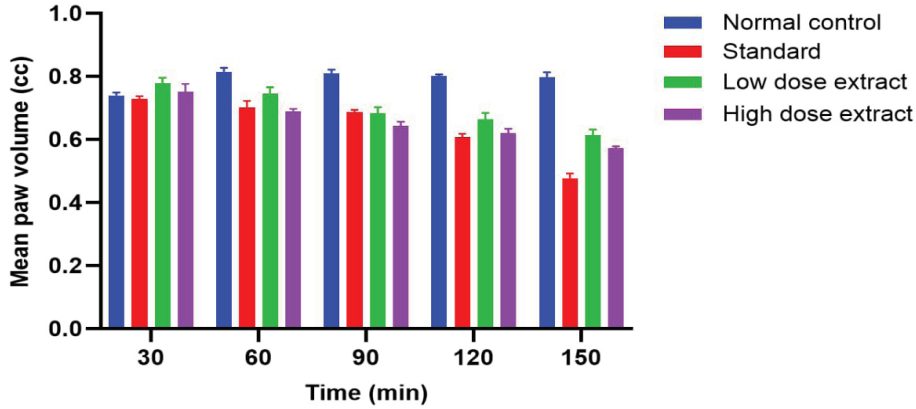

Figure 6. Comparison between groups vs. the mean paw volume of inflammation

DISCUSSION

The literature survey revealed that the herb *I. hederifolia* has been used as a traditional medicine for the treatment of inflammation (Kurian et al., 2023). The herb contains alkaloids, flavonoids, tannins, steroids, *etc* phytoconstituents which are responsible for analgesic and anti-inflammatory activity (Fan et al., 2014; Ijeoma et al., 2011). Thus, the present study was designed to evaluate traditional claims for the above-mentioned use of *I. hederifolia*.

The results showed significant analgesic and anti-inflammatory effects, which can be attributed to the presence of bioactive compounds within the extract. These effects were evaluated through *in vitro* assays, including protein denaturation and antioxidant assay, complemented by phytochemical screening, GC-MS analysis, *in vivo* study of analgesic effect evaluated by hot plate method and acetic acid induced writhing method, and anti-inflammatory effect evaluated by carrageenan-induced paw edema method.

The phytochemical analysis of ethanolic IHE revealed the presence of various bioactive compounds such as carbohydrates, alkaloids, tannins, saponins, flavonoids, cardiac glycosides, anthraquinone, steroids, phenol, triterpenoids, and glycosides. The quantitative study of IHE shows tannins 8.33%, flavonoids 8.02%, phenolics 4.93%, and alkaloids 7.18%, respectively. These results indicated that the ethanolic extract contains a high % of tannins and flavonoids.

The percentages of phenolics and flavonoids were found to be higher than those reported previously for the leaves of the plant (Suchiang, Devi, Zuala, & Shantabi, 2024). According to the literature, alkaloids, flavonoids, steroids, and tannins show analgesic and anti-inflammatory activity (Fan et al., 2014; Ijeoma et al., 2011). The presence of these compounds supports the pharmacological activities of the extract. Flavonoids and steroids act by inhibiting prostaglandin production, which inhibits pain and inflammation (Rafieian-kopaei, Shakiba, Sedighi, & Bahmani, 2017). GCMS analysis showed that most of the compounds present in IHE possess antioxidant, analgesic, and anti-inflammatory activity. Previous phytochemical studies have reported that *I.* hederifolia contains several bioactive constituents, including the pyrrolizidine alkaloids ipanguline and isoipanguline; the nortropane alkaloids calystegines B1 and B2, known for their glycosidase inhibitory activity; as well as cyanogenic glycosides, ergoline derivatives, isoenzymes, and hederifolic acids A–D, which are peracetylated glycosidic acid derivatives classified as resin glycosides (Ravikumara, Kaveri, & Umesha, 2025). The synergistic action of these phytochemicals may contribute to the overall analgesic and anti-inflammatory activity of the IHE (Fan et al., 2014; Ijeoma et al., 2011).

Oxidative stress is a major contributor to inflammation and pain, as excessive free radical generation

amplifies inflammatory signalling and tissue injury. Antioxidants play a crucial role in mitigating oxidative stress by neutralizing these reactive species. The ethanolic extract of IHE exhibited dose-dependent DPPH free radical scavenging activity comparable to that of ascorbic acid. However, since this study employed only the DPPH assay, the observed results represent the extract's potential for DPPH radical neutralization and do not reflect its overall antioxidant capacity, which would require evaluation through additional assays based on different mechanisms. The IC_{50} value obtained for the extract was higher than that reported by Suchiang et al. (2024), which may be attributed to variations in plant collection, extraction methods, and processing conditions. The antioxidant property of *I. hederifolia* may contribute to its analgesic and anti-inflammatory effects by reducing oxidative stress and preventing cellular damage. These protective effects, together with possible direct protein stabilization mechanisms (Hossain et al., 2022), may underlie the extract's efficacy in managing pain and inflammation. Future studies employing assays such as ABTS, FRAP, or ORAC, which operate through different antioxidant mechanisms, are warranted to provide a more comprehensive evaluation of the antioxidant potential of *I. hederifolia*.

