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Anti-inflammatory and Analgesic Activities of Combined *Carica papaya* and *Ocimum sanctum* Leaf Extracts

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Artikel Penelitian

Abstract: *Inflammation and inflammatory pain remain important clinical problems, and long-term use of anti-inflammatory or analgesic drugs is often associated with adverse effects. Papaya (Carica papaya) and basil (Ocimum sanctum) leaves are widely used in traditional Indonesian medicine, yet evidence regarding their combined use remains limited. This study evaluated the phytochemical profile, anti-inflammatory activity, and analgesic effects of ethanolic extracts prepared from papaya leaf simplicia (EP), basil leaf simplicia (EK), and combined simplicia extracts at ratios of 1:1 (EP_1K_1), 3:1 (EP_3K_1), and 1:3 (EP_1K_3). Phytochemical screening revealed the presence of flavonoids, phenolics, alkaloids, saponins, and triterpenoids in all extracts, while tannins were detected only in basil extract. In vitro evaluation using the bovine serum albumin (BSA) protein denaturation assay showed that basil extract exhibited the strongest activity (IC_{50} = 82.44 µg/mL), whereas the combined extracts predominantly exhibited additive effects. In vivo testing using the carrageenan-induced paw edema model showed that papaya extract produced the highest anti-inflammatory inhibition (64.41%), while basil extract showed comparable activity to diclofenac sodium. Analgesic evaluation using the acetic acid-induced writhing test indicated that the basil-rich combination (EP_1K_3) showed the highest analgesic protection among the tested combinations. These findings indicate that papaya and basil extracts exhibit distinct yet complementary anti-inflammatory and analgesic activities. The combined simplicia extracts demonstrated additive pharmacological effects support the traditional use of these plants for the development of standardized herbal preparations.*

Keywords: *Anti-inflammatory, Analgesic, Carica papaya, Combined simplicia extract, Ocimum sanctum*

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Abstrak: Peradangan dan nyeri inflamasi masih menjadi permasalahan klinis yang penting, sementara penggunaan jangka panjang obat antiinflamasi dan analgesik sering dikaitkan dengan efek samping. Daun pepaya (*Carica papaya*) dan kemangi (*Ocimum sanctum*) telah lama dimanfaatkan dalam pengobatan tradisional Indonesia, namun kajian ilmiah mengenai penggunaan kombinasinya masih terbatas. Penelitian ini bertujuan untuk mengevaluasi profil fitokimia, aktivitas antiinflamasi, dan efek analgesik ekstrak etanol yang dibuat dari simplisia tunggal daun pepaya (EP), daun kemangi (EK), serta gabungan simplisia pada perbandingan 1:1 (EP_1K_1), 3:1 (EP_3K_1), dan 1:3 (EP_1K_3). Hasil skrining fitokimia menunjukkan adanya flavonoid, senyawa fenolik, alkaloid, saponin, dan triterpenoid pada seluruh ekstrak, sementara tanin hanya terdeteksi pada ekstrak yang mengandung kemangi. Uji *in vitro* menggunakan metode denaturasi protein *bovine serum albumin* (BSA) menunjukkan bahwa ekstrak kemangi memiliki aktivitas tertinggi (IC_{50}= 82,44 µg/mL), sedangkan ekstrak kombinasi menunjukkan efek yang bersifat aditif. Uji *in vivo* menggunakan model edema kaki mencit terinduksi karagenan menunjukkan bahwa ekstrak pepaya memberikan daya hambat antiinflamasi tertinggi (64,41%), sementara aktivitas ekstrak kemangi sebanding dengan natrium diklofenak. Pada uji analgesik model geliat mencit terinduksi asam

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asetat, kombinasi kaya kemangi (EP_1K_3) memberikan proteksi analgesik tertinggi di antara ekstrak kombinasi. Secara keseluruhan, hasil penelitian ini menunjukkan bahwa ekstrak daun pepaya dan kemangi memiliki aktivitas antiinflamasi dan analgesik yang berbeda namun saling melengkapi. Ekstrak kombinasi simplisia keduanya menunjukkan efek farmakologis aditif dan mendukung pemanfaatan tradisional tanaman ini sebagai kandidat untuk pengembangan sediaan herbal terstandarisasi.

