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Assessment of phytochemical composition and anti-inflammatory activity of methanolic roots extract of *Gnidia kraussiana* (Thymeleaceae)

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Inflammation plays a significant role in many chronic health conditions, posing a major threat to public health, especially in developing countries. Various medicinal plants and formulations have been proven to exhibit anti-inflammatory properties. *Gnidia kraussiana* is a plant used in traditional African medicine to treat inflammatory diseases such as gout, rheumatism, and jaundice. The present study was undertaken to explore the anti-inflammatory potential of the methanolic root extract of *Gnidia kraussiana* (MREGK). Material and Methods: Phytochemical characterization studies and *in vitro* antioxidant activity through DPPH, ABTS, and FRAP tests were achieved on MREGK. The safety of MREGK was evaluated by an acute oral toxicity test in conformity with OECD 423 guidelines. The *in vitro* anti-inflammatory activity of MREGK was evaluated utilizing the protein denaturation inhibition assay. The anti-inflammatory effect of MREGK (25, 50, and 100 mg/kg) was explored *in vivo* in a carrageenan-induced acute inflammation mouse model. The anti-inflammatory activity was assessed by measuring the percentage inhibition of edema. Phytochemical screening revealed varying levels of total phenolic compounds, flavonoids, and tannins. *In vitro* antioxidant tests showed antiradical capacity against DPPH free radical (426.36 mmol T.Eq/100 g) and ABTS (349.14 mmol T.Eq/100 g) as well as a strong iron reducing power (75.05 mg vitC.Eq/100 g). The acute oral toxicity evaluation of MREGK revealed a LD_{50} greater than 2000 mg/kg. The MREGK significantly inhibited protein denaturation comparable to the standard drug diclofenac. A significant inhibition of paw oedema was noted *in vitro* with MREGK comparable to diclofenac. Results suggest that *Gnidia kraussiana* exhibits significant antioxidant and anti-inflammatory activity. Consequently, its chemical constituents may hold promise for use as drugs with antioxidant and anti-inflammatory properties.

Keywords: *Gnidia kaussiana*, carrageenan, antioxidant, anti-inflammatory, protein denaturation**.**

Inflammation is a complex set of interactions among different types of cells forming a communication network involving numerous pro- or anti-inflammatory chemical mediators that can perpetuate, or modify a response[1]. Its primary function is to eliminate, or isolate the aggressor agent from the rest of the organism, and to facilitate tissue repair as quickly as possible[2]. However, when the body fails to regulate it, this inflammation persists and becomes chronic, leading to severe tissue damage accompanied by the development of certain pathophysiological processes and diseases such as cancer, atherosclerosis, osteoarthritis, Alzheimer's disease, rheumatoid arthritis, chronic asthma, sclerosis, and Crohn's disease. These diseases are prevalent worldwide and constitute one of the greatest challenges in current conventional medicine[3]. Nonsteroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids are commonly used to treat inflammatory conditions. These medications play a crucial role in reducing inflammation and disease progression[4]. However, they have several disadvantages such as short

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duration of action and long-term adverse effects including nephrotoxicity, gastrointestinal tract irritation, hematological disorders, increased risk of cardiovascular diseases, muscle weakness, cataracts, etc[5]. All these adverse effects have shifted research concerns regarding inflammation towards safer and less toxic indigenous medicines.

Medicinal plants have long been recognized as valuable sources of therapeutic agents because of their rich diversity of specialized secondary metabolites[6,7]. Among these bioactive constituents are phenolic compounds, phenolic acids, flavonoids, tannins, terpenoids, and other phytochemicals that exhibit a broad range of biological activities[8].

Increasing evidence suggests that these phytochemicals constitute an important natural reservoir of antioxidants, capable of scavenging reactive oxygen species, enhancing the body's antioxidant defense system, and protecting against oxidative stress-related disorders[8,9]. These antioxidant properties are believed to contribute significantly to their therapeutic potential and their protective effects against numerous chronic diseases.[10]

Gnidia kraussiana, is a plant employed in Central African folk medicine in the treatment of various ailments including edema, fever, bronchitis, hepatitis, snakebites, earworms, leprosy, and acute gout[11,12]. The phytochemical analysis of this plant has revealed substantial levels of flavonoids, coumarins, and terpenes[13]. Other studies have shown that phenolic compounds present in this plant extract promote immunoglobulin synthesis in case of immune-deficient infections, pediatric pulmonary infections, chronic bronchitis in adults, HIV/AIDS, leukemia and cancer[12,14]. The current work aims to study the ability of the MREGK roots to inhibit protein denaturation and its effect on carrageenan-induced inflammation in mice.

