PHARMACOLOGICAL STUDY OF COFFEE-MANGO LEAF EXTRACT COMBINATION AS ANTI-INFLAMMATORY IN RATS

SUKANDI, H. P.[1] – SETIANI, L. A.[1*] – HERLINA, N.[1]

[1] *Faculty of Mathematics and Natural Sciences, Pakuan University, West Java, Indonesia.*

**Corresponding author*
e-mail: lusi.setiani[at]unpak.ac.id

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Abstract. Inflammation occurs as a result of cell-damaging stimuli, causing free arachidonic acid to trigger inflammatory symptoms. Coffee leaves (Coffea arabica) and mango leaves (Mangifera indica) contain phenols, tannins, flavonoids, alkaloids, saponins, terpenoids, and steroids, which have been reported to possess anti-inflammatory activity, giving them great potential and enhancing anti-inflammatory effects when combined. The research aimed to evaluate the anti-inflammatory potential and optimal dosage of a combined extract of coffee and mango leaves (CLMLE). The anti-inflammatory potential was tested through carrageenan induction on the paw of the feet of Sprague Dawley rats in 6 groups of oral doses: negative control (CMC-Na 1%), positive control (Diclofenac Sodium 0.9 mg/200 g BW), CLMLE dose 1 (60 mg/kg BW and 100 mg/kg BW), CLMLE dose 2 (120 mg/kg BW and 200 mg/kg BW), CLMLE dose 3 (240 mg/kg BW and 200 mg/kg BW), and CLMLE dose 4 (120 mg/kg BW and 400 mg/kg BW). The results indicated that all CLMLE doses exhibited significant anti-inflammatory activity compared to the negative control ($P < 0.05$). Dosage 2 had the highest anti-inflammatory potential, with an inhibition percentage of 76.3431%. The combination of CLMLE in this research was judged to have anti-inflammatory potential, and the findings can be used to produce anti-inflammatory medicines and formulations.
Keywords: *anti-inflammatory, coffea arabica, mangifera indica, rattus norvegicus*

Introduction

The term "inflammation" is derived from the Latin word "inflamao, inflamare" which translates to "to ignite, to kindle." This natural protective response serves as a protective mechanism of the body by eliminating inflammatory stimulants, thereby facilitating the healing process (Oronsky et al., 2022; Jain et al., 2015). Inflammation is caused by physical trauma, noxious chemicals, or microbiological agents that trigger a protective response (Gusev and Zhuravleva, 2022; Oronsky et al., 2022; McCutcheon, 2016). The following symptoms are indicative of inflammation: redness (erythema and rubor); swelling (edema or tumor); heat (calor); pain (dolor); and loss of function (functio laesa) (Stone et al., 2024). Inflammation treatment aims to relieve pain caused by visible symptoms while also slowing and limiting tissue damage. Nonsteroidal anti-inflammatory drugs (NSAIDs) and steroidal anti-inflammatory drugs (SAIDs) are frequently employed in the pharmacological treatment of inflammation (Pramitaningastuti and Anggraeny, 2017; Ramadhani and Sumiwi, 2016). NSAIDs generally reduce inflammation by inhibiting the enzyme cyclooxygenase (COX), whereas steroids inhibit prostaglandins through a central mechanism and downregulate several important inflammation-mediated actions (Kim et al., 2016).

The use of anti-inflammatory medications over an extended period has been demonstrated to engender a multitude of deleterious and hazardous side effects. The use of systemic steroid medications as anti-inflammatory agents has been observed to result in a range of adverse effects, including the suppression of endogenous glucocorticoid synthesis, the development of peptic ulcers, the onset of osteoporosis, the atrophy of muscle and fat tissue, the elevation of intraocular pressure, the development of diabetes, the diminution of the body's immune response to infection, the exacerbation of osteoporosis, the manifestation of moonface, and the occurrence of hypertension (Hodgens and Sharman, 2022). The use of non-steroidal anti-inflammatory drugs (NSAIDs) can also lead to various gastrointestinal disorders, including peptic ulcers, analgesic nephropathy, and platelet dysfunction, as well as potential complications such as bleeding, kidney damage, anemia, and infertility (Puppala and Reddy, 2020). Carrageenan-induced leg edema is a well-known, widely used, and highly reproducible model of acute inflammation for screening new natural and synthetic anti-inflammatory compounds (Karim et al., 2019; Sarkhel, 2016). Carrageenan is a galactan polysaccharide that functions as an intercellular matrix in red seaweed or marine algae from the class Rhodophyta (Thakur and Thakur, 2016). The sulfate sugars present in carrageenan have been identified as the critical mediators responsible for activating the complement system and inducing inflammatory responses (Patil et al., 2019).

