Trop J Nat Prod Res, January 2026; 10(1): 7026 - 7035

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Anti-ulcer Effect of the Methanol Fraction of *Cuminum cyminum* Leaves against Diclofenac-induced Gastric Mucosal Damage

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ARTICLE INFO

Article history:
Received 14 September 2025
Revised 05 November 2025
Accepted 12 November 2025
Published online 01 February 2026

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ABSTRACT

The present study investigated the anti-ulcer effect of the methanol fraction of *Cuminum cyminum* leaves (MFCCL) on diclofenac-induced gastric mucosal damage in Wistar rats. Phytochemical screening of the crude extract and fractions revealed the presence of saponins, tannins, flavonoids, glycosides, terpenoids, steroids, phenols, and alkaloids in varying proportions. In vitro antioxidant analyses showed a dose-dependent increase in DPPH radical-scavenging activity, ferric reducing power, and total antioxidant capacity, with the methanol fraction exhibiting significantly higher activity than the other fractions. GC-FID analysis of MFCCL identified ten bioactive flavonoids. An acute toxicity study showed no signs of toxicity or mortality up to 5000 mg/kg body weight. MFCCL at 200 and 400 mg/kg significantly reduced gastric juice volume and ulcer index and increased gastric juice pH compared to the diclofenac-treated group, with effects comparable to ranitidine (100 mg/kg). Histopathological examination revealed that MFCCL, especially at 400 mg/kg, efficiently arrested diclofenac-induced mucosal damage, smooth muscle distortion, and hemorrhage. MFCCL significantly decreased lipid peroxidation and increased the activities of SOD, CAT, and GST compared with the diclofenac-treated group. The anti-ulcer effects of MFCCL may be attributed to its antioxidant properties and the presence of bioactive constituents. These findings suggest that MFCCL has significant gastroprotective potential against NSAID-induced gastric ulcers.

Keywords: *Cuminum cyminum*, Antioxidant, GC-FID, Diclofenac, Ulcer index, Histopathological

Introduction

Peptic ulcer disease is a prevalent gastrointestinal disorder characterized by mucosal damage resulting from pepsin and gastric acid secretion[1]. It typically manifests as gastric ulcers in the stomach or duodenal ulcers in the proximal duodenum[2,3,4]. This condition arises from an imbalance between defensive factors (e.g., mucus production, bicarbonate, prostaglandins) and aggressive factors (e.g., gastric acid, pepsin, Helicobacter pylori, and nonsteroidal anti-inflammatory drugs)[5]. Non-steroidal anti-inflammatory drugs (NSAIDs), including diclofenac, are widely used, but can cause gastric mucosal damage and ulceration[5, 6]. Although effective anti-ulcer medications exist, they often lead to adverse effects and relapse, prompting interest in natural alternatives[7]. Plant-based remedies have shown promise in treating gastric ulcers, with species such as Buchholzia coriacea and Cnidoscolus aconitifolius demonstrating anti-ulcer properties[8]. *Cuminum cyminum* L. (cumin), an annual herbaceous plant belonging to the Apiaceae family, has a long history of medicinal use[9].

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Citation: Uzoagulu CH, Chinwuba P, Attama AA, Ujam IB. Anti-ulcer Effect of the Methanol Fraction of *Cuminum cyminum* Leaves against Diclofenac-induced Gastric Mucosal Damage. Trop J Nat Prod Res. 2026; 10(1): 6922 – 6931 https://doi.org/10.26538/tjnpr/v10i1.79

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Previous studies have reported various pharmacological effects of *C. cyminum*, including antidiabetic, antimicrobial, analgesic, antioxidant, and anti-inflammatory properties[10]. Cumin is widely used to treat stomach ailments in central Asia and Xinjiang, China. Furthermore, its essential oil has been found to effectively treat gastric ulcers.[11] However, its potential gastroprotective effects against NSAID-induced ulcers have not been fully explored[12]. The novelty of this study lies in investigating the anti-ulcer effects of the methanol fraction of *Cuminum cyminum* leaves (MFCCL) on diclofenac-induced gastric mucosal damage in Wistar rats. This study aimed to elucidate the gastroprotective potential of MFCCL, its underlying mechanisms, and possible use as a natural alternative for the prevention and treatment of NSAID-induced gastric ulcers.

This study's focus on MFCCL's gastroprotective effects against diclofenac-induced ulcers addresses a significant gap in the current understanding of *C. cyminum*'s medicinal properties of C. cyminum. By exploring the mechanisms through which MFCCL exerts its anti-ulcer effects, this study could provide valuable insights into the development of novel plant-based treatments for NSAID-induced gastric damage. Furthermore, the findings of this study may have broader implications for the management of peptic ulcer disease, potentially offering a safer and more natural alternative to conventional antiulcer medications.

Materials and Methods

This study was conducted at the Faculty of Pharmacy, State University of Medical and Applied Sciences, Igbo-Eno, Enugu State. The *Cuminum cyminum* leaves used as plant material were sourced from Ihe-Achi in Oji-River L.G.A., Enugu State, Nigeria. Alfred Ozioko from the International Centre for Ethnomedicine and Drug Development (InterCEDD) in Nsukka, Enugu State, Nigeria, performed

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identification and authentication. The voucher number for *Cuminum cyminum* was Intercedd/16319.

