



International Journal of Research in Pharmacology & Pharmacotherapeutics (IJRPP)

IJRPP | Vol.14 | Issue 3 | Jul - Sept -2025

www.ijrpp.com

ISSN: 2278-2648

DOI : <https://doi.org/10.61096/ijrpp.v14.iss3.2025.631-639>

Research

Evaluation of Anti-Inflammatory and Analgesic Activities of Ethanolic Stem Extract of *Abutilon crispum*: An In Vitro Study

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	Abstract
Published on: 27 Sept 2025	Inflammation and pain are interrelated physiological responses that contribute to the pathogenesis of various acute and chronic diseases. This study evaluates the anti-inflammatory and analgesic potential of the ethanolic stem extract of <i>Abutilon crispum</i> (EEAC), a plant traditionally recognized for its medicinal properties, though its stem remains largely unexplored scientifically. The cytotoxicity profile of EEAC was assessed using the MTT assay on LPS-stimulated macrophages. EEAC exhibited high cell viability (>82%) even at the highest tested concentration (100 µg/mL), indicating strong cytocompatibility and suggesting mitochondrial protection. Anti-inflammatory activity was evaluated by quantifying nitric oxide (NO) levels using the Griess method. LPS-induced NO production (20.19 ± 0.22 µM/mL) was significantly reduced following EEAC treatment (17.45 ± 0.19 µM/mL), implying suppression of inducible nitric oxide synthase (iNOS) and potential modulation of the NF-κB signaling pathway. Furthermore, EEAC demonstrated notable analgesic activity by inhibiting prostaglandin E₂ (PGE₂) production. PGE₂ levels in LPS-induced cells (2206.2 ± 19.45 pg/mL) were reduced to 1101.4 ± 13.49 pg/mL upon EEAC treatment, suggesting inhibition of cyclooxygenase-2 (COX-2) activity. Although less potent than indomethacin (189.4 ± 3.39 pg/mL), EEAC exhibited a moderate yet promising analgesic effect. Overall, the extract's bioactivity appears to involve antioxidant defense mechanisms, suppression of key inflammatory mediators (NO and PGE₂), and cellular protection. These findings support the potential of EEAC as a natural therapeutic agent with dual anti-inflammatory and analgesic properties, warranting further investigation for plant-based interventions in inflammatory disorders.
Published by: Futuristic Publications	
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Keywords: <i>Abutilon crispum</i> ; Anti-inflammatory; Analgesic; Nitric oxide; Prostaglandin E ₂ ; COX-2	

1. INTRODUCTION

Inflammation and pain are two fundamental biological responses of the body to harmful stimuli such as pathogens, damaged cells, or irritants. Although they are vital for tissue repair and protection, chronic or excessive inflammation and pain can contribute to the pathogenesis of several diseases, including arthritis, cardiovascular disorders, neurodegenerative diseases, and cancers [1]. Despite the widespread availability of synthetic drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) and opioid analgesics, their prolonged use is associated with severe side effects, including gastrointestinal ulcers, renal toxicity, and dependence [2]. This has intensified the global search for safer, cost-effective, and naturally derived alternatives, particularly from medicinal plants, which have historically served as vital sources of pharmacologically active compounds.

Medicinal plants have played an integral role in traditional healthcare systems across cultures. Their use in managing inflammation and pain is well documented in systems such as Ayurveda, Traditional Chinese Medicine (TCM), Siddha, and Unani [3-5]. In Ayurvedic medicine, inflammation is often described as a result of imbalances in the *doshas* particularly *Pitta* and is treated using herbs known for their *shothahara* (anti-inflammatory) and *vedanasthapana* (analgesic) properties [6]. Plants like *Ashwagandha* (*Withania somnifera*) [7], *Guggulu* (*Commiphora mukul*) [8], *Shallaki* (*Boswellia serrata*) [9], and *Turmeric* (*Curcuma longa*) [10] have been traditionally prescribed for inflammatory conditions. These herbs have since been investigated for their bioactive phytoconstituents such as alkaloids, flavonoids, steroids, and terpenoids, which are known to modulate inflammatory mediators like prostaglandins, cytokines, and reactive oxygen species.