Coffee leaves (C. arabica) contain a variety of bioactive phytochemicals, including alkaloids, flavonoids, terpenes, tannins, xanthonoids, phenolic acids, phytosterols, amino acids, and carotenoids, which contribute to various pharmacological effects (Chen, 2019). The properties and efficacy of these substances are derived from the active chemicals and polyphenols they contain (Abdulwahab et al., 2022). Specifically, phenolic acids such as caffeic, chlorogenic, p-coumaric, ferulic, and sinapic acids, along with flavonoids like rutin, quercetin, kaempferol, and isoquercitrin, have been identified in Arabica coffee leaves (Patay et al., 2016). C. arabica leaf extract has been shown to possess antioxidant activity, which interferes with the process of free radicals. It has been demonstrated that C. arabica leaf extract exerts an anti-inflammatory effect by impeding several processes, including increased blood vessel permeability, vasodilation, edema formation, leukocyte migration, and ROS formation. Furthermore, it has been demonstrated that C. arabica leaf extract exerts a suppressive effect on the response of histamincyclooxygenase-2 (COX2) and cytokines (Wenas et al., 2020; Segheto et al., 2018). The active constituents of mango leaves (M. indica) consist of polyphenols, flavonoids and triterpenoids. Mangiferin compound is a compound that was first isolated from mango leaves. M. indica contains high levels of phenolic compounds, with mangiferin being responsible for various pharmaceutical activities. Mango leaves are a source of secondary metabolites, including alkaloids, flavonoids, tannins, saponins, total phenols, and cardiac glycosides (Yadav et al., 2022; Ali et al., 2020; Batool et al., 2018). Mango leaves are rich in phenolics that have shown various bioactivities, such as antioxidant, antidiabetic, antimicrobial, immunomodulatory, antipyretic, anti-inflammatory and analgesic (Pan et al., 2018). Several natural polyphenols in mango have been reported exhibit anti-inflammatory properties, achieved through the suppression of nuclear factor kappa-B (NF-αB), as well as the inhibition of nitric oxide (NO), nitric oxide synthase (NOS), and cyclooxygenase 2 expression (Agrawal, 2021; Batool et al., 2018). The anti-inflammatory activity of flavonoids is associated with the inhibition of cyclooxygenase and lipoxygenase pathways, as well as with the inhibition of histamine and leukocyte accumulation. Additionally, flavonoids have been observed to impede the secretion of arachidonic acid and lysosomal enzymes (Hidayati et al., 2022).

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Materials and Methods

The materials used include distilled water, hydrochloric acid (HCl) (Merck®), iron (III) chloride ($FeCl_3$), coffee leaves (Coffea arabica L.), mango leaves (Mangifera indica), 70% ethanol, 10% gelatin, and 0.5-2% carrageenan irritant solution (kappa type 1%) (Indo Food Chem®), rat food and drink, sodium chloride 0,9% (NaCl) (SatoriaFarma®), standard drug (Sodium Diclofenac) (Bernofarm®), carrier (CMC-Na 1%), Bouchardart reagent (glacial CH_3COOH + H_2SO_4), Dragendorff reagent ($K(BiI_4)$), Mayer reagent ($K_2(HgI_4)$), magnesium (Mg) and zinc (Zn) powder (Merck®), young and healthy adult male Sprague-Dawley strain rats. The tools used include mesh sieves, dark glass bottles, crucibles, porcelain cups, steam cups, glass funnels (Pyrex®), Erlenmeyer flasks (Pyrex®), beakers (Pyrex®), measuring cups (Pyrex®), watch glasses (Pyrex®), experimental animal cages, mortars (mortar & pestle), analytical balances, ovens (Memert®), stirrers, glassware (Pyrex®, Iwaki®, Normax®), porcelain equipment, cleaning equipment, plethysmometers, test tube racks, rotary evaporators (Ika®), wood shavings. oral probes, markers (Snowman®), syringes (OneMed®), injection syringes (OneMed®), stopwatches, test tubes (Pyrex®), furnaces (Daihan®), rat food and drink containers, scales, animal scales, extract containers, maceration containers (Merck®).