Animals
Adult male Wistar rats, weighing 150 ± 11 g in weight, were obtained from the Animal House of the Department of Pharmacology and Toxicology at the State University of Medical and Applied Sciences, Igbo-Eno, Enugu State. They were housed in a metal cage for at least one week prior to the start of the experiment. The rats were kept under standard environmental conditions with a 12-hour light-dark cycle. The animals had access to standard food pellets and clean water ad libitum. The use of laboratory animals adhered to the laboratory practice regulations and principles of humane animal care, as documented by Zimmermann (1983). The study was approved by the Institutional Ethics and Biosafety Committee of the Faculty of Biological Sciences, University of Nigeria, Nsukka (approval no. UNN/FBS/EC/1078)

Preparation of Extract
The leaves of the plants were washed with tap water and subsequently air-dried at room temperature (25-30 °C). They were then pulverized using a mechanical grinder to achieve coarse consistency, and weighed using an electronic balance. Pulverized leaves were macerated in methanol for 48 h. The resulting filtrate was concentrated using a rotary evaporator to obtain a crude methanol extract, which was subsequently weighed to determine the percentage yield. The extract was stored in a refrigerator until further use[8,13].

Determination of the Percentage Yield of the Methanol Extract of Cuminum cyminum Leaves
The percentage yield of the methanol extract was determined from the weight of the dried pulverized leaves before maceration and the weight of the crude extract after concentration[8,9,14].

Partitioning of Cuminum cyminum Leaves
The methanol extract of *Cuminum cyminum* leaves underwent solvent-solvent partitioning using a modified method from Emiran et al.[15]. The crude extract (40 g) was dissolved in aqueous methanol (20:80 v/v) to create a stock solution, which was then partitioned using n-hexane and ethyl acetate. Initially, the crude extract (40 g) was dissolved in 200 ml of aqueous methanol (20:80 v/v) and carefully placed in a separating funnel. Next, n-hexane (400 ml) was added to the aqueous methanol extract and shaken vigorously. The mixture was allowed to separate into layers, and the n-hexane layer was collected and labelled as the *Cuminum cyminum* leaf n-hexane fraction. This process was repeated until no observable color change was observed in the stock solution. Ethyl acetate was used to repeat the process. The physical appearance and quantity of the fractions after partitioning were noted. The resulting plant fractions were dried by evaporation of the respective solvents using a rotary evaporator[8,16]. The fractions were stored at 4°C in airtight containers[17]. Phytochemical analyses of the *Cuminum cyminum* (*C. cyminum*) leaf extract and fractions were conducted using the method described by Trease and Evans[18].

In Vitro Antioxidant Properties
The *in vitro* antioxidant properties of the methanol extract and its fractions were assayed using the following formulae:

- DPPH (2, 2-Diphenyl-2-picrylhydrazyl (DPPH) assay: DPPH free radical scavenging ability of the crude extract and its fractions [19].
- Ferric reducing antioxidant power (FRAP) assay: The reducing power of the crude extract and its fractions [20].
- TAC (Total Antioxidant Capacity): Total antioxidant capacity of the crude extract and its fractions was estimated using the phosphomolybdate method [21]

Identification and Structural Elucidation of the Potent Fraction of C. cyminum leaves
Gas chromatography-flame ionization detector (FID) analysis was used to identify the bioactive compounds present in the methanol fraction of *Cuminum cyminum* leaves (MFCCL)[22].

In Vivo Studies
The Median Lethal Dose (LD_{50}) Study
The median lethal dose (LD_{50}) study was conducted using Lorke's method. Eighteen albino mice were used, and the test comprised of two phases. In the first phase, the animals were divided into three groups, each containing three mice, and administered 10, 100, and 1000 mg/kg b. w. of the extract. Because no deaths occurred, the second phase was initiated. In this phase, 1600, 2900, and 5000 mg/kg b.w were orally administered to three groups of three mice each via the oral route[23,24].

Anti-ulcerogenic Study
The anti-ulcerogenic effect of the most potent fraction, the methanol fraction of *Cuminum cyminum* leaves (MFCCL), was evaluated in Wistar rats as described by Roslida et al.[25]. Before the test, the rats were fasted for 24 h, but had free access to water. Thirty Wistar rats were used for this study. The rats were weighed and randomly divided into six groups of five rats each. Group 1 served as the normal control (without pretreatment or induction) and received normal saline (2 ml/kg body weight) orally. Group 2, the positive control (without pretreatment, but induced), also received normal saline (2 ml/kg body weight) orally. Group 3, the standard control, was pretreated with the standard drug ranitidine (100 mg/kg b.w) orally before induction. Groups 4, 5, and 6 were test groups pretreated with varying doses of the methanol fraction of *Cuminum cyminum* leaf extract (100, 200, and 400 mg/kg body weight) orally before induction. After a 14-day pretreatment period, ulcers were induced in the rats by oral administration of diclofenac (150 mg/kg b.w) suspended in normal saline (2 ml/kg) to all groups except group 1, following the method described by Roslida et al. (2010). Group 2 received 2 ml/kg b.w of normal saline + 150 mg/kg b.w of diclofenac (positive control), Group 3 received 100 mg/kg b.w of ranitidine + 150 mg/kg b.w of diclofenac (standard control), Group 4 received 100 mg/kg b.w of MFCCL + 150 mg/kg b.w of diclofenac; Group 5 received 200 mg/kg b.w of MFCCL + 150 mg/kg b.w of diclofenac, while Group 6 received 400 mg/kg b.w of MFCCL + 150 mg/kg b.w of diclofenac. Six hours after induction, the rats were sacrificed by inhalation of chloroform in an airtight plastic container, and their stomach tissues were dissected to determine biochemical indices, gastric ulcer index, gastric juice volume, and gastric juice pH. Subsequently, a portion of the stomach from one rat in each group was taken for histopathological studies[1,26].