Among the vast array of plant species used traditionally, the genus *Abutilon* (Family: Malvaceae) holds a notable place in folk and Ayurvedic medicine. *Abutilon crispum*, commonly referred to as “bladdermallow” or “Indian mallow,” is an herbaceous plant widely distributed across India, Sri Lanka, and parts of Southeast Asia [11]. The species is known for its ethnomedicinal applications, and several parts of the plant have been used in the treatment of fever, ulcers, wounds, and inflammatory disorders [12]. Previous phytochemical studies have revealed that the plant contains bioactive constituents such as flavonoids, phenolic compounds, sterols, and mucilage, which are commonly associated with anti-inflammatory and analgesic properties [12].

Pharmacological exploration of *Abutilon crispum* has primarily focused on its leaves, which have shown promising results in various biological activities including antimicrobial, antioxidant, hepatoprotective, and anti-inflammatory actions [13]. However, despite these promising leads, no substantial scientific investigation has yet been directed toward evaluating the pharmacological potential of its stems. The stem of a plant often harbors a unique composition of secondary metabolites that may differ from those present in leaves or roots, and such parts are often underexplored despite their potential therapeutic value. The rationale for the present study is rooted in this gap in knowledge. Given the traditional use of *Abutilon crispum* and the bioactive potential demonstrated by other parts of the plant, it is plausible that the stems may also possess significant anti-inflammatory and analgesic properties. This study aims to investigate the anti-inflammatory and analgesic activities of the ethanolic extract of *Abutilon crispum* stems using standard in vivo models. Through this exploration, the study seeks to validate the traditional claims associated with the plant and to potentially uncover a new plant-based therapeutic candidate for inflammation and pain management.

2. MATERIALS AND METHODS

2.1. Collection, authentication, and preparation of *Abutilon crispum* extract

The fresh stems of *Abutilon crispum* were collected from their natural habitat, ensuring they were free from disease or damage. The plant material was authenticated by a Dr. P Radha, Research Office (Botany), Sci II i/c, Siddha Medicinal Plants Garden, CCRS, Ministry of Ayush, Tamilnadu, and a voucher specimen was deposited for future reference (A260325272C). The collected stems were washed to remove dust and shade-dried at room temperature (25–30°C) for 10–15 days. Once dried, the stems were cut into small pieces and ground into a coarse powder using a mechanical grinder, then sieved through a 40-mesh sieve. For extraction, 100 g of the powdered stem was soaked in 1 L of ethanol in a sealed glass container and left to macerate at room temperature for 72 hours with intermittent shaking. The extract was then filtered using Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator at 40–50°C. The resulting semi-solid extract (EEAC) was further dried in a vacuum desiccator and stored in an air-tight container at 4°C for future use. The percentage yield of the extract was calculated based on the initial weight of the plant material and the final weight of the dried extract [14].

2.2. In-vitro analgesic and anti-inflammatory activity

2.2.1. MTT assay

The RAW 264.7 murine macrophage cell line was maintained in DMEM supplemented with 10% FBS, 10,000 µg/mL streptomycin, and 1% penicillin (10,000 units/mL) at 37 °C under a humidified atmosphere containing 5% CO₂. Cell viability was assessed using the MTT colorimetric assay. The RAW 264.7 cells were

seeded into plates and exposed to varying concentrations (1.5–100 µg/mL) of *A. crispum* ethanol extract. After 24 hours of incubation, the culture medium was removed, and MTT solution (0.5 mg/mL) was added to each well, followed by a further 2-hour incubation at 37 °C. Post-incubation, the medium was discarded, and 100 µL of isopropanol was added to each well to solubilize the formazan crystals. Absorbance was measured at 570 nm using a microplate reader. The percentage of cell viability was calculated using the following formula [15]:

$$\text{Viability \%} = \frac{\text{Absorbance}_{\text{treatment}} - \text{Absorbance}_{\text{background}}}{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{background}}} \times 100\%$$

2.2.2. In-vitro anti-inflammatory activity

The anti-inflammatory activity of *Abutilon crispum* ethanol extract was assessed by quantifying nitric oxide (NO) metabolite levels, specifically nitrite, in the cell culture supernatant using the Griess reagent method [15]. RAW 264.7 cells were seeded into a 48-well plate at a density of 1×10^6 cells/mL and incubated at 37 °C in a humidified atmosphere containing 5% CO₂ for 24 hours. The cells were pre-treated for 2 hours with non-toxic concentrations of the *Abutilon crispum* ethanol extract. Following this, they were stimulated with 1 µg/mL lipopolysaccharide (LPS) from *Escherichia coli* for an additional 22 hours. After incubation, 50 µL of the collected culture supernatant was mixed with 50 µL of Griess reagent (0.1% *N*-(1-naphthyl)ethylenediamine dihydrochloride in 5% phosphoric acid and 1% sulfanilamide) and incubated in the dark at room temperature for 10 minutes. The absorbance was measured at 540 nm using a microplate reader. Nitrite concentration was determined based on a sodium nitrite standard curve, and indomethacin (100 µM) was used as the positive control [15].