The research was conducted from January to May of 2024 at the Pharmacology Laboratory of the Pharmacy Study Program, which is part of the Faculty of Mathematics and Natural Sciences at Pakuan University in Bogor. The use permission of test animals in this study was authorized by the Ethics Committee Decree No. 37/KEPHP-UNPAK/12-2023 from the Ethics Committee for the Use of Experimental Animals, Faculty of Mathematics and Natural Sciences, Pakuan University, Bogor City. The plant materials used in the research were Arabica coffee leaves (Coffea arabica) from the Anacardiaceae family and Arumanis mango leaves (Mangifera indica) from the Rubiaceae family, as determined by the results of plant identification number 153/UN2.F3.11/PDP.02.00/2024. Both plant materials were obtained from Jalan Tentara Pelajar No. 3, Menteng Asri Village, West Bogor District, Bogor City, West Java, 16111. The plant materials were identified at the University of Indonesia (UI) through the Department of Biology, FMIPA UI and Herbarium Depokensis (UIDEP), Jalan Lingkar Kampus Raya, Pondok Cina, Beji District, Depok City, West Java 16424.

Anti-inflammatory test

The research used 30 male white rats (Rattus norvegicus) of the Sprague Dawley strain, exhibiting good health and body weights ranging from 100 to 200 grams. The rats were acclimatized for approximately one week in the Pakuan University Pharmacy Laboratory, allowing them to adjust to their environment. During the acclimatization period, the rats were provided with food and water ad libitum, and their general condition was routinely observed. The standard feed administered to the rats was pellets (Citrafeed Ratbio, PT Citra Ina Feedmill). The test animals were subjected to a fasting period of approximately 18 to 24 hours, either overnight or for a day with constant access to drinking water. The rat's feet were marked with a marker on the ankle. Subsequent to this, the volume of the feet and the weight of the test animals were measured (V0). The experimental design incorporated a positive control (Na Diclofenac 0.9 mg/200 g BW), a negative control (CMC Na 1%), and four distinct doses (60 mg/kg BW and 100 mg/kg BW, 120 mg/kg BW and 200 mg/kg BW, 240 mg/kg BW and 200

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mg/kg BW, and 120 mg/kg BW and 400 mg/kg BW). The doses were containing coffee leaf and mango leaf extracts (CLMLE) in a sequential manner (*Table 1*).

***Table 1**. Phytochemical test results.*

Group	Treatment
Positive contro	Na-Diklofenak 0,9 mg/200 g BB tikus
Negative control	CMC Na 1% (Induksi)
Dose 1	Coffee leaf extract 60 mg/kg BW and mango leaf extract 100 mg/kg BW
Dose 2	Coffee leaf extract 120 mg/kg BW and mango leaf extract 200 mg/kg BW
Dose 3	Coffee leaf extract 240 mg/kg BW and mango leaf extract 200 mg/kg BW
Dose 4	Coffee leaf extract 120 mg/kg BW and mango leaf extract 400 mg/kg BW

Each rat was administered the test material orally for each group. Subsequently, 30 minutes after the oral solution (carrier solution/test preparation/standard drug) was induced by 0.1 mL of 1% carrageenan solution subplantarly on the sole of the left foot of the test animal by injection, the volume of fluid expelled from the rat's pedal extremity was measured. The increase in foot volume was measured using a plethysmometer. Edema in the soles of the feet was measured every 30 minutes for a period of 6 hours following the injection of carrageenan (Vt). The data obtained were processed using a formula to become a curve of the edema volume of the feet of the test animals (BPOM, 2021) (Eq. (1)

$$V_e = V_t - V_0 \qquad \text{Eq. (1)}$$

$$Inflamation\ percentage = \frac{V_e}{V_0} X\ 100\% \qquad \text{Eq. (2)}$$

$$Inhibition\ percentage = \frac{a-b}{a} X100\% \qquad \text{Eq. (3)}$$

Where, Ve=rat paw edema volume; Vt=rat paw volume after carrageenan induction; V_0=initial volume of rat paw before carrageenan induction; a=percentage of edema in the negative control group; b=percentage of edema in the test drug group. The percentage data of edema inhibition obtained were processed statistically using the SPSS application. The analysis of rat paw edema data in this study employed the Randomized Block Design (RBD) method, subsequently followed by the Duncan test.

Preparation of crude extract

Coffee leaves and mango leaves, with a weight of approximately 5 kilograms (wet weight), undergo wet sorting and were meticulously washed with clean, running water to ensure removal of any dirt or contamination. Thereafter, the leaves were drained and chopped, and subsequently dried in an oven set at a temperature of 30-40°C. Subsequent to the drying process, the ingredients were sorted dry and ground into powdered simplicia. The extraction of the active principles was achieved through maceration with 70% alcohol, using 400 grams of the powdered simplicia. The extraction process involves soaking the simplicia in alcohol for a period of 3-5 days, with regular stirring to ensure uniform distribution of the solvent throughout the material. The extraction process was repeated once more, employing the same type of solvent but reducing the volume by half compared to the initial extraction. The macerates were subsequently