Protein denaturation is a process in which proteins lose their tertiary and secondary structures, leading to loss of function. Inflammation involves the denaturation of proteins, but bioactive compounds present in IHE may inhibit protein denaturation and therefore, can be considered as potential anti-inflammatory agents (Osman, Sidik, Awal, Adam, & Rezali, 2016). The ethanolic IHE shows dose-dependant protein denaturation as compared with the standard drug diclofenac. The protein denaturation inhibition percentage of IHE was found to be 78.52% to 89.20% and standard diclofenac was 81.30% to 95.19%, respectively. This indicates that the IHE contains bioactive compounds that may stabilize proteins and prevent their denaturation, thereby exhibiting anti-inflammatory properties. It suggests that IHE may show therapeutic potential in treating inflammatory conditions.

The hot plate test measures the paw licking and jumping response to thermal pain, which indicates central analgesic activity. The IHE significantly (P<0.05) increased the pain threshold in low-dose (200mg/kg) animals at 30-minutes times compared to the normal control group. Similarly, all other low and high dose of IHE at 30, 60, 90, 120 minutes shows very significant (p<0.001) analgesic activity that suggesting the involvement of central mechanisms like activation of μ-receptor at supraspinal sites, $_{K}$-receptor within the spinal cord and δ-receptor involved at both spinal and supraspinal level (Fan et al., 2014; Faujdar, Sharma, Sati, Pathak, & Paliwal, 2016). At a 90-minute time interval, the low-dose extract shows significant (p<0.05) analgesic activity when compared with the standard drug, and the high-dose extract shows very significant (p<0.001) analgesic activity. The maximum possible analgesia at a 90-minute time interval was less than 50% analgesic activity in low-dose extract, while the high-dose extract showed 75.54% activity. Similarly, standard drug tramadol showed 82.42% analgesic activity at a 120-minute time interval. It suggests that IHE showed time-dependent analgesic activity at a specific time level, but after a certain time, there is a decrease in activity.

The acetic acid-induced writhing test measures the number of writhes in response to an irritant, which is indicative of peripheral analgesic activity. This activity may be mediated through the inhibition of COX or lipoxygenase enzyme (Faujdar et al., 2016). Both low (200 mg/kg) and high (400 mg/kg) dose of IHE shows dose-dependent reduction in the number of writhes, implying that it possesses peripheral analgesic properties compared to the normal control group. Both doses of IHE show significant results as compared to the normal control group at P<0.05. Standard drug shows a significant result as compared to the low-dose extract at P<0.05.

The analgesic effects observed in IHE may be due to the modulation of endogenous opioid pathways, inhibition of pain mediators, or anti-inflammatory properties that reduce the underlying cause of pain (Tracey & Mantyh, 2007). It suggests that both the hot plate method and the acetic acid-induced writhing test method show the analgesic effect of ethanolic IHE. According to the study observation in both studied models, IHE shows a significant analgesic effect. From this, we conclude that the extract has central as well as peripherally acting analgesic effects.

The carrageenan-induced paw edema model is used to evaluate acute inflammation. The IHE significantly reduced paw edema in treated rats, which indicates its effectiveness in reducing acute inflammatory responses. Inflammation is inhibited by COX-2 inhibitors, which leads to inhibition of prostaglandins (Wani et al., 2012). This reduction suggests the inhibition of mediators like histamine, serotonin, and prostaglandins, which are involved in the early phase of inflammation (Kaushik et al., 2012; Rafieian-kopaei et al., 2017). According to these mechanisms, our extract may show an anti-inflammatory effect.

Previous studies on other *Ipomoea* species, such as *Ipomoea pes-caprae* (Vieira et al., 2013) and *Ipomoea involucrate* (Ijeoma et al., 2011), have reported significant antinociceptive activity and anti-inflammatory activities. These findings support the possibility that similar bioactive constituents present in *I. hederifolia* may contribute to its observed analgesic and anti-inflammatory effects in the present study.

The overall study of ethanolic IHE shows no toxicity and promising analgesic and anti-inflammatory activity.

CONCLUSION

The present study provides compelling scientific evidence supporting the traditional use of *I. hederifolia* for pain and inflammation management. The ethanolic extract demonstrated significant antioxidant, analgesic, and anti-inflammatory activities, which were attributed to its rich phytochemical composition, including tannins, phenolics, alkaloids, and flavonoids. GC-MS analysis identified 23 bioactive compounds that likely contribute to these pharmacological effects.

The extract exhibited dual analgesic mechanisms (both central and peripheral) in rodent models and showed dose-dependent anti-inflammatory activity in the carrageenan-induced paw edema test. Importantly, acute toxicity studies revealed an excellent safety profile at doses up to 2000 mg/kg, suggesting its potential therapeutic window.

These findings validate the ethnomedicinal claims of *I. hederifolia* and highlight its potential as a source of novel bioactive compounds for developing natural analgesic and anti-inflammatory agents. Future studies should focus on isolating and characterizing the active constituents, elucidating their molecular mechanisms, and evaluating clinical efficacy.

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AUTHOR CONTRIBUTION STATMENT

Developing hypothesis (K.K.M., S.A.S), experimenting and statistics (K.K.M., B.D.J.), study text (B.D.J), literature research (S.A.S), interpretation of the data and reviewing (N.H.S., R.J.D.).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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