2.2.3. In-vitro analgesic activity

For the evaluation of analgesic activity, the concentrations that exhibited significant anti-inflammatory effects were selected. The levels of Prostaglandin E2 (PGE2) in the collected cell culture supernatants were measured using a commercially available quantitative enzyme-linked immunosorbent assay (ELISA) kit, following the manufacturer's protocol [15].

2.2.4. Statistical analysis

The statistical analysis for all experimental assays was performed using GraphPad Prism software. Data were expressed as mean ± standard deviation (SD) from at least three independent experiments conducted in triplicate. Comparisons between groups were carried out using one-way analysis of variance (ANOVA).

3. RESULTS

3.1. Collection, authentication and preparation of plant extract

The plant material of *Abutilon crispum* was collected from its natural habitat, ensuring healthy, undamaged, and disease-free specimens. Botanical identification was confirmed by Dr. P. Radha, Siddha Medicinal Plants Garden, CCRS, Ministry of Ayush, Tamil Nadu, and a voucher specimen (A260325272C) was deposited for future reference and reproducibility. The stems were shade-dried at ambient temperature (25–30°C) for 10–15 days to preserve thermolabile compounds, then pulverized into coarse powder to enhance solvent penetration. Extraction was performed by macerating 100 g of powdered material in 1 L of ethanol for 72 hours with intermittent shaking. Maceration was preferred to avoid degradation of heat-sensitive phytochemicals, while ethanol was chosen as a safe, broad-spectrum solvent compatible with traditional medicinal systems. Following extraction, the solution was filtered (Whatman No. 1) and concentrated using a rotary evaporator under reduced pressure at 40–50°C. The semi-solid extract was further dried in a vacuum desiccator to ensure stability for storage and pharmacological studies.

The process yielded 23.18% extract, indicating efficient recovery and a high content of ethanol-soluble phytoconstituents such as flavonoids, alkaloids, tannins, phenolics, and glycosides—compounds associated with significant pharmacological potential. The high extractive value demonstrates that minimal raw material can produce sufficient extract for bioassays, standardization, and future formulation, reducing resource requirements and environmental impact.

This efficient extraction protocol validates the richness of *A. crispum* in bioactive compounds and establishes a foundation for further phytochemical profiling, biological activity evaluation, and potential development as an evidence-based phytotherapeutic agent.

3.2. MTT assay

The cytotoxic profile of the ethanol extract of *A. crispum* (EEAC) was assessed using the MTT assay, which evaluates mitochondrial metabolic activity as an indicator of cell viability. Cells were treated with increasing concentrations of EEAC (1.5 to 100 µg/mL), and the percentage of viable cells was determined relative to untreated controls.

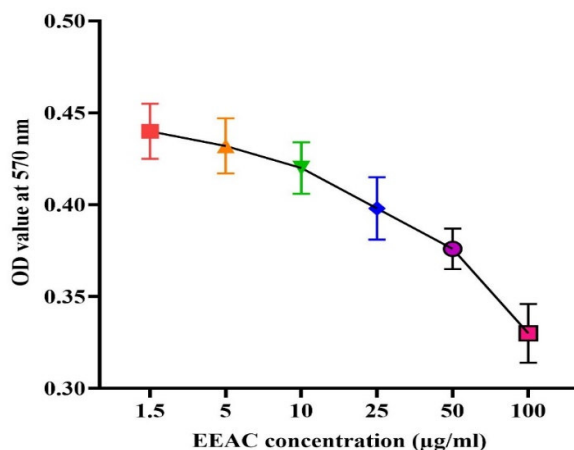


Fig 1: OD value of % cell viability of EEAC at 570 nm

The data showed a concentration-dependent decrease in cell viability. The mean viability at the lowest concentration (1.5 µg/mL) was $98.87 \pm 0.25\%$, indicating negligible cytotoxicity. As the concentration increased, a progressive reduction in viability was observed, with values of $97.10 \pm 0.20\%$ (5 µg/mL), $94.87 \pm 0.15\%$ (10 µg/mL), $91.00 \pm 0.20\%$ (25 µg/mL), $87.23 \pm 0.23\%$ (50 µg/mL), and finally, $82.00 \pm 0.30\%$ at the highest tested concentration of 100 µg/mL. Despite this downward trend, cell viability remained above 80% at all concentrations, indicating that EEAC exerts low cytotoxic effects within the studied dose range.