Biochemical Assay
Six hours after diclofenac administration, all rats were euthanized by chloroform inhalation in an airtight plastic container. Stomach tissues were removed and rinsed with normal saline. Gastric juices were collected in test tubes. Stomach tissues were opened along the greater curvature, washed with normal saline, pinned flat on a cork board, and examined for gastric ulcers using a magnifying lens (×10). Each ulcer was assigned a severity rating according to Main and Whittle (1975), as follows: ≤ 1 mm = 1, 1 mm < ulcer ≤ 2 mm = 2, and 2 mm < ulcer ≤ 3 mm = 3. The total score was divided by 10 to determine the ulcer index (UI). The percentage of ulcer inhibition (percentage ulcer protection) for each group was calculated using the equation provided by Agrawal et al.[1,26,27]:

Percentage protection = (Uc-Ut/Uc) x 100Uc: Ulcer index in control
Ut: Ulcer index in test

Gastric Juice Volume
Gastric juices collected from all rats were centrifuged at 500 rpm for 5 min, after which they were separated, and their volumes were measured using a graduated cylinder[28].

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ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Gastric Juice pH
The pH of gastric juice was determined using a digital pH meter (HI 9021)[29].

Non- Antioxidant Enzymes
Lipid Peroxidation
Malondialdehyde (MDA) levels were determined. The results were expressed as nM MDA/mg of protein.

Antioxidant Enzymes
Superoxide dismutase (SOD) activity was estimated, and results were expressed as U/mg of protein. Catalase (CAT) activity was measured, and the results were expressed as M of hydrogen peroxide decomposed per milligram of protein. GSH-S-transferase (GST) activity was assessed spectrophotometrically, with enzyme activity calculated as nM of the CDNB-GSH conjugate formed per minute per mg of protein[30].

Histopathological examination
The stomachs of the scarified rats were collected and immersed in a 10 % formalin solution. The fixed specimens were trimmed, washed, and dehydrated using ascending grades of alcohol. Subsequently, the specimens were cleared in xylol, embedded in paraffin, sectioned at a thickness of 4-6 microns, and stained with Hematoxylin and Eosin to examine the stomach[31].

Statistical Analysis
The results were expressed as mean ± SD and analyzed using Statistical Product and Service Solutions (SPSS) version 20. Statistical significance was assessed through Analysis of Variance (ANOVA), with p-values less than 0.05 considered statistically significant.

Results and Discussion

Percentage Yield of the Methanol Extract of Cuminum cyminum Leaves
The extraction of 1200 g of the pulverized *Cuminum cyminum* leaves with methanol gave an extract yield of 88.05g which represents 7.34 % of the starting plant material as shown in Table 1

Percentage Yield of the Crude Extract of Cuminum cyminum Leaves and Its Fractions
The Solvent partitioning of 40 g of the crude extract from *Cuminum cyminum* leaves using n-hexane, ethyl acetate, and methanol yielded different weights and percentage yields for the fractions n-hexane (4.97 g at 12.43%), ethyl acetate (2.75 g at 6.88%), and methanol (5.84 g at 14.6%), as detailed in Table 2.

Phytohemical Composition of Methanol Extract of Cuminum cyminum Leaves and Its Fractions
Qualitative Phytochemical Constituents of Methanol Extract of Cuminum cyminum Leaves and Its Fractions
Qualitative phytochemical analysis of the methanol extract from *Cuminum cyminum* leaves and its fractions revealed the presence of saponins, tannins, flavonoids, glycosides, terpenoids, steroids, phenols, and alkaloids (Table 3). The methanol extract contained saponins, tannins, flavonoids, glycosides, phenols, alkaloids, terpenoids, and steroids. The n-hexane fraction contained saponins, tannins, flavonoids, glycosides, phenols, steroids, and alkaloids. The ethyl acetate fraction contained tannins, glycosides, terpenoids, steroids, saponins, flavonoids, phenols, and alkaloids. The methanol fraction also contains saponins, tannins, flavonoids, alkaloids, glycosides, terpenoids, steroids, and phenols.

Quantitative Phytochemicals Composition of Methanol Extract of Cuminum cyminum Leaves and Its Fractions
As illustrated in Table 4, the quantitative phytochemical profile of the methanol extract of *Cuminum cyminum* leaves and its fractions revealed the presence of varying proportions of phytochemical constituents. Saponins were the most concentrated in the crude extract and the least concentrated in the ethyl acetate fraction. The methanol fraction contained the highest amount of tannins, whereas n-hexane had the lowest amount. Flavonoid content was highest in the methanol fraction, with ethyl acetate having the lowest. Glycoside content was higher in the methanol fraction, whereas the crude extract had the lowest content. Terpenoids were abundant in n-hexane, with the methanol fraction having the lowest concentration. The alkaloid content was higher in the methanol fraction than in the other fractions, and the ethyl acetate fraction had the lowest alkaloid content. Phenols were the most abundant in the ethyl acetate fraction, with the methanol fraction containing the least. The steroid content was the highest in the ethyl acetate fraction, whereas the crude extract had the lowest.

Table 1: Percentage yield of methanol extract of *Cuminum cyminum* leaves

Extract	Weight of Plant Sample (g)	Yield of Extract (g)	Percentage Yield (%)
Methanol	1200	88.05	7.34

Table 2: Percentage yield of crude extract of *Cuminum cyminum* leaves and its fractions after partitioning

Solvent Yield	Weight of plant sample (g)	Yields of Fractions (g)	Percentage Yield (%)
n-Hexane	40	4.97	12.43
Ethylacetate	40	2.75	6.88
Methanol	40	5.84	14.6

Median lethal dose (LD_{50}) of the Methanol Extract of Cuminum cyminum Leaves
The acute toxicity study of the methanol extract from *Cuminum cyminum* leaves revealed no fatalities, signs of toxicity, or behavioral changes, even at the highest dose of 5000 mg/kg body weight (Table 5).