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collected and subjected to evaporation using a rotary evaporator until a viscous extract is obtained. The yield was calculated as the percentage of weight (b/b), where b is the weight of the extract and b is the weight of the simplicia powder used (Eq. (4).

$$Yield\ (\%) = \frac{Extract\ mass}{Simplicial\ powder\ mass}\ X\ 100\% \qquad \text{Eq. (4)}$$

Phytochemical qualitative analysis

Phytochemical screening carried out consisted of alkaloids, phenols, flavonoids, tannins, terpenoids, steroids, and saponins. In determination of alkaloids, 0.5 g of sample were meticulously measured. Subsequently, 1 mL of 2N HCl and 9 mL of water were added to the flask, which was then subjected to heating in a water bath. Following this, the flask was allowed to cool, and the contents were filtered to produce a filtrate that was subsequently divided for identification. Bouchardat positive results were indicated by the formation of a brown to black precipitate. Conversely, Mayer positive results were indicated by the formation of a white precipitate. Dragendroff positive results were indicated by the formation of an orange-brown precipitate (Hanani, 2015). Meanwhile, for phenols, 0.5 g of sample were initially dissolved in methanol. A portion of the solution was taken, followed by the addition of three drops of 1% FeCl3 reagent. The color change of the solution to green, blue, or purple serves as an indication of the presence of phenol compounds (Hanani, 2015). Countinously in flavonoids, 0.5 g of sample were extracted with dichloromethane for a duration of 15 minutes. The mixture was subsequently filtered to ensure the separation of the components. The filtrate was then subjected to a process of evaporation, resulting in a dry residue to dissolved in 50% methanol, if necessary by heating on a water bath. Subsequently, a small quantity of magnesium metal or zinc powder was added, along with 5-6 drops of concentrated hydrochloric acid. The mixture was then heated for several minutes on a water bath. The blue-green color that appears indicates the presence of flavonoid compounds (Hanani, 2015).

In determining tannins, 0.5 g of the sample were extracted using 70-95% ethanol for a duration of 15 minutes, followed by filtration. The filtrate obtained was then subjected to evaporation in a water bath, followed by the addition of hot distilled water to the residual evaporation and stirring. Subsequent to cooling, the solution undergoes centrifugation. The upper liquid was separated by decantation, and the filtrate is used for identification. The presence of tannins was indicated by the formation of a white precipitate. 1% gelatin solution in 10% NaCl (1:1 ratio) was used to identify tannin, which is indicated by the formation of a precipitate. Finally, the presence of tannin was indicated by the emergence of a blue-green to blackish color in a 3% iron (III) chloride solution. For terpenoids and steroids, 0.5 grams of sample were dissolved with n-hexane. Subsequently, a small volume of the mixture was taken, followed by the addition of 1 mL of glacial CH3COOH and 1 mL of concentrated H2SO4 solution. The appearance of a reddish brown ring at the interface between the two solvents was indicative of the presence of terpenoids, whereas the presence of a blue or green ring signifies the presence of a steroid compound group (Hanani, 2015). Lastly, to determine saponin, 0.5 grams of sample in a test tube was added to 10 mL of hot water, followed by cooling. Following this, the test tube was vigorously shaken for 10 seconds. The presence of saponins was indicated by the formation of stable foam when hydrochloric acid was added to the mixture (Hanani, 2015).

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Results and Discussion

The extract obtained from each 400 g of powdered simplicia was 97.56 g (24.39%) on coffee leaves and 123.00 g (30.75%) on mango leaves (*Table 2*). The characterization of both extracts revealed that they were thick-textured. The coffee leaf extract manifested a brown hue and possessed a moderately intense, discernible aroma. In contrast, the mango leaf extract manifested a dark green hue and a subtle, non-distinctive aroma (*Figure 1*). Phytochemical tests through screening have revealed the presence of phenol, tannin, flavonoid, alkaloid, terpenoid, and steroid compounds, as well as saponin, in both coffee and mango leaves. This finding aligns with the extant literature, which documents the presence of flavonoid, saponin, triterpenoid, tannin, and alkaloid compounds in coffee and mango leaves (Hidayati et al., 2022; Wenas et al., 2020) (*Figure 2*). The experimental animals were acclimatized for adaptation, so it is expected that they will not experience stress during the procedure due to the transfer from their previous cage. The duration of acclimatization has been demonstrated to mitigate stress levels in animals. The coefficient of variance (CV) of the mice's weight prior to acclimatization was 7.94%. Subsequent to acclimatization, a CV value of 6.47% was obtained, which was deemed to meet the stipulated criteria due to the experimental animals exhibiting relative homogeneity with a value of <15% (Stępniak, 2011). It is noteworthy that a decrease in CV value corresponds to increased homogeneity in the data (*Figure 3*).