Table 1: OD value of % cell viability of EEAC at 570 nm

S. No.	Concentration of EEAC	OD value at 570 nm			Mean $\pm$ SD
1	Control	0.460	0.443	0.427	0.443 ± 0.016
2	1.5 µg/ml	0.456	0.438	0.426	0.440 ± 0.015
3	5 µg/ml	0.448	0.430	0.418	0.432 ± 0.015
4	10 µg/ml	0.436	0.418	0.406	0.420 ± 0.014
5	25 µg/ml	0.414	0.396	0.384	0.398 ± 0.017
6	50 µg/ml	0.392	0.374	0.362	0.376 ± 0.011
7	100 µg/ml	0.346	0.328	0.316	0.330 ± 0.016

All values are expressed in Mean with SD

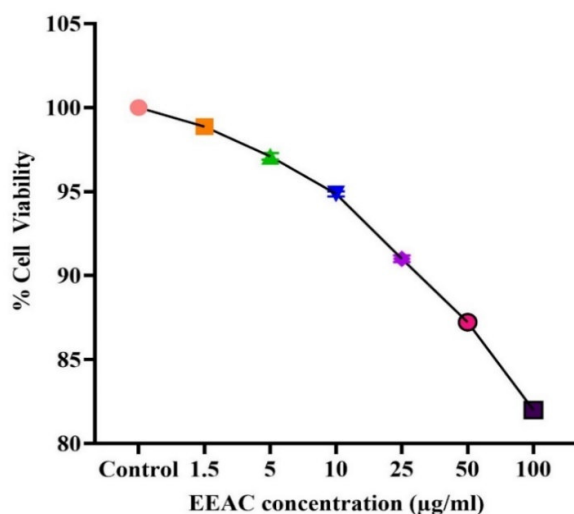


Fig 1: % cell viability of EEAC

Table 2: % cell viability of EEAC

S. No.	Concentration of EEAC	Cell viability (%)			Mean $\pm$ SD
1	Control	100	100	100	100.00
2	1.5 μ g/ml	98.6	99.1	98.9	98.87 $\pm$ 0.25
3	5 μ g/ml	97.3	96.9	97.1	97.10 $\pm$ 0.2
4	10 μ g/ml	95.0	94.7	94.7	94.87 $\pm$ 0.15
5	25 μ g/ml	91.2	90.8	91.0	91.00 $\pm$ 0.2
6	50 μ g/ml	87.5	87.2	87.0	87.23 $\pm$ 0.23
7	100 μ g/ml	82.3	82.0	81.7	82.00 $\pm$ 0.3

All values are expressed in Mean with SD

The MTT assay is a colorimetric assay that measures the enzymatic reduction of MTT by mitochondrial succinate dehydrogenase in viable cells. A reduction in MTT activity, reflected by decreased formazan formation, can result from various cellular events such as mitochondrial dysfunction, metabolic suppression, or the initiation of cell death pathways.

In the present study, treatment with EEAC produced a mild, concentration-dependent suppression of mitochondrial activity, as evidenced by the gradual decline in cell viability. However, the observed reduction was minimal and did not indicate overt cytotoxicity or cellular damage. This suggests that EEAC, even at higher concentrations, does not severely compromise mitochondrial function or cell membrane integrity.

The slight reduction in cell viability may be attributed to bioactive phytochemicals in the ethanol extract of *A. crispum*, such as flavonoids, phenolic acids, and terpenoids. These compounds are known to interact with cellular membranes and modulate intracellular signaling pathways. While many of these phytochemicals exhibit antioxidant properties, under certain conditions or at higher doses, they can display pro-oxidant effects, temporarily increasing reactive oxygen species (ROS) levels and altering mitochondrial redox states. Such shifts may moderately suppress mitochondrial respiration without causing apoptosis or necrosis, which aligns with the modest reduction in viability observed here.