In-vitro Antioxidant Properties of Methanol Extract of Cuminum cyminum Leaves and Its Fractions
DPPH Scavenging Activity of Methanol Extract of Cuminum cyminum Leaves and Its Fractions
Table 6 presents the DPPH scavenging activity of the methanol extract of *Cuminum cyminum* leaves and their fractions. The results indicated a concentration-dependent increase in the DPPH scavenging activities of both the crude extract and its fractions as the concentration increased from 2 mg/mL to 16 mg/ml. Notably, the methanol fraction exhibited significantly ($p < 0.05$) higher scavenging activity than the other fractions did. At the highest tested concentration (16 mg/ml), the standard (vitamin C) demonstrated a significantly ($p < 0.05$) greater scavenging activity than both the crude extract and fractions.

Ferric Reducing Antioxidant Power of Methanol Extract of Cuminum cyminum Leaves and Its Fractions
Table 7 presents the ferric reducing power of the methanol extract from *Cuminum cyminum* leaves and its fractions, illustrating a concentration-dependent ferric reducing power in both the crude extract and *C. cyminum* fractions. The methanol fraction exhibited a significantly higher ferric reducing power ($p < 0.05$) than the other fractions and the crude extract, whereas the ethyl acetate fraction showed the lowest value. The standard (FeSO4) demonstrated a significantly higher ferric reducing power ($p < 0.05$) than all fractions at a concentration of 10 mg/ml.

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ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 3: Qualitative phytochemical composition of methanol extract of *Cuminum cyminum* leavesand its fractions

Phytochemical Constituents	Methanol Extract	n-Hexane Fraction	Ethyl-acetate Fraction	Methanol Fraction
Saponins	+	+	+	+
Tannins	+	+	+	+
Flavonoids	+	+	+	+
Glycosides	+	+	+	+
Terpenoids	+	+	+	+
Steroids	+	+	+	+
Phenols	+	+	+	+
Alkaloids	+	+	+	+

KEY
- Not detected
+ Present

Table 4: Quantitative composition of methanol extract of *Cuminum cyminum* leaves and its fractions

Fractions	**Saponins (%)**	**Tannins (mg/100g)**	**Flavonoids (mg/100g)**	**Glycosides (mg/100g)**	**Terpenoids (mg/100g)**	**Alkaloids (%)**	**Phenols (%)**	**Steroids (Mg/100g)**
Crude extract	46.02 ± 1.25^{a}	905.00 ± 3.60^{b}	32.64 ± 0.91^{b}	51.73 ± 2.06^{a}	433.76 ± 3.22^{b}	18.35 ± 0.250^{c}	12.74 ± 0.31^{c}	0.60 ± 0.08^{a}
n-Hexane	$13.51 \pm .41^{b}$	491.00 ± 3.00^{a}	21.82 ± 0.90^{a}	95.99 ± 2.21^{b}	898.15 ± 7.29^{d}	8.50 ± 0.38^{b}	9.23 ± 0.47^{b}	0.71 ± 0.047^{b}
Ethyl acetate	5.57 ± 0.13^{c}	1151.67 ± 3.06^{c}	20.94 ± 1.69^{a}	192.32 ± 1.24^{c}	497.75 ± 2.08^{c}	2.27 ± 0.14^{a}	17.60 ± 0.33^{d}	1.21 ± 0.08^{c}
Methanol	30.94 ± 0.62^{d}	3846.67 ± 1.08^{d}	53.11 ± 0.28^{c}	268.39 ± 1.45^{d}	77.07 ± 0.95^{a}	27.07 ± 0.96^{d}	2.09 ± 0.09^{a}	0.97 ± 0.13^{d}

Results are expressed in Means ± SD (n = 3)
Mean values with different superscripts down the column are considered significant at $p < 0.05$

Table 5: Acute toxicity study of the methanol extract of *Cuminum cyminum* leaves on albino mice

Groups of deaths	**Dose (mg/kg b. w)**	**Number**
PHASE I		
Group 1	10	Nil
Group 2	100	Nil
Group 3	1000	Nil
PHASE II		
Group 1	1600	Nil
Group 2	2900	Nil
Group 3	5000	Nil

Total Antioxidant Capacity of Methanol Extract of Cuminum cyminum Leaves and Its Fractions

Table 8 presents the total antioxidant capacity of the methanol extract of *Cuminum cyminum* leaves and its fractions. The total antioxidant capacities (TAC) of the samples were found to be concentration-dependent; as the concentration increased from 2.5 to 20 mg/ml, the total antioxidant capacity of the samples also increased. At the highest tested concentration (20 mg/ml), the crude extract exhibited a significantly higher ($p < 0.05$) antioxidant capacity than the other fractions, followed by the methanol fraction, with the ethyl acetate fraction showing the lowest capacity. The standard (α-tocopherol) demonstrated a significantly higher ($p < 0.05$) antioxidant capacity than the other samples.

Gas Chromatography - Flame Ionization Detector (GC-FID) of the methanol fraction of Cuminum cyminum leaves

GC-FID analysis of the methanol fraction of *Cuminum cyminum* leaves revealed different concentrations of anthocyanins, chalcones, isoflavones, flavonones, and Flavan-3-ol, Flavone, glycone, gallocatechin, catechin, and epicatechin (Table 9, Figure 1). The concentration of the phytocompound Isoflavones (25.02ug/ml) was the highest, while catechin (2.98ug/ml) was the lowest.