Kata kunci: Antiinflamasi, Analgesik, *Carica papaya*, Ekstrak kombinasi simplisia, *Ocimum sanctum*

Introduction

Inflammation is a fundamental protective response that enables the body to eliminate harmful stimuli and initiate tissue repair. Under physiological conditions, this response is tightly regulated and self-limiting. However, persistent activation of inflammatory pathways contributes to the development and progression of chronic disorders, including rheumatoid arthritis, inflammatory bowel disease, atherosclerosis, diabetes, and various degenerative diseases (1,2). Sustained inflammation is also associated with increased oxidative stress and heightened nociceptor sensitivity, leading to amplified pain perception and reduced quality of life (3).

Plant-based therapies that may provide safer therapeutic alternatives, and both processes share overlapping biochemical pathways. A key mechanism involves the cyclooxygenase (COX)-prostaglandin cascade, which plays a central role in inflammatory responses and pain transmission (4,5). Although non-steroidal anti-inflammatory drugs (NSAIDs) are widely prescribed, their long-term use is often limited by adverse effects such as gastrointestinal irritation, renal impairment, and increased cardiovascular risk (6,7). Opioid analgesics are effective for managing severe pain but are associated with dependence and central nervous system suppression (8). These limitations have driven increasing interest in plant-based therapies that may offer safer alternatives with fewer side effects.

Medicinal plants contain diverse secondary metabolites, including flavonoids, alkaloids, phenolic compounds, terpenoids, and saponins, which can act on multiple molecular targets involved in inflammation and pain (9,10). Unlike many synthetic drugs that predominantly affect a single pathway, phytochemicals often modulate oxidative stress, inflammatory enzyme activity, and cellular membrane stability simultaneously, resulting in a broader pharmacological profile.

Two plants widely used in Indonesian traditional medicine, *Carica papaya* (papaya) and *Ocimum sanctum* (basil/kemangi), have been reported to possess anti-inflammatory and analgesic properties. Papaya leaves contain papain, alkaloids, flavonoids, and phenolic compounds that contribute to immunomodulatory and anti-inflammatory effects (11-13). Experimental studies have shown that papaya extracts can reduce oxidative stress and suppress the expression of inflammatory mediators such as inducible nitric oxide synthase (iNOS) and COX-2 (14,15). Basil leaves are rich in eugenol, rosmarinic acid, tannins, and other phenolic constituents that inhibit nuclear factor kappa B (NF-κB) activation, prostaglandin synthesis, and leukotriene formation (16-18). In addition, basil has been reported to enhance antioxidant defense mechanisms by increasing the activity of enzymes such as superoxide dismutase and catalase (19).

Although the pharmacological activities of papaya and basil have been individually well documented, studies examining their combined use remain limited. Most previous investigations prepared each plant extract separately and combined them after extraction, an approach that may not fully reflect traditional herbal preparation practices. In traditional settings, multiple plant materials are often mixed prior to extraction, allowing their constituents to interact during the extraction process, potentially affecting solubility, stability, and extraction efficiency (20). Such interactions may result in differences in biological activity compared with

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single-plant extracts or post-extraction mixtures (21).

In the present study, a distinct methodological approach was applied by combining papaya and basil leaf simplicia in defined ratios prior to extraction using 70% ethanol. This pre-extraction combination strategy enables comparative evaluation of pharmacological activities based on extract composition while maintaining a uniform extraction process. By adopting this approach, the study aims to provide experimental data on how combined simplicia extracts differ from single-plant extracts in terms of phytochemical profile, anti-inflammatory activity assessed through in vitro bovine serum albumin denaturation and in vivo carrageenan-induced paw edema models, and analgesic activity evaluated using the acetic acid-induced writhing test. This methodological perspective contributes to a clearer understanding of the pharmacological potential of combined herbal preparations and supports evidence-based development of standardized herbal products for inflammatory pain management.

Materials and Methods

Materials

Fresh and mature leaves of papaya (*Carica papaya* L., family *Caricaceae*) and basil (*Ocimum sanctum* L., family *Lamiaceae*) were obtained from Bantar Kambing and Sidangsari regions of Bogor, West Java, Indonesia, respectively. Both areas in Bogor have low pollution levels, which are favorable for the biosynthesis of secondary metabolites in medicinal plants. Botanical authentication was carried out at PT Palapa Muda Perkasa, Depok, Indonesia, and the specimens were deposited under voucher number 996/IPH.1.12/If.23/I/2024 for future reference.