***Table 2**. Phytochemical test results.*

Phytochemical content	Coffee leaf extract	Mango leaf extract	Result
Fenol	+	+	Blue Purple
Tanin	+	+	Blackish green
Flavanoid	+	+	Yellowish orange
Alkaloid			
(Mayer)	+	+	White sediment
(Dragendrof)	+	+	Reddish brown sediment
(Bourchard)	+	+	Brown sediment
Terpenoid dan Steroid	+	+	Green ring
Saponin	+	+	Foam is formed

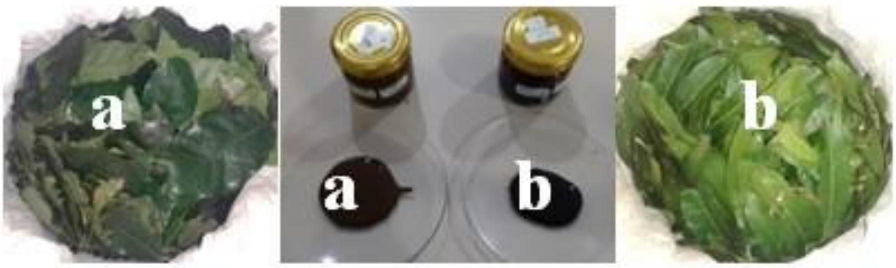

***Figure 1.** (a) Coffee Leaves and Leaf Extract and (b) Mango Leaves and Leaf Extract.*

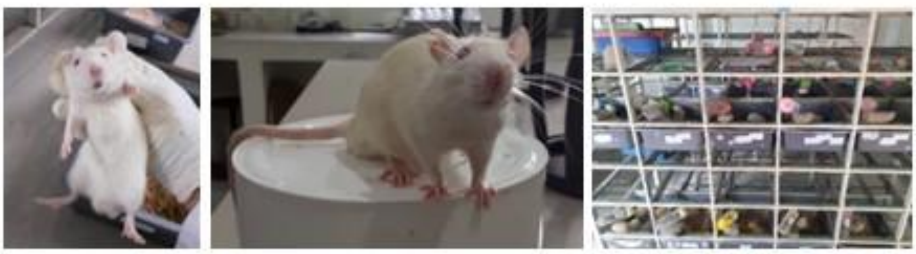

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Figure 2. *Male White Rat (Rattus norvegicus) Sparague dawley strain.*

Figure 3. *Anti-inflammatory test.*

The method of inducing artificial edema on the soles of rat's feet by using carrageenan as an inducer is a widely accepted and employed test method due to its high sensitivity, reproducibility, and popularity (Dzoyem et al., 2017; Sarkhel, 2016). The inflammatory response is assessed by measuring the increase in the size of the rat's paw using a plethysmometer, a tool that allows for observation of the development of the inflammatory response and measurement of the effectiveness of anti-inflammatory agents in reducing endemic conditions (Paw Edema) (Krishna and Maurya, 2018). The underlying principle of the plethysmometer is rooted in Archimedes' Law (Mohazzab, 2017). The anti-inflammatory test response exhibited the optimal anti-inflammatory potential from the dose variations identified at dose 2 CLMLE (120 mg/kg BW and 200 mg/kg BW). The volume of inflammation was measured using a Plethysmometer after the induction of 1% carrageenan, and the data were processed to obtain the percentage of edema, inflammation, and inhibition. The percentage of inflammation data indicates the extent of edema, while the percentage of inhibition or edema inhibition signifies the capacity of the test material to impede edema, thereby enabling the assessment and determination of its anti-inflammatory potential (*Table 3*).

Table 3. *Percentage (%) inflammation.*

Group	Average percentage (%) of inflammation in rat paws										
	A	B	C	D	E	F	G	H	I	J	$\bar{x}$
Negative control	37,99	43,94	60,30	77,04	86,55	129,61	110,12	128,05	108,65	108,05	89.03
Positive control	20,82	30,84	23,00	49,10	35,58	97,66	72,87	63,23	36,22	29,13	45.84
Dose 1	35,14	34,96	56,30	61,31	79,73	101,51	73,96	80,57	76,87	56,45	65.68
Dose 2	17,60	16,07	35,65	40,97	33,59	68,64	66,09	50,94	34,38	25,40	38.93
Dose 3	22,29	27,34	53,10	49,56	70,98	97,18	81,88	66,81	57,21	41,80	56.82
Dose 4	22,56	36,57	56,86	56,73	83,76	97,60	81,46	67,66	61,70	51,75	61.67

Note: A=0 min; B=30min; C=60min; D=90min; E=120min; F=150min; G=180min; H=240min; I=300min; J=360min.