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ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 6: DPPH scavenging activity of methanol extract of *Cuminum cyminum* leaves and its fractions

Samples	DPPH Concentrations (mg/ml)				
	2	4	8	12	16
Crude extract	5.71±0.15^{c}	11.60±0.45^{c}	24.20±0.33^{c}	37.92±0.74^{b}	51.40±0.78^{b}
n-hexane.	2.53±0.31^{a}	4.71±0.18^{a}	14.94±0.44^{a}	39.64±1.01^{c}	54.63±0.63^{c}
Ethyl acetate.	4.66±0.29^{b}	8.60±0.29^{b}	18.05±0.21^{b}	26.27±0.40^{a}	35.05±0.55^{a}
Methanol.	8.70 ±0.16^{d}	17.80±0.60^{d}	35.27±0.88^{d}	52.53±0.98^{d}	68.02±0.50^{d}
Vitamin C	41.47±0.64^{e}	82.08±0.62^{e}	84.55±0.40^{e}	87.59±0.34^{e}	89.72±0.38^{e}

Results are expressed in Means ± SD (n = 3)
Mean values with different superscripts down the column are considered significant at $p < 0.05$

Table 7: Ferric reducing antioxidant power of methanol extract of *Cuminum cyminum* leaves and its fractions FRAP ($FeS0_4$ Standard mg/100g)

Samples	Concentrations (mg/ml)			
	10	7.5	5	2.5
Crude extract	291.33±3.05^{b}	228.33±4.04^{b}	220.67±5.69^{b}	127.00±5.29^{b}
n-Hexane fr.	493.00±3.61^{c}	422.00±2.00^{c}	374.00±3.61^{c}	214.3±3.06^{c}
Ethyl acetate fr.	168.00±2.53^{a}	141.67±2.52^{a}	126.33±2.31^{a}	88.33±3.51^{a}
Methanol fr.	932.67±3.51^{d}	883.67±2.52^{d}	702.33±1.53^{d}	448.00±3.00^{d}
$FeS0_4$ (STD)	1419.33±8.62^{e}	1250.33±19.60^{e}	894.67±4.04^{e}	643.67±6.66^{e}

Results are expressed in Means ± SD (n = 3)
Mean values with different letters as superscripts down the column are considered significant at $p < 0.05$
KEY: fr. = fraction

Effect of Methanol Fraction of Cuminum cyminum Leaves on the Gastric Juice pH, Gastric Juice Volume, Gastric Ulcer Index and Percentage Ulcer Inhi bition (Percentage Ulcer Protection) of the Diclofenac-Induced Ulcerogenic Rats

Table 10 presents the antiulcerogenic properties of MFCCL in diclofenac-induced gastric ulcers in Wistar rats. It displays the mean gastric juice pH for various groups of rats at different extract doses as well as single doses of ranitidine, normal saline, and diclofenac. A significant ($p < 0.05$) decrease in the mean gastric juice pH was observed in groups 2 and 4, with pH values of 4.62±0.45^{a} and 5.98±1.01^{a}, respectively, compared to the control. In contrast, there was no significant ($p > 0.05$) reduction in the mean gastric juice pH of rats in groups 3, 5, and 6, with pH values of 8.32±1.91^{b}, 7.74±1.08^{b}, and 8.02±1.09^{b}, respectively, compared with the control (group 1). The table also shows the mean gastric juice volume for the various groups of rats at different extract doses, as well as single doses of ranitidine, diclofenac, and normal saline. There was a significant ($p < 0.05$) increase in the gastric juice volume of groups 2 and 4, by 2.92±0.72^{d} and 2.00±0.37^{c}, respectively, compared to the control. However, there was no significant ($p > 0.05$) increase in the gastric juice volume of groups 3, 5, and 6, by 1.26±0.13^{ab}, 1.62±0.46^{abc}, and 1.78±0.31^{bc}, respectively, compared to the control (group 1). The data in Table 10 also showed that the mean ulcer index was significantly ($p < 0.05$) higher in groups 2, 4, and 5, with values of 1.68±0.56^{c}, 0.68±0.72^{b}, and 0.48±0.17^{ab}, respectively, compared to the control. Groups 4 and 5 had percentage ulcer protection of 60.0% and 71.0%, respectively. Meanwhile, there was no significant ($p > 0.05$) increase in the mean ulcer index of groups 3 and 6, by 0.30±0.10^{ab} and 0.32±0.28^{ab}, with percentage ulcer protection of 82.0% and 81.0%, respectively, compared to the control.

Effects of methanol fraction of Cuminum cyminum leaves on the antioxidant status of diclofenac-induced gastric mucosal damage in wistar rats

The findings indicated that The MDA concentration in animals administered diclofenac was significantly ($p < 0.05$) higher than that in the treatment groups. Additionally, MFCCL significantly ($p < 0.05$) enhanced the activities of SOD, CAT, and GST in the treatment groups compared to those in group 2 (positive).

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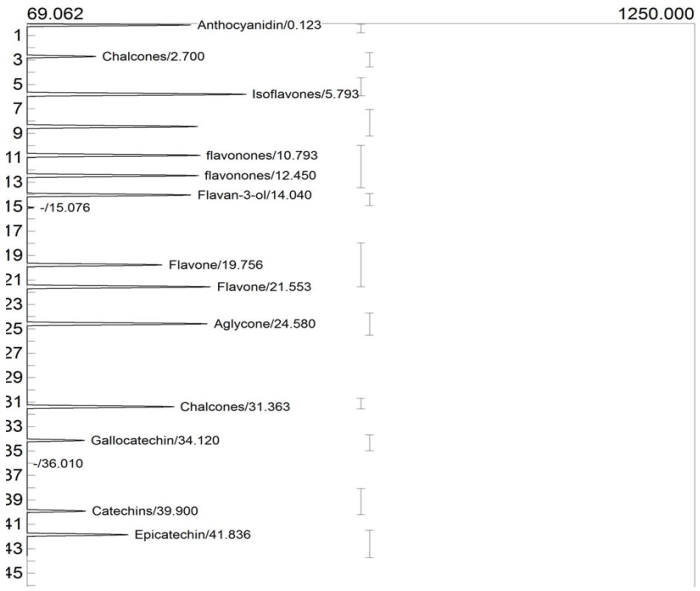
ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)