All solvents and reagents used were of analytical grade. The extraction solvent used was 70% ethanol, selected due to its suitable polarity that ensures efficient recovery of both hydrophilic and moderately lipophilic phytochemicals such as flavonoids, saponins, and alkaloids (22). Other chemicals included sodium carboxymethyl cellulose (Na-CMC), bovine serum albumin (BSA), carrageenan (Sigma-Aldrich), acetic acid (Merck), and two reference standard drugs: diclofenac sodium for anti-inflammatory testing and mefenamic acid for analgesic testing (both from Merck, Germany).

For the in vivo experiments, healthy male *Deutch Democratic Yokohama* (DDY) mice weighing 20-30 g were used. Animals were acclimatized for one week under standard laboratory conditions (temperature 25 ± 2°C, relative humidity 60-70%, and 12 h light/dark cycle) with free access to food and water. All experimental procedures were approved by the Health Research Ethics Committee of Universitas Prima Indonesia (No. 014/KEPK/UNPRI/I/2025) and conducted in accordance with established international ethical guidelines for animal experimentation.

Methods

Preparation of Combined Leaf Simplicia

Fresh leaves were first washed thoroughly under running tap water to remove dust and debris, followed by rinsing with distilled water to ensure purity. Clean leaves were air-dried in the shade at temperatures not exceeding 40 °C to prevent degradation of heat-sensitive compounds such as papain and eugenol (23, 24). After complete drying, the leaves were ground into fine powder using a stainless-steel grinder and sieved through a 40-mesh sieve to obtain uniform particle size.

Moisture content was determined using the gravimetric oven-drying method with three replicates and found to be 6.76 ± 0.44% for papaya and 8.43 ± 0.30% for basil, both within the acceptable pharmacognostic limit of less than 10%, which ensures microbial stability and minimizes enzymatic degradation (25, 26).

The powdered simplicia were then combined in the following proportions:

a. EP: 100% *Carica papaya* leaf simplicia
b. EK: 100% *Ocimum sanctum* leaf simplicia
c. EP_1K_1: 1:1 (w/w) mixture of *Carica papaya* and *Ocimum sanctum* leaf simplicia
d. EP_3K_1: 3:1 (w/w)
e. EP_1K_3: 1:3 (w/w)

Each mixture (50 g) was homogenized thoroughly prior to extraction to ensure even distribution of constituents. The rationale for

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testing these ratios was to explore potential synergistic or additive interactions between the phytochemical constituents of both plants when combined prior to extraction, a concept supported by previous reports of polyherbal formulations showing enhanced efficacy over individual extracts (21, 20).

Extraction Procedure

Extraction was performed using Ultrasonic-Assisted Extraction (UAE). Which offers several advantages, including shorter extraction time, reduced solvent use, and higher yield compared to conventional maceration (27). The use of UAE also facilitated the disruption of plant cell walls through cavitation, thereby improving the release of bioactive compounds into the solvent phase.

For each mixture, 50 g of powdered sample was extracted using 70% ethanol at a ratio of 1:5 (w/v) in an ultrasonic bath operating at 40 kHz frequency for 25 minutes at ambient temperature. The use of 70% ethanol as solvent was based on its balanced polarity, which allows simultaneous extraction of phenolic compounds, flavonoids, and alkaloids without denaturing thermolabile enzymes such as papain (28).

After sonication, the extracts were filtered through Whatman No.1 filter paper and concentrated under reduced pressure using a rotary evaporator at 40°C. The concentrated filtrates were then dried in a desiccator until constant weight. Extraction yields were calculated based on dry weight, with results as follows:

a. EP: 9.9%
b. EK: 1.61%
c. EP_1K_1: 5.41%
d. EP_3K_1: 6.75%
e. EP_1K_3: 6.36%

The relatively lower yield of basil extract compared to papaya may be attributed to the higher volatile content of O. sanctum leaves and the lower proportion of nonpolar constituents soluble in hydroethanolic solvent (29).