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The percentage of edema or peak inflammation at 150 minutes with the negative control (CMC Na 1%), which was only given a carrier and induced by carrageenan, showed the highest percentage of visible edema (129.61%). The presence of a peak indicates that carrageenan works optimally because carrageenan theoretically causes edema in the induction method on the feet of mice. The doses 2 CLMLE of 120 mg/kg BW and 200 mg/kg BW, along with the positive control group (Diclofenac Sodium 0.9 mg/200 g BW), demonstrated the lowest levels of inflammation compared to the other treatment groups. The positive control group (Na Diclofenac 0.9 mg/200 g BW) exhibited a lower percentage of inflammation compared to the negative control (CMC Na 1%), thereby validating the efficacy of the standard drug and substantiating the robustness of the test method. The percentage of inflammation exhibited an inverse proportionality to the anti-inflammatory capacity, with higher percentages of inflammation corresponding to reduced anti-inflammatory efficacy. The percentage of inflammation data was further analyzed using Duncan (*Figure 4*). The negative control (CMC Na 1%)" was identified as the most significant (89.03%) and was found to be significantly different "(P<0.05)" from all treatments, including both positive controls and the four CLMLE dose variants. This finding indicates that all treatments exhibited the potential to reduce inflammation. The positive control group (Na Diclofenac 0.9 mg/200 g BB) was also significantly different (P<0.05) from other treatments according to Duncan's test, thereby confirming the validity of the test method based on statistical results. The second CLMLE dose (120 mg/kg BB and 200 mg/kg BB) exhibited the lowest average percentage of inflammation (38.93%) and, according to Duncan's further test, was significantly different (P<0.05) from other CLMLE dose variants but not significantly different from the positive control (Na Diclofenac 0.9 mg/200 g BB).

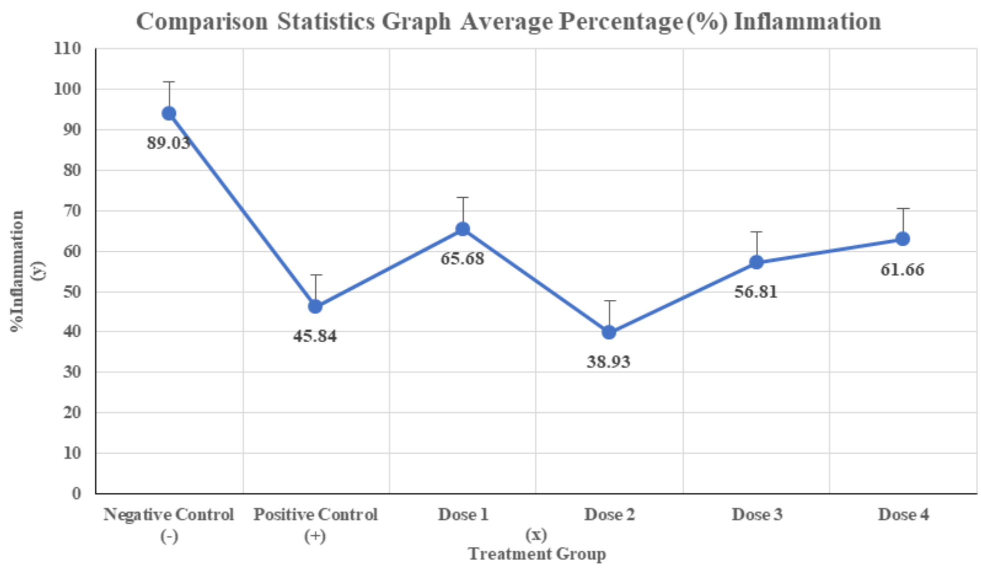

Figure 4. *Comparison statistics graph average percentage (%) inhibition.*
Note: a, b, and c indicate significant differences between treatment columns (P<0.05).

The percentage of inhibition was calculated, with the highest percentage (76.34%) observed at dose 2 CLMLE (120 mg/kg BW and 200 mg/kg BW) after 360 minutes. In contrast, the lowest percentage (48.11%) was recorded at CLMLE dose variant 1, which included 60 mg/kg BW and 100 mg/kg BW. The observed anti-inflammatory effect of the test material is indicated by a reduction in swelling (inhibition) of 50% or more. The