Component	Retention	Area	Height	External	Units
Anthocyanidin	0.123	8081.7916	360.197	11.5200	ug/ml
Chalcones	2.700	2436.2256	191.759	3.2387	ug/ml
Isoflavones	5.793	5819.4134	456.746	25.0192	ug/ml
flavonones	10.793	4775.5038	375.479	6.2905	ug/ml
flavonones	12.450	4743.7844	372.795	6.2487	ug/ml
Flavan-3-ol	14.040	4579.5053	360.005	7.6559	ug/ml
Flavone	19.756	3930.6483	309.146	3.8411	ug/ml
Flavone	21.553	5025.1523	394.848	3.8411	ug/ml
Aglycone	24.580	4953.9757	389.494	7.6800	ug/ml
Chalcones	31.363	4199.9884	329.743	2.3974	ug/ml
Gallocatechin	34.120	2174.5382	171.250	11.5320	ug/ml
Catechins	39.900	2206.4649	173.478	2.9755	ppm
Epicatechin	41.836	3159.5558	248.559	4.9642	ug/ml
		56086.5477		97.2042	

Figure 1: GC-FID Chromatogram of the methanol fraction of *Cuminum cyminum* leaves

Table 8: Total antioxidant capacity of methanol extract of *Cuminum cyminum* leaves and its fractions TAC (α-Tocopherol Standard mg/100g)

Samples	Concentrations (mg/ml)			
	20	10	5	2.5
Crude extract	62.35±0.49^{d}	23.59±0.53^{d}	4.74±0.26^{b}	2.46± 0.12^{b}
n-hexane.	23.38±0.65^{b}	8.26±0.25^{b}	2.50±0.25^{a}	1.45±0.10^{a}
Ethyl acetate.	6.64±0.19^{a}	5.81±0.23^{a}	5.31±0.08^{b}	2.66± 0.05^{b}
Methanol.	45.02±0.13^{c}	11.35±0.03^{c}	8.37±0.32^{c}	4.43±0.36^{c}
α-tocopherol (STD)	126.83±1.26^{e}	80.41±0.31^{e}	45.22±0.59^{d}	19.81±0.47^{d}

Results are expressed in Means ± SD (n = 3)
Mean values with different letters as superscripts down the column are considered significant at $p < 0.05$

Table 9: Chemical compounds identified in the methanol fraction of *Cuminum cyminum* leaves using Gas Chromatography-Flame Ionization Detector (GC-FID)

Components	Concentration (ug/ml)	% Concentration
Anthocyanins	11.52	11.85
Chalcones	5.64	5.8
Isoflavones	25.02	25.74
Flavonones	12.54	12.9
Flavan-3-ol	7.66	7.88
Flavones	7.68	7.9
Aglycones	7.68	7.9
Gallocatechins	11.53	11.86
Catechins	2.96	3.06
Epicatechins	4.96	5.11
Total	97.2	100

The present study explored the anti-ulcer effects of the methanol fraction of *Cuminum cyminum* leaves (MFCCL) on diclofenac-induced gastric mucosal damage in Wistar rats. These results indicate that MFCCL has significant gastroprotective potential. Phytochemical screening has identified various bioactive compounds in the crude extract and fractions, including saponins, tannins, flavonoids, glycosides, terpenoids, steroids, phenols, and alkaloids. These phytochemicals, especially flavonoids and phenolic compounds, are known for their antioxidant and anti-inflammatory properties, which may contribute to the observed gastroprotective effects[20,32]. In vitro antioxidant analyses demonstrated a dose-dependent increase in DPPH radical scavenging activity, ferric reducing power, and total antioxidant capacity. The methanol fraction showed significantly higher antioxidant activity than the other fractions, suggesting its potential as a source of natural antioxidants. GC-FID analysis of MFCCL identified ten bioactive flavonoids, with isoflavones being the most abundant. Flavonoids have been reported to possess gastroprotective properties through various mechanisms, including free radical scavenging, enhancement of mucosal defensive factors, and inhibition of inflammatory mediators[33]. An acute toxicity study confirmed the safety of MFCCL, with no signs of toxicity or mortality observed up to 5000 mg/kg body weight, indicating a wide margin of safety for potential therapeutic use. In the anti-ulcer study, MFCCL at doses of 200 and 400 mg/kg significantly reduced gastric juice volume and ulcer index, while increasing gastric juice pH compared to the diclofenac-treated group. These effects were comparable to those of ranitidine (100 mg/kg), suggesting that MFCCL may have similar gastroprotective mechanisms. Histopathological examination revealed that MFCCL, particularly 400 mg/kg, effectively prevented diclofenac-induced mucosal damage, smooth muscle distortion, and hemorrhage (Plate 1-6). This further supports the gastroprotective potential of MFCCL and its ability to maintain the gastric mucosal integrity. Antioxidant status analysis showed that MFCCL significantly decreased lipid peroxidation (MDA levels) and increased the activities of antioxidant enzymes (SOD, CAT, and GST) compared to the diclofenac-treated group. This suggests that the gastroprotective effect of MFCCL may be partly mediated through its antioxidant properties, which help to neutralize free radicals and reduce oxidative stress-induced damage to the gastric mucosa. The observed anti-ulcer effects of MFCCL may be attributed to its antioxidant properties and the presence of bioactive constituents,