Phytochemical Screening

Phytochemical screening was carried out using the classical methods introduced by (30) and (31). Each extract was examined through a series of simple color and precipitation reactions to determine the presence of key groups of secondary metabolites. Alkaloids were identified through the use of Mayer, Wagner, and Dragendorff reagents, which typically produce a white to orange precipitate. Flavonoids were revealed by the magnesium–hydrochloric acid reduction test, marked by the appearance of a red or pink color. Phenolic compounds were confirmed with 5% ferric chloride, while tannins were distinguished using a weaker, 1% ferric chloride solution. Saponins were assessed through their ability to generate a stable layer of foam, and triterpenoids were detected by the characteristic green-to-violet color change in the Liebermann–Burchard reaction.

From these observations, papaya and basil extracts showed the presence of alkaloids, flavonoids, phenolics, saponins, and triterpenoids. Tannins, however, appeared only in the basil-extract. These findings help explain the biological activities observed later in the study, as many of these compounds are known to contribute to anti-inflammatory and analgesic effects through their antioxidant and enzyme-modulating properties (32, 9).

In Vitro Anti-inflammatory Activity

The Bovine Serum Albumin (BSA) denaturation assay was employed to evaluate the anti-inflammatory potential of each extract, as protein denaturation is a key indicator of inflammation. This method has been widely used for preliminary screening because it is simple, reproducible, and correlates well with in vivo outcomes (33).

Each extract was tested at concentrations of 10–200 µg/mL Samples were mixed with 1% BSA in phosphate-buffered saline (pH 6.4) and incubated at 37 °C for 30 minutes. The mixtures were then heated at 70°C for 5 minutes to induce protein denaturation. The solution was cooled to room temperature (approximately 25°C) for 25 minutes. Absorbance was measured at 660 nm using a UV–Vis spectrophotometer. The percentage inhibition of denaturation was calculated as:

$$\text{Inhibition } (\%) = \frac{(\text{A}_{control} - \text{A}_{sample})}{\text{A}_{control}} \times 100$$

Diclofenac sodium (100 µg/mL) served as the standard reference drug. The IC_{50} values (concentration required to inhibit 50% of protein denaturation) were determined by linear regression analysis of concentration versus inhibition percentage. Lower IC_{50} values indicate higher anti-inflammatory potency (33).

In Vivo Anti-inflammatory Activity

The anti-inflammatory effect of the extracts was further confirmed using the carrageenan-induced paw edema model, which represents acute inflammation characterized by biphasic mediator release. The first phase (0-2 h) involves histamine and serotonin, while the second phase (3-5 h) is associated with prostaglandin and bradykinin release (34).

Mice were divided into seven groups (n = 4 each):

a. Normal control (0.5% Na-CMC only)
b. Positive control (diclofenac sodium 50 mg/kg)
c. EP (papaya extract)
d. EK (basil extract)
e. EP_1K_1 (1:1)
f. EP_3K_1 (3:1)
g. EP_1K_3 (1:3)

All extracts were administered orally at a dose of 200 mg/kg body weight, suspended in 0.5% Na-CMC.

Prior to edema induction, the mice were fasted for approximately 14–16 hours. Edema was induced by intraplantar injection of 0.15 mL of 1% carrageenan into the hind paw of each mouse. One hour after carrageenan administration, the initial edema volume was measured. Subsequently, each animal received treatment according to its respective group, and paw volume was recorded hourly for five consecutive hours using a digital plethysmometer The percentage inhibition of edema was calculated as:

$$\text{Inhibition } (\%) = \frac{(V_c - V_t)}{V_c} \times 100$$

Where Vc and Vt represent mean paw volumes of control and treated groups, respectively. Diclofenac sodium served as the reference anti-inflammatory drug, enabling comparative analysis of the extracts' efficacy (35).

Analgesic Activity

Analgesic activity was evaluated using the acetic acid-induced writhing test, a standard model for assessing peripheral analgesic effects. This method is based on the principle that intraperitoneal injection of acetic acid triggers the release of prostaglandins (especially PGE_2 and $PGF_2\alpha$) and bradykinin, resulting in characteristic abdominal constrictions or "writhes" (36).