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anti-inflammatory effect exhibited a direct proportionality to the increasing percentage of inflammation inhibition (Istiqomah et al., 2023). The inhibition percentage of the negative control (CMC Na 1%) was either absent or negligible due to its use as a comparison and the absence of anti-inflammatory substances, resulting in an inhibition value of zero. The percentage of inhibition exhibited a direct proportionality to the anti-inflammatory capacity, with higher percentages of inhibition corresponding to greater anti-inflammatory efficacy. The data from the percentage of inhibition or edema inhibition ability can be used to determine the anti-inflammatory ability of the test material, thereby assessing its potential to be an anti-inflammatory agent (*Table 4*). The positive control (Na Diclofenac 0.9 mg/200 g BB) as well as the CLMLE dose variants based on Duncan's further test were all significantly different ($P<0.05$) from the negative control (CMC Na 1%), which was considered to have anti-inflammatory effects. Dose 2 CLMLE (120 mg/kg BB and 200 mg/kg BB) had the highest anti-inflammatory potential and was significantly different ($P<0.05$) from the other doses by Duncan's multiple comparison test and as the highest average percentage of inhibition (57.33%). Additional statistical tests helped interpret the data to identify and sort out treatments that were considered effective and efficient. Dose 2 CLMLE (120 mg/kg BB and 200 mg/kg BB) was significantly different ($P<0.05$) in the inflammation percentage and inhibition percentage data than other CLMLE dose variants marked with different alphabetical letters, indicating differences in position or placement in the statistics (*Figure 5*).

***Table 4**. Percentage (%) inhibition.*

Group	Average percentage (%) of inflammation in rat paws										
	A	B	C	D	E	F	G	H	I	J	x̄
Negative control	0	0	0	0	0	0	0	0	0	0	0
Positive control	40,37	30,89	54,92	38,16	51,20	25,45	36,39	52,44	66,76	73,46	47.00
Dose 1	-6,44	20,50	13,61	26,77	4,33	24,93	32,63	36,76	29,35	48,11	23.06
Dose 2	48,83	62,82	48,38	49,36	68,59	50,79	40,29	59,85	68,15	76,34	57.34
Dose 3	45,73	36,08	8,49	31,55	18,96	24,46	23,75	46,34	48,01	61,52	34.49
Dose 4	39,26	16,99	2,21	24,41	2,19	25,15	24,67	43,68	43,96	52,06	27.46

Note: A=0 min; B=30min; C=60min; D=90min; E=120min; F=150min; G=180min; H=240min; I=300min; J=360min.

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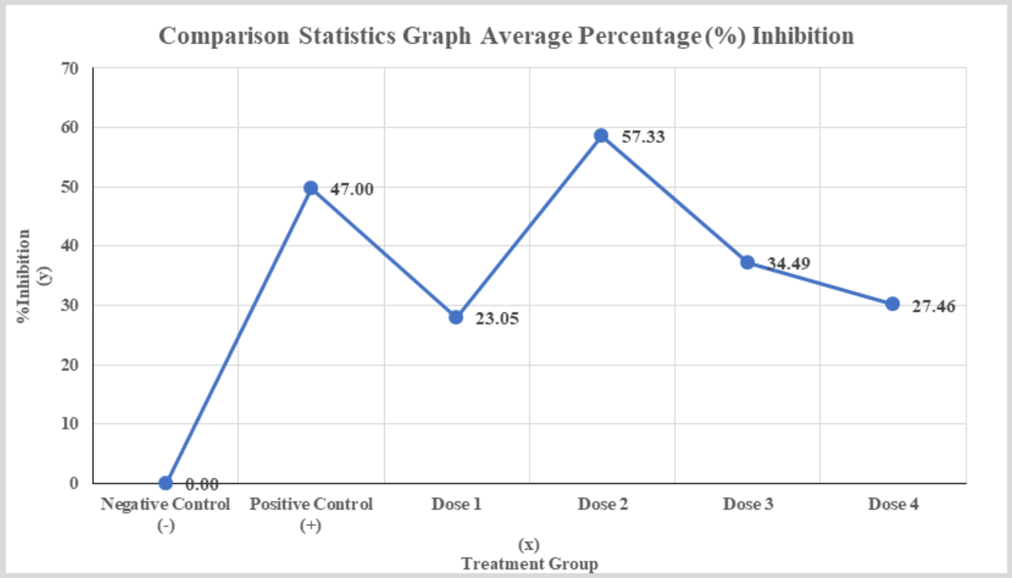

Figure 5. *Comparison statistics graph average percentage (%) inhibition.*
Note: a, b, c, d, and e indicate significant differences between treatment columns (P<0.05).