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ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 10: Results of the effect of MFCCL on the Gastric Juice pH, Gastric Juice Volume, Gastric Ulcer Index and Percentage Ulcer Inhibition (Percentage Ulcer Protection) of the Diclofenac-Induced Ulcerogenic Rats

Group	Treatment	pH of Gastric Juice (GJH)	Volume of Gastric Juice (ml) (GJV)	Ulcer Index	(%) of Ulcer Protection
1	Normal saline (2 ml/kg)	8.36±1.11^{b}	1.18±0.08^{a}	0.00±0.00^{a}	-
2	Normal saline (2 ml/kg) + diclofenac (150 mg/kg)	4.62±0.45^{a}	2.92±0.72^{d}	1.68±0.56^{c}	-
3	Ranitidine (100 mg/kg) + diclofenac (150 mg/kg)	8.32±1.91^{b}	1.26±0.13$_{ab}$	0.30±0.10^{ab}	82.0%
4	MFCCL (100 mg/kg) + diclofenac (150 mg/kg)	5.98±1.01^{a}	2.00±0.37^{c}	0.68±0.72^{b}	60.0%
5	MFCCL (200 mg/kg) + diclofenac (150 mg/kg)	7.74±1.08^{b}	1.62±0.46^{abc}	0.48±0.17^{ab}	71.0%
6	MFCCL (400 mg/kg) + diclofenac (150 mg/kg)	8.02±1.09^{b}	1.78±0.31^{bc}	0.32±0.28^{ab}	81.0%

Results are presented as Mean ± S.D (n = 5)
Mean values with different letters as superscripts down the column are considered significant at $p < 0.05$

Table 11: Effects of methanol fraction of *Cuminum cyminum* leaves on the antioxidant status of diclofenac-induced gastric mucosal damage in wistar rats

Groups	MDA (mg/dl)	SOD (IU/L)	CAT (IU/L)	GST (IU/L)
Group 1-Normal control (2 ml/kg distilled water)	3.45 ± 0.29^{a}	10.10 ± 0.64^{e}	0.68 ± 0.04^{e}	64.41±3.81^{b}
Group 2- positive control (150 mg/kg diclofenac)	6.98 ± 3.99^{b}	4.23 ± 0.511^{a}	0.18 ± 0.05^{a}	39.21±2.93^{a}
Group 3 - Standard control (200 mg/kg ranitidine + 150 mg/kg diclofenac)	4.01 ± 0.43^{a}	9.28 ± 0.08^{d}	0.61 ± 0.04^{d}	68.45±4.02^{bcd}
Group 4 - 100 mg/kg MFCCL + 150 mg/kg diclofenac	4.73 ± 0.41^{ab}	8.43 ± 0.13^{c}	0.55 ± 0.03^{c}	64.38±3.01^{b}
Group 5 - 200 mg/kg MFCCL+ 150 mg/kg diclofenac	4.15 ± 0.49^{a}	9.10 ± 0.41^{d}	0.59 ± 0.03^{cd}	66.53±2.83^{bc}
Group 6 - 400 mg/kg MFCCL + 150 mg/kg diclofenac	3.25 ± 1.90^{a}	9.78 ± 0.44^{de}	0.63 ± 0.04^{de}	72.64±2.82^{d}

Results are expressed in Means ± SD (n = 5)
Mean values with different superscripts down the column are considered significant at $p < 0.05$

particularly flavonoids. These compounds may act synergistically to protect the gastric mucosa through various mechanisms, including free radical scavenging, enhancement of mucosal defense factors, and modulation of inflammatory responses[34].

Conclusion

This study explored the anti-ulcer effects of the methanol fraction of *Cuminum cyminum* leaves (MFCCL) on diclofenac-induced gastric damage in Wistar rats. The results showed that MFCCL has significant gastroprotective potential owing to its antioxidant properties and bioactive constituents, particularly flavonoids. Phytochemical screening identified compounds, including saponins, tannins,

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ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

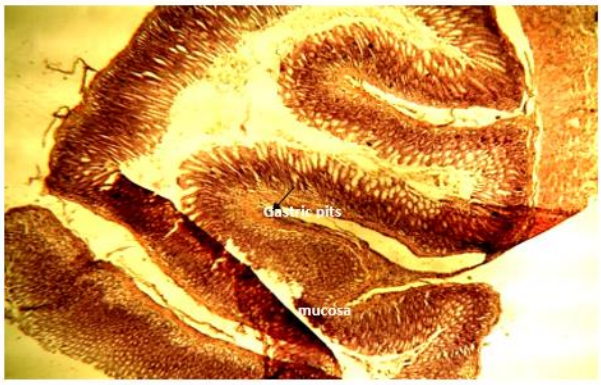

Plate 1: (Normal stomach): This photomicrograph of the stomach reveals intact gastric pits (black arrow) and mucosa, with no observed
damage.

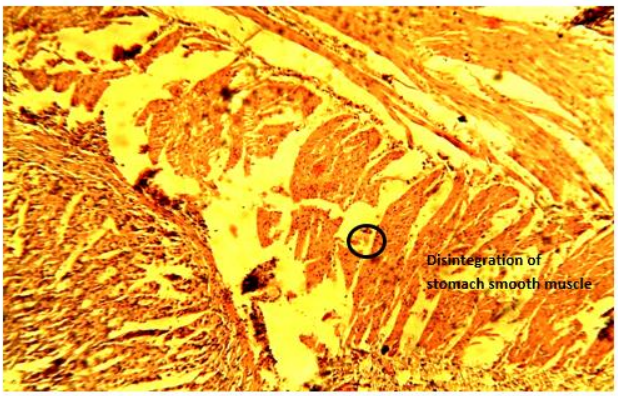

Plate 2: Photomicrograph of the stomach reveals (1) severe mucosal damage and distortion of smooth muscle, and (2) spotty hemorrhage (circle), suggesting possible hemorrhaging due to blood vessel rupture.