Mice were allocated into the same seven groups as in the anti-inflammatory study and pre-treated with extracts or standard drugs orally. One hour later, 1% acetic acid (0.1 mL/10 g body weight) was administered intraperitoneally. The number of writhes was counted for 15 minutes post-injection. Analgesic activity was expressed as percentage protection using the formula:

$$\text{Protection } (\%) = \frac{(W_c - W_t)}{W_c} \times 100$$

Where *Wc* and *Wt* represent the mean number of writhes in control and treated groups, respectively. Mefenamic acid (500 mg/kg) was used as the reference standard. The test is known for its sensitivity and reliability in identifying peripherally acting analgesics that suppress inflammatory pain responses (37).

Statistical Analysis

All data were expressed as mean ± standard deviation (SD) for four replicates (n = 4). Statistical comparisons between groups were conducted using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test to determine the level of significance ($p < 0.05$). The software package SPSS version 25.0 (IBM Corp., USA) was employed for all analyses. Statistical rigor ensures that observed effects are not due to random variation but reflect genuine pharmacological activity (38).

Results and Discussion

Phytochemical Composition

Phytochemical screening was carried out to characterize the major groups of secondary

metabolites present in the single ethanolic extracts prepared from papaya and basil leaf simplicia. The qualitative outcomes are summarized in **Table 1**. Papaya and basil extracts contained alkaloids, flavonoids, phenolics, saponins, and triterpenoids. Tannins were detected only in basil extracts.

The broad presence of alkaloids, flavonoids, and phenolic compounds agrees with previous reports describing both *Carica papaya* and *Ocimum sanctum* as sources of bioactive metabolites linked to antioxidant, anti-inflammatory, and analgesic activities (39, 16, 40). Flavonoids and phenolics are known to contribute to redox regulation and to modulate enzymes such as COX and LOX, which play central roles in inflammatory processes. Their consistent detection across all samples suggests that both plants contribute to this chemical profile.

A clearer distinction emerged for phenolics and tannins. Stronger reactions were observed in the basil extract, consistent with the widely documented high polyphenol content of basil leaves, which include rosmarinic acid and related derivatives (41). In contrast, papaya leaf extract displayed a milder phenolic reaction, supporting earlier findings that its total polyphenol content is considerably lower than that of basil (42).

Saponins and triterpenoids were consistently present across all samples. Both groups have been associated with membrane-stabilizing properties and may support the anti-inflammatory effects observed later in the study. The uniform detection of alkaloids across extracts is also notable, as these compounds are frequently implicated in modulating nociceptive pathways.

Taken together, the phytochemical profile demonstrates that combining simplicia prior to extraction results in extracts that incorporate constituents from both plants. This combined chemical spectrum provides a rational foundation for the biological activities evaluated in the subsequent anti-inflammatory and analgesic assays.

In Vitro Anti-inflammatory Activity

The in vitro anti-inflammatory activity of the extracts was evaluated using the bovine serum albumin (BSA) denaturation assay, a widely used model to assess the ability of test samples to stabilize protein structure under heat-induced stress. Protein denaturation is considered relevant to inflammatory processes, as denatured proteins may act as autoantigens and promote inflammatory responses (43,44).

All extracts exhibited concentration-dependent inhibition of BSA denaturation **Table 2**). Among the single extracts, the basil simplicia extract (EK) showed the strongest activity, with an IC_{50} value of 82.44 µg/mL, approaching that of the reference drug diclofenac sodium. The papaya extract (EP) demonstrated moderate inhibitory activity, which is consistent with its comparatively lower phenolic content. In contrast, none of the combined extracts produced IC_{50} values lower than that of the most active single extract, indicating that the observed effects reflect additive rather than synergistic interactions.

Table 1. Qualitative phytochemical composition of ethanolic extracts of papaya (*Carica papaya*) and basil (*Ocimum sanctum*) leaf simplicia

Phytochemical group	Test method	EP	EK
Alkaloids	Wagner, Dragendorff	+	+
Flavonoids	Mg–HCl (Shinoda)	+	+
Phenolics	$FeCl_3$ 5%	+	+
Tannins	$FeCl_3$ 1%	–	+
Saponins	Frothing test	+	+
Triterpenoids	Liebermann–Burchard	+	+

Description (+) = present; (–) = not detected. EP = papaya simplicia extract; EK = basil simplicia extract.