Furthermore, cellular adaptation mechanisms in response to plant extract exposure, such as activation of stress response pathways (e.g., Nrf2/Keap1), may transiently reduce proliferation or energy metabolism, also contributing to the subtle decline in MTT activity. However, the preservation of >80% cell viability across all concentrations confirms the biocompatibility and non-toxic nature of EEAC in vitro.

This low cytotoxicity profile is promising, particularly for future pharmacological assays where the functional benefits of the extract such as antioxidant, anti-inflammatory, or metabolic modulatory activities can be evaluated without the confounding effects of cell death or toxicity.

3.3. Anti-inflammatory effect by NO inhibition

The Griess assay is a widely adopted, reliable colorimetric method to measure nitrite levels, the stable end product of NO degradation in aqueous solutions. It serves as an indirect but robust estimate of NO production.

- The control group (untreated cells) exhibited a baseline NO production with a mean total nitrite concentration of 9.78 ± 0.15 μ M/ml.
- Treatment with LPS (1 μ g/mL) significantly increased NO production, with a mean nitrite level of 20.19 ± 0.22 μ M/ml, indicating successful induction of inflammation.
- Cells treated with EEAC (100 μ g/mL) showed a notable decrease in nitrite concentration to 17.45 ± 0.19 μ M/ml, indicating inhibition of NO production.
- The standard drug Indomethacin (100 μ M/ml) demonstrated stronger NO inhibition, with a reduced nitrite level of 13.18 ± 0.18 μ M/ml.

Table 3: OD value of Total nitrite concentration (μ M/ml)

Group	OD value at 540 nm	Total nitrite concentration (μ M/ml)	Mean $\pm$ SD
Control	0.536	9.41	9.78 $\pm$ 0.15
	0.541	9.50	
	0.549	9.71	
LPS (1 μ g/mL)	0.907	20.32	20.19 $\pm$ 0.22
	0.914	20.47	
	0.929	20.75	
EEAC (100 μ g/mL)	0.762	16.37	17.45 $\pm$ 0.19
	0.774	16.61	

	0.789	16.74	
	0.644	12.48	
Indomethacin (100 µM/ml)	0.651	12.57	13.18 ± 0.18
	0.663	12.83	

All values are expressed in Mean with SD

In this study, stimulation with LPS (lipopolysaccharide) significantly elevated NO production, as evidenced by increased nitrite concentrations (20.19 ± 0.22 µM/ml). LPS binds to TLR4 (Toll-like receptor 4) on macrophages, activating downstream transcription factors like NF-κB and AP-1, which in turn upregulate iNOS gene expression. The ethanolic extract of *A. crispum* (EEAC), when administered at 100 µg/mL, significantly reduced NO production to 17.45 ± 0.19 µM/ml, compared to the LPS group, suggesting inhibition of inflammatory mediators and molecular pathways responsible for NO biosynthesis.

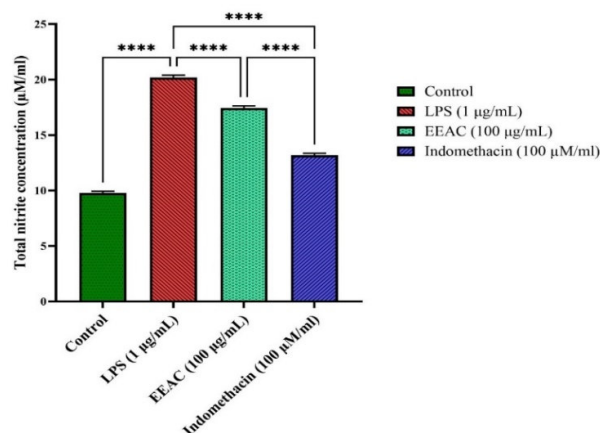


Fig 2: In-vitro nitric oxide inhibition assay

3.4. In-vitro analgesic activity

In the present study, PGE₂ levels in the untreated control group remained within the basal physiological range (176.0 ± 3.92 pg/mL), indicative of normal cellular homeostasis. However, treatment with lipopolysaccharide (LPS), a potent pro-inflammatory endotoxin derived from Gram-negative bacterial membranes, resulted in a robust increase in PGE₂ synthesis (2206.2 ± 19.45 pg/mL). This elevation reflects LPS-induced upregulation of COX-2 expression, along with downstream inflammatory mediators like TNF-α and IL-1β, which are known to drive PGE₂ production.