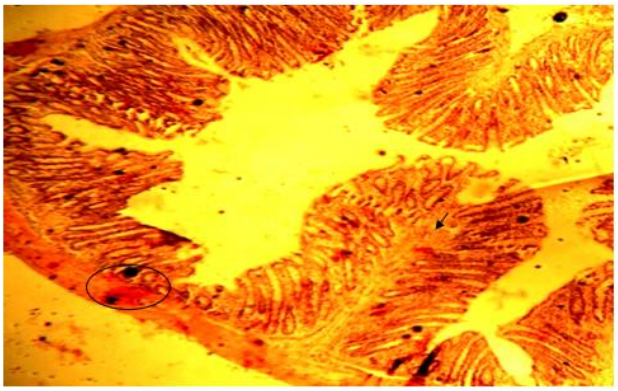

Plate 3: (standard drug) Photomicrograph of stomach showing minor hemorrhage (circles) although the stomach architecture is intact with intact gastric pits.

flavonoids, glycosides, terpenoids, steroids, phenols, and alkaloids. In vitro antioxidant analyses showed dose-dependent increases in DPPH radical-scavenging activity, ferric reducing power, and total antioxidant

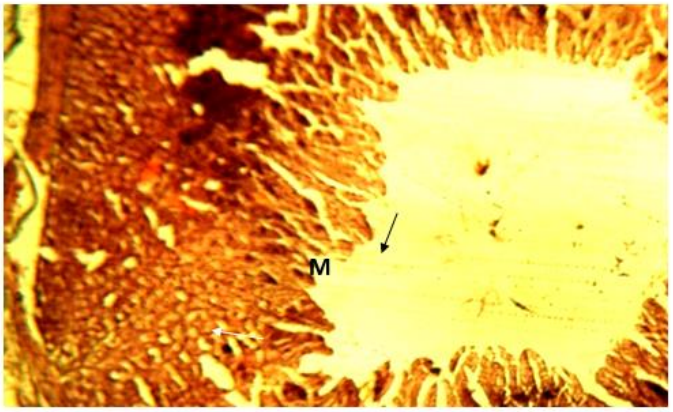

Plate 4: Photomicrograph of the stomach reveals (1) erosion of the gastric mucosa (M), with epithelial cells typically exhibiting an inflammatory reaction (black arrow); (2) the presence of chief cells (white arrow); and (3) an absence of cellular debris in the lumina.

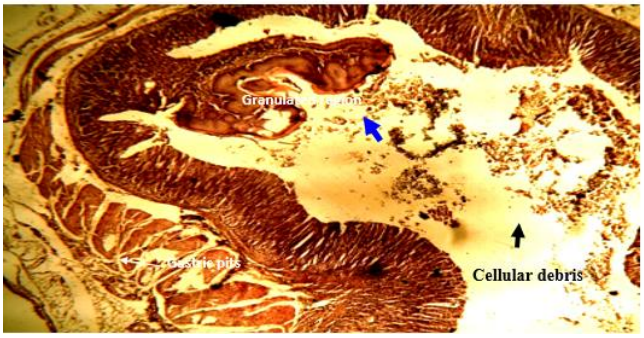

Plate 5: Photomicrograph of stomach showing (1) typical stomach region with granulation of tissue (blue arrow) characterized by the appearance of bumpy thickened structure in the mucosa. Presence of granulation tissue is a good sign of deep ulcer repair after a severe injury, (2). the gastric pits (white arrow) are intact with good formation. (3). Cellular debris is also found around the superficial zone with numerous degenerate polymorphs (black arrow).

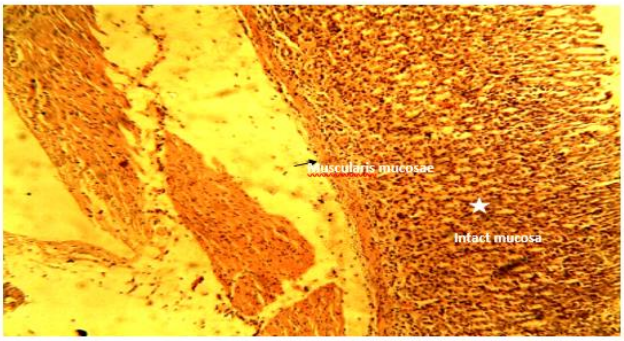

Figure 6: Photomicrograph of the stomach showing (1) no observed damage with the mucosa remaining intact (star). (2) The muscularis mucosae (MM) are also intact (black arrow).

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capacity, with the methanol fraction showing higher activity than the other fractions. An acute toxicity study confirmed MFCCL's safety up to 5000 mg/kg body weight. In the anti-ulcer study, 200 and 400 mg/kg MFCCL reduced gastric juice volume and ulcer index while increasing gastric pH compared to the diclofenac-treated group. These effects were comparable with those of ranitidine (100 mg/kg). Histopathological examination showed that 400 mg/kg MFCCL prevented diclofenac-induced mucosal damage, smooth muscle distortion, and hemorrhage. Antioxidant status analysis showed that MFCCL decreased lipid peroxidation and increased antioxidant enzyme activity compared with the diclofenac group. This study provides evidence of MFCCL's gastroprotective potential of MFCCL against NSAID-induced ulcers. These findings suggest that MFCCL may be a promising natural alternative for gastric ulcer treatment. Further studies are needed to elucidate these mechanisms and to evaluate their clinical potential.

Conflict of Interest

The author declares no conflicts of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors express their gratitude for the TETFUND Institutional Based Research (IBR) Grant 2023/2024.

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ISSN 2616-0684 (Print)
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