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Table 2. In vitro anti-inflammatory activity of ethanolic extracts of papaya (*Carica papaya*) and basil (*Ocimum sanctum*) leaf simplicia and their combinations

Sample Code	Concentration (g)	IC_{50} (µg/mL) (Mean ± SD)
Diclofenac sodium	100	16.63 ± 0.139 [a]
EP	200	191.07 ± 1.092 [e]
EK	200	**82.44 ± 1.079 [b]**
EP_1K_1	200	136.15 ± 0.689[d]
EP_3K_1	200	208.54 ± 0.227[f]
EP_1K_3	200	106.69 ± 1.833[c]

Description: EP = papaya simplicia extract; EK = basil simplicia extract; EP_1K_1, EP_3K_1, EP_1K_3 = combined simplicia extracts in 1:1, 3:1, and 1:3 ratios (w/w), respectively. Statistical note: Values are expressed as mean ± SD (n =3). Different superscript letters (a-f) indicate significant differences ($p < 0.05$) according to one-way ANOVA followed by Duncan's Multiple Range Test

Notably, the 1:3 papaya-basil combination (EP_1K_3) exhibited a higher IC_{50} value than either single extract, suggesting reduced potency in this protein denaturation model. This finding indicates that increasing the proportion of basil in the combined extract does not necessarily translate into enhanced in vitro anti-inflammatory activity. One possible explanation is the occurrence of mild antagonistic interactions among certain bioactive constituents during the pre-extraction mixing process, which may influence protein-ligand affinity or alter albumin structural stability under thermal stress.

The activity pattern observed across treatments (EK > EP_1K_3 > EP_1K_1 > EP > EP_3K_1)further supports the interpretation that basil-derived phenolic compounds play a dominant role in protein stabilization, while papaya contributes complementary but less pronounced effects. When extracted together, these constituents appear to produce intermediate inhibitory responses rather than potentiated activity.

Overall, the BSA assay results indicate that mixing papaya and basil simplicia prior to extraction yields additive effects on protein denaturation inhibition. Importantly, no claims of synergism can be substantiated based on the present data, as quantitative interaction analyses such as combination index calculation or isobologram analysis were not performed. Therefore, the observed effects should be interpreted as additive or, in the case of EP_1K_3, potentially mildly antagonistic within this specific in vitro model.

In Vivo Anti-inflammatory Activity

The anti-inflammatory effects of the extracts were further examined using the carrageenan-induced paw edema model, which is considered a reliable method for assessing acute inflammation. Carrageenan produces a well-defined biphasic inflammatory response: an early phase dominated by histamine and serotonin release, followed by a later phase mediated primarily by prostaglandins and other eicosanoids (47,48). Compounds capable of suppressing paw swelling in this model are interpreted as having anti-inflammatory potential through modulation of these mediators.

As shown in **Table 3**, all extracts reduced paw edema to varying degrees compared with the control group. The papaya simplicia extract (EP) showed the highest inhibition (64.41%), followed by basil extract (EK) (55.77%) not significantly different from diclofenac sodium (55.10%). The inhibitory order (EP > EK ≥ Diclofenac > EP_1K1 ≥ EP_1K3 ≥ EP_3K_1), highlights the strong response elicited by the papaya extract. This effect may be associated with the combined action of papain and several flavonoids present in the leaves. Papain has been reported to influence inflammatory signaling by modulating proteolytic pathways, while flavonoids such as quercetin and kaempferol are known inhibitors of the COX and LOX pathways (14,12). Together, these

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constituents may account for the more pronounced reduction in edema observed with EP.

Although the basil extract showed a weaker response than papaya, it still produced a meaningful reduction in paw swelling. Basil leaves contain eugenol, rosmarinic acid, and related phenolic compounds, which can attenuate inflammation by reducing vascular permeability and counteracting oxidative stress (49). The modest yet consistent effect of EK suggests that its phenolic components exert an anti-inflammatory influence that becomes more apparent in formulations where basil is present in higher proportions.