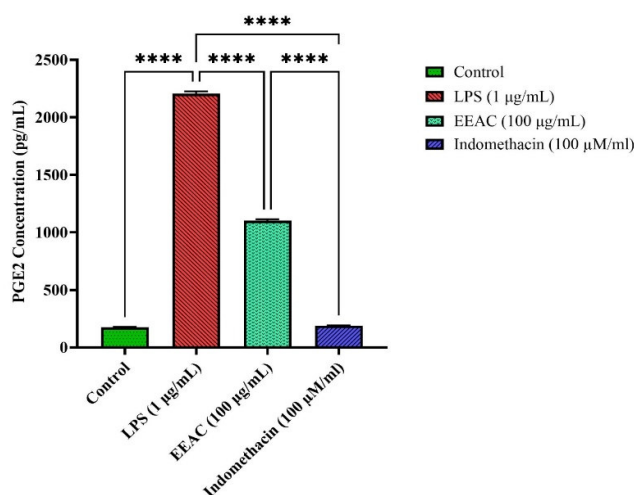


Fig 3: In-vitro analgesic activity of EEAC

Table 4: OD values of PGE₂ Concentration (pg/mL)

Group	OD value at 405 nm	PGE ₂ Concentration (pg/mL)	Mean ± SD (pg/mL)
Control	0.235	172.4	176.0 ± 3.92
	0.241	180.3	
	0.239	175.3	
LPS (1 µg/mL)	0.837	2210.4	2206.2 ± 19.45
	0.829	2195.2	
	0.841	2213.0	
EEAC (100 µg/mL)	0.619	1092.3	1101.4 ± 13.49
	0.631	1114.8	
	0.624	1097.1	
Indomethacin (100 µM/ml)	0.275	192.2	189.4 ± 3.39
	0.271	185.4	
	0.279	190.6	

Upon treatment with EEAC (100 µg/mL), a significant decrease in PGE₂ levels was observed (1101.4 ± 13.49 pg/mL), representing a ~50% inhibition compared to the LPS group. Although not as profound as the suppression observed with the standard NSAID indomethacin (189.4 ± 3.39 pg/mL), this reduction still signifies a meaningful attenuation of the inflammatory response. Indomethacin, as a potent non-selective COX inhibitor, serves as a benchmark for prostaglandin suppression, confirming the responsiveness of the system used.

4. DISCUSSIONS

Inflammation is a fundamental physiological response to injury and infection. However, its dysregulation is central to the pathogenesis of many chronic conditions, including arthritis, inflammatory bowel disease, and cardiovascular disorders. One of the molecular hallmarks of inflammation is the overproduction of nitric oxide (NO), particularly mediated by inducible nitric oxide synthase (iNOS) in activated macrophages. Nitric oxide, while essential for host defense and signaling, becomes detrimental when produced in excess, contributing to oxidative stress, DNA damage, and modulation of inflammatory cytokines [16].

Several bioactive compounds commonly reported in *A. crispum*, such as flavonoids (e.g., apigenin, luteolin), phenolic acids (e.g., caffeic acid), and essential oils, have been implicated in anti-inflammatory mechanisms [17]. The observed NO inhibitory effect of EEAC can be mechanistically explained through multiple biological actions. The suppression of iNOS mRNA and protein expression is a key mechanism underlying reduced NO production. Compounds such as apigenin and quercetin, often present in medicinal plants, are known to interfere with the transcriptional activation of the iNOS gene by inhibiting the nuclear translocation of NF-κB. In its active state, NF-κB binds to promoter regions of inflammatory genes, including iNOS. EEAC may inhibit upstream signaling pathways (e.g., MAPKs, PI3K/Akt), leading to reduced NF-κB activation and downstream iNOS suppression [18, 19]. Apart from gene regulation, polyphenolic constituents of EEAC might directly scavenge NO radicals. These compounds possess electron-donating groups capable of neutralizing free radicals and reactive nitrogen intermediates. This antioxidant property reduces cellular damage and helps maintain redox homeostasis during inflammation.