The investigation of anti-inflammatory potential that has been conducted is suspected that the combination of coffee leaves and mango leaves has an additive effect and there is no evidence of antagonistic or opposing effects produced. Synergy occurs when the combined effect of the substances exceeds their individual additive effects, while antagonism is observed when the combination produces an effect smaller than or opposite to the additive effect, or no effect at all (Niu et al., 2019). Based on the composition of the ingredients, it is known that coffee leaf extract is dominant, because dose 3 CLMLE (240 mg/kg BW and 200 mg/kg BW) has a greater percentage of inhibition than dose 4 CLMLE (120 mg/kg BW and 400 mg/kg BW). One of the factors in the effect of the test material or drug ingredients are active ingredients, the anti-inflammatory effect is directly proportional to the increasing number of active ingredients contained in the ingredients (Zunnita and Auliya, 2024). Active substances or phytochemical compounds, including phenols, tannins, flavonoids, alkaloids, terpenoids, and steroids, as well as saponins, were positive in the phytochemical analysis of coffee and mango leaves, which was consistent with the literature used (Hidayati et al., 2022; Wenas et al., 2020). According to previous studies, coffee leaves are thought to inhibit pro-inflammatory mediators, prostaglandin biosynthesis, leukotrienes, leukocyte accumulation, and neutrophil degranulation (Wenas et al., 2020). Mango leaves are thought to inhibit COX-2, arachidonic acid secretion, and histamine accumulation (Hidayati et al., 2022).

Flavonoids have been demonstrated to reduce inflammation by inhibiting the lipoxygenase (LOX) enzyme, which plays a role in leukotriene biosynthesis. In addition, flavonoids have been observed to reduce prostaglandin production by inhibiting cyclooxygenase (COX) activity and arachidonic acid metabolism. Alkaloids, classified as anti-inflammatories, have been shown to histamine release from mast cells and reduce the secretion of interleukin-1 by monocytes and platelet-activating factor (PAF) in platelets (Dwitiyanti et al., 2022; Setianto et al., 2020). Tannins have been shown to possess antioxidant activity, which contributes to their anti-inflammatory effects in several ways, including the inhibition of oxidant production (O2) by

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neutrophils, monocytes, and macrophages. Phenolics have been observed to capture free radicals and inhibit the cyclooxygenase enzyme. Saponins are known to interact with various lipid membranes, including phospholipids, which are precursors of prostaglandins and other inflammatory mediators (Batmomolin et al., 2022). Steroids and terpenoids have been shown to inhibit the oxidation of arachidonic acid, resulting in analgesic and anti-inflammatory effects. Terpenoids have also been shown to stimulate the biosynthesis of lipomodulin proteins, which can inhibit the enzymatic activity of phospholipase, the enzyme responsible for the release of arachidonic acid. In addition, terpenoids can block the cyclooxygenase and lipoxygenase pathways, further contributing to their anti-inflammatory effects.

Preliminary phytochemical test results indicate the presence of phenol, tannin, flavonoid, alkaloid, terpenoid, steroid, and saponin compounds in coffee and mango leaves. It is hypothesized that the majority of these compounds function by impeding the activation of arachidonic acid through the suppression of cyclooxygenase and lipoxygenase enzymes. The inhibition of these enzymes has been demonstrated to impede the formation of inflammatory mediators, thereby contributing to a reduction in inflammation. The study demonstrated that a combination of coffee leaves and mango leaves exhibited significant anti-inflammatory properties, suggesting their potential as natural anti-inflammatories. The observed efficacy of this combination in mitigating inflammation and edema supports the hypothesis that these natural products may offer a promising avenue for addressing inflammatory-related health concerns. The observed effect is known to be more pronounced or potent than the individual form of coffee leaves, which has been documented at a dose of 180 mg/kg BB (71.66%). The study found that coffee leaf extract exhibited a greater inhibitory effect compared to mango leaves, with higher doses of coffee leaf extract (240 mg/kg BW and 200 mg/kg BW) demonstrating a more significant inhibitory response compared to lower doses (120 mg/kg BW and 400 mg/kg BW). The study observed the highest inhibition percentage of 76.34% at dose 2 CLMLE (120 mg/kg BW and 200 mg/kg BW), indicating a substantial difference in response ($P<0.05$) between the treatment and the negative control, suggesting its potential as an anti-inflammatory agent. These findings underscore the significance of coffee leaf extract in the development of herbal anti-inflammatory drugs and their formulations and preparations.

Conclusion

The combination of coffee leaf and mango leaf extracts demonstrates significant potential as an anti-inflammatory agent. The optimal anti-inflammatory dosage is identified as variant 2 CLMLE dose (120 mg/kg BW and 200 mg/kg BW), which exhibited the highest inhibition percentage of 76.34% at 360 minutes. Furthermore, a statistical analysis, namely Duncan's test, revealed that this dosage was significantly different from other dose variants.

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Conflict of interest

The authors confirm that there is no conflict of interest involve with any parties in this research study.

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