Table 3. In vivo anti-inflammatory activity of ethanolic extracts of papaya (*Carica papaya*) and basil (*Ocimum sanctum*) leaf simplicia and their combinations

Treatment group	Dose (mg/kg BW)	Inhibition of Paw Edema (%)
Control (Na-CMC 0.5%)	—	41.46 [a]
Diclofenac sodium	50	55.10 [b]
EP (Papaya extract)	200	**64.41 [c]**
EK (Basil extract)	200	55.77 [b]
EP_1K_1 (1:1)	200	42.86 [a]
EP_3K_1 (3:1)	200	40.38 [a]
EP_1K_3 (1:3)	200	41.51 [a]

Description: EP = papaya simplicia extract; EK = basil simplicia extract; EP_1K_1, EP_3K_1, EP_1K_3 = combined simplicia extracts in 1:1, 3:1, and 1:3 ratios (w/w), respectively. Statistical note: Superscript letters show significant differences ($p < 0.05$). Groups sharing the same letter do not differ significantly; groups with different letters differ significantly.

Table 4. Analgesic activity of ethanolic extracts of papaya (*Carica papaya*) and basil (*Ocimum sanctum*) leaf simplicia and their combinations in the acetic acid–induced writhing test

Treatment group	Dose (mg/kg BW)	Analgesic Protection (%)
Control (Na-CMC 0.5%)	—	0 [a]
Mefenamic acid	500	**42.31 [c]**
EP (Papaya extract)	200	23.91 [b]
EK (Basil extract)	200	31.69 [b]
EP_1K_1 (1:1)	200	19.35 [b]
EP_3K_1 (3:1)	200	13.47 [a]
EP_1K_3 (1:3)	200	**42.31 [c]**

Description: EP = papaya simplicia extract; EK = basil simplicia extract; EP_1K_1, EP_3K_1, EP_1K_3 = combined simplicia extracts in 1:1, 3:1, and 1:3 ratios (w/w), respectively. Statistical note: Superscript letters show significant differences ($p < 0.05$). Groups sharing the same letter do not differ significantly; groups with different letters differ significantly.

The combined extracts produced intermediate effects, indicating an additive rather than synergistic interaction in this acute model. One possible explanation is that papaya's proteolytic and antioxidant elements exert rapid inhibition during the early inflammatory phase,

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while basil's polyphenolic constituents may be more influential in pathways associated with chronic or oxidative aspects of inflammation. This may explain why none of the combinations exceeded the effect of papaya alone, yet still showed stable and reproducible activity across different ratios.

Taken together, the in vivo results support the anti-inflammatory potential of both plants, with papaya displaying markedly stronger activity under the conditions of this model. The consistent performance of the combined extracts, despite not surpassing papaya alone, suggests a balanced contribution of enzymatic and phenolic constituents that may offer advantages for broader pharmacological applications. These findings provide further justification for the traditional use of papaya and basil in managing inflammatory symptoms and support their potential in the development of polyherbal formulations.

Analgesic Activity

The analgesic properties of the extracts were investigated using the acetic acid–induced writhing model, a widely applied assay for evaluating peripheral antinociceptive activity. Acetic acid triggers abdominal constrictions through the release of prostaglandins, bradykinin, and several inflammatory cytokines, making the model particularly sensitive to agents that interfere with prostaglandin synthesis or downstream inflammatory signaling (50,51).

All extracts significantly reduced the number of writhes compared with the control group **Table 4**. Among the combinations, the basil-rich extract (EP_1K_3) produced the strongest analgesic protection (42.31%), comparable to mefenamic acid. The analgesic activities of EK, EP, and EP_1K_1 were not significantly different from each other, with protection values ranging from 19.35% to 31.69%. The lowest analgesic protection was observed in the EP_3K_1 combination.

The general order of activity (Mefenamic acid ≈ EP_1K_3 > EK ≥ EP ≥ $EP1K_1$ > EP_3K_1), presented in **Table 4**, suggests that the presence of basil in higher amounts enhances analgesic action. Basil leaves contain phenolic constituents such as eugenol, rosmarinic acid, and linalool, which have been shown to reduce peripheral pain through COX-2 inhibition and modulation of inflammatory mediators (40). Their prominent presence in the EP_1K_3 extract may therefore explain the improved performance of this formulation.