LPS-stimulated macrophages release cytokines such as TNF-α, IL-1β, and IL-6, which further amplify the inflammatory response and iNOS expression [20]. EEAC likely exerts a down-modulatory effect on these cytokines, indirectly reducing the NO burst. The anti-inflammatory effect of EEAC is potentially enhanced by its antioxidant activity, as flavonoids and phenolic compounds contribute to neutralizing superoxide anions (O₂⁻) and reducing lipid peroxidation. This dual antioxidant-anti-inflammatory action synergistically reduces the oxidative stress-induced NO production in inflamed cells. Indomethacin, a well-characterized NSAID, acts by inhibiting cyclooxygenase (COX) enzymes, thereby suppressing prostaglandin synthesis [21]. In this study, it significantly reduced NO levels to 13.18 ± 0.18 µM/ml, validating its potent anti-inflammatory action. Although EEAC was less effective than indomethacin, its natural origin, lower toxicity profile, and multitargeted action make it a promising candidate for further exploration as a plant-based anti-inflammatory agent.

The modulation of prostaglandin E₂ (PGE₂) synthesis plays a pivotal role in the pathogenesis and resolution of pain and inflammation. PGE₂ is one of the most abundant prostaglandins produced at sites of inflammation and is synthesized from arachidonic acid through the action of cyclooxygenase (COX) enzymes, particularly COX-2, which is inducible under inflammatory conditions such as bacterial infection, tissue damage, or immune cell activation [22]. Phytochemical constituents in *A. crispum*, particularly flavonoids, tannins, and

triterpenoids, are well-documented for their ability to modulate the expression and enzymatic activity of COX-2. Flavonoids such as quercetin, kaempferol, and apigenin (which are commonly found in plants of the Apiaceae family) can inhibit the transcriptional activation of COX-2 by interfering with the NF- κ B and AP-1 signaling pathways [23]. By suppressing the expression of COX-2 at the mRNA or protein level, these compounds reduce the conversion of arachidonic acid to PGE₂.

The inflammatory process is tightly linked with oxidative stress, where reactive oxygen species (ROS) further potentiate COX-2 expression and prostaglandin biosynthesis. EEAC may exert its effect indirectly by reducing oxidative stress through the action of antioxidant phytochemicals such as phenolic acids, flavonoids, and vitamin-like compounds. By quenching ROS, the extract limits redox-sensitive signaling cascades involved in COX-2 activation [24]. EEAC may interfere with intracellular pathways such as the MAPK/ERK and JAK/STAT cascades, which are essential in transmitting inflammatory stimuli that upregulate prostaglandin production. These pathways converge on transcription factors like NF- κ B, which is a master regulator of many pro-inflammatory genes, including COX-2. Suppression of NF- κ B nuclear translocation is another plausible mechanism for PGE₂ downregulation [25].

Unlike synthetic drugs, plant extracts typically contain a spectrum of bioactive molecules that act synergistically. The observed PGE₂ inhibition could result from the combined effects of several minor compounds acting at different targets within the inflammatory cascade, thereby providing a more balanced and potentially safer anti-inflammatory response. The implications of these findings suggest that EEAC holds significant potential as an analgesic and anti-inflammatory agent, particularly for conditions where elevated PGE₂ plays a pathogenic role, such as arthritis, muscle soreness, and other pain-related disorders. Although the extract's activity is not as potent as indomethacin, its natural origin and lower likelihood of causing side effects (e.g., gastrointestinal irritation, renal dysfunction) make it an attractive candidate for further development.

5. CONCLUSION

The ethanol extract of *Abutilon crispum* exhibited notable anti-inflammatory and analgesic potential. It significantly reduced nitric oxide (NO) production in LPS-stimulated macrophages by downregulating iNOS expression through NF- κ B inhibition and modulation of MAPK/PI3K-Akt pathways. EEAC's flavonoids and phenolic acids further contributed to direct NO scavenging, reducing oxidative stress and maintaining redox homeostasis. Additionally, EEAC suppressed prostaglandin E₂ (PGE₂) synthesis by interfering with COX-2 expression, a key mediator of pain and inflammation. This dual action highlights its potential as both an anti-inflammatory and analgesic agent. Although less potent than indomethacin, EEAC offers the benefits of a natural origin, multi-targeted mechanisms, and a lower risk of adverse effects. The findings validate the traditional use of *A. crispum* for inflammatory and pain-related disorders and warrant further studies on active components, in vivo efficacy, and clinical relevance.

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