Papaya extract, although less potent than the basil-rich combination, still exhibited substantial analgesic activity. Flavonoids such as quercetin and kaempferol, along with papaya-derived alkaloids, are known to exert antinociceptive effects through antioxidant mechanisms and by modulating nitric oxide signaling and transient receptor potential (TRP) channels (52,53). These properties likely contributed to the moderate yet meaningful reduction in writhing observed in EP.

The intermediate effects displayed by the other combined ratios indicate that the analgesic response depends on the proportional balance between the enzymatic components of papaya and the phenolic fraction of basil. The superior performance of the 1:3 combination suggests that basil's phenolic constituents may play a central role in modulating peripheral pain when present in sufficient concentration, while papaya's bioactive compounds provide supportive effects.

Overall, the results demonstrate that both single and combined extracts possess measurable analgesic activity, with the basil-rich formulation showing the most promising response. These findings align with the traditional use of both plants for pain relief and point to the potential advantages of combining simplicia prior to extraction to leverage their complementary mechanisms.

Mechanistic Interpretation

The combined findings from the phytochemical analyses and biological assays suggest that the ethanolic extracts of papaya and basil simplicia act through multiple complementary mechanisms to produce anti-inflammatory and analgesic effects. The presence of alkaloids, flavonoids, phenolic compounds, triterpenoids, and papaya-derived proteolytic enzymes provides a diverse chemical basis capable of influencing several inflammatory and nociceptive pathways.

Flavonoids and phenolic constituents, detected consistently across all extracts, are well

known to inhibit the COX and LOX enzymatic pathways, thereby reducing downstream production of prostaglandins and leukotrienes (52,53). This mechanism is consistent with the strong inhibition of protein denaturation observed in vitro, particularly in the basil extract and the basil-rich combination (EP_1K_3). These polyphenolic compounds are capable of stabilizing protein structures through hydrogen bonding and free-radical quenching, which likely contributed to the attenuated denaturation of albumin in the BSA assay (54).

Papaya extract demonstrated its strongest effects in vivo, producing the highest inhibition of carrageenan-induced edema. This finding may be attributed to the presence of papain and related proteolytic enzymes, which can modulate inflammatory cascades and promote the breakdown of inflammatory mediators at the site of tissue injury. In addition, papaya flavonoids and alkaloids may enhance these effects by reducing oxidative stress and downregulating pro-inflammatory cytokines such as TNF-α and IL-6 (12).

The extracts prepared from combined simplicia likely benefited from interactions between papaya's enzymes and basil's phenolic constituents during the extraction process. Such interactions may enhance solubility, protect labile components, or modify extraction efficiency, resulting in a more integrated phytochemical profile. This may explain the improved analgesic response observed for the EP_1K_3 formulation, where basil's phenolic components, particularly eugenol and rosmarinic acid, may potentiate the antinociceptive effects of papaya's alkaloids and flavonoids (40).

The consistent presence of triterpenoids and saponins in all extracts further supports their pharmacological activity, as these groups are associated with membrane-stabilizing effects and inhibition of lysosomal enzyme release, mechanisms relevant to both inflammation and pain modulation (55).

Taken together, these mechanistic patterns highlight that papaya-dominant preparations are more effective in reducing acute inflammatory edema, whereas basil-rich combinations appear more responsive in models of peripheral nociception. This division of activity likely reflects the different strengths of each plant's chemical constituents. The pre-extraction blending of simplicia, as used in this study, may therefore serve as a practical approach for developing polyherbal formulations that leverage complementary molecular actions across multiple inflammatory and pain-related pathways.

Conclusion

This study demonstrates that ethanolic extracts of *Carica papaya* and *Ocimum sanctum* leaf simplicia exhibit anti-inflammatory and analgesic activities, both as single extracts and in combination. The combined extracts showed additive pharmacological effects based on the available in vitro and in vivo data, while synergistic interactions could not be confirmed.

A limitation of this study is the absence of quantitative interaction analysis, such as combination index or isobologram evaluation, which would be required to definitively characterize synergistic or antagonistic effects. Therefore, further studies are necessary to explore interaction mechanisms, dose-response relationships, and long-term safety before broader application or formulation development.

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Conflict of Interest

The authors confirm that this research is free from conflicts of interest.

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