Materials and Methods

Preparation of plant material and extraction

The roots of *Gnidia kraussiana* were sourced from the 'TOKOMBERE' subdivision (Far-North region of Cameroon: longitude E 14°08'35"; latitude N 10°52'18"; altitude 746 m) after the approval of the research project by the University of Maroua (Faculty of Science). The freshly collected material was conveyed in polystyrene bags to the Laboratory of Biochemistry and Biological Chemistry within the Faculty of Science at the University of Maroua, where it was identified and authenticated by Professor Tchopsala, botanist at the same University. A voucher specimen was submitted to the Cameroon National Herbarium for comparison with specimen number 322643/HNC. The roots were rinsed three times with tap water and air-dried at room temperature. The dried materials were ground into a powder, sieved (0.5 mm), and stored prior to extraction.

Extraction involved soaking 300 grams of the powder in 3000 mL of 80% methanol for 72 hours with continuous stirring at room temperature[15]. The mixture was passed through Whatman filter paper (N°. 1) to obtain the filtrate, which was then concentrated using a rotary evaporator operating at 70°C. The resulting residue was stored at -4°C.

Experimental animals

The research involved male albino Swiss mice weighing between 25 and 30 g, obtained from LANAVET ('Laboratoire National Vétérinaire, Garoua'). All animals aged 4 months were reared in the animal breeding facility within the Department of Biological Sciences (University of Maroua). During 14 days of acclimatization, conditions of room temperature, natural light/dark cycle, free access to water and food were met for animals housed in polyacrylic cages (n=5). The animals were treated in compliance with the guidelines of the Cameroonian Bioethics Committee (reg N° FWAIRB00001954) and adhered to the *NIH Guide for the Care and Use of Laboratory Animals* (*8th edition).*

Phytochemical analysis

Total polyphenol content

The total polyphenol content of the MREGK was determined using the Folin-Ciocalteu colorimetric method described by Singleton *et al*.[16]. Briefly, a mixture of 0.2 mL of the sample diluted in 80% methanol and 1 mL of 10% Folin-Ciocalteu reagent was prepared in a test tube. Following 5 minutes of stirring, 0.8 mL of a 7.5% sodium bicarbonate solution was introduced. After a 30-minute incubation period at room temperature, absorbance was recorded at 745 nm using a UV-vis spectrophotometer (PRIM Light & Advanced, Germany). Polyphenol quantification was based on a gallic acid standard curve (0-250 µg/mL; R^2 = 0.9921), with results reported as mg GAE/100 g (milligrams of gallic acid equivalent per 100 grams of dry weight).

$$TPC\left(\frac{\text{mgGAE}}{100\text{ g}}\right)\text{DE} = \frac{\text{OD} * \text{df} * \text{V}}{\text{TP} * \alpha}$$

TPC= total polyphenol content; mg GAE= milligrams of gallic acid equivalent; DE= dry extract; OD= optical density; df= dilution factor; V= total extraction volume (10 mL); TP= test portion; α = slope of the calibration.

Flavonoids content

Flavonoid content in the MREGK was assessed following the method outlined by Mimica-Dukic *et al*.[17]. 1 mL of the extract was mixed with 1 mL of $AlCl_3$ reagent (prepared by dissolving 130 mg of $AlCl_3$ and 400 mg of sodium acetate in 100 mL of methanol) in a tube. After stirring for 5 minutes, two drops of acetic acid were introduced into the mixture. The absorbance of the resulting solution was recorded at 430 nm. The outcome was reported as milligrams of quercetin equivalent per 100 grams of dry weight (mg QE/100 g), utilizing a standard curve correlating optical density with quercetin concentration (0-100 µg/mL; R^2 = 0.9924).

$$FLAC\left(\frac{\text{mgQE}}{100\text{ g}}\right)\text{DE} = \frac{\text{OD} * \text{df} * \text{V}}{\text{TP} * \alpha}$$

Tannins content

The tannin content was assessed following the method described by Bainbridge *et al*.[18]. In the experimental procedure, 200 µL of the MREGK and 2 mL of vanillin solution (1 g vanillin/ 100 mL of HCl conc.) were added to a test tube. The mixture was then incubated in darkness for 30 minutes. After incubation, the absorbance of the resulting solution was measured at 500 nm. A calibration curve was constructed using catechin (0-50 µg/mL; R^2 = 0.9879), and the results were expressed as milligrams of catechin equivalent per 100 grams of dry weight (mg CE/100 g).

$$TANC\left(\frac{\text{mgCE}}{100\text{ g}}\right)\text{DE} = \frac{\text{OD} * \text{df} * \text{V}}{\text{TP} * \alpha}$$

Evaluation of the in-vitro antioxidant activities of the MREGK

The FRAP assay, as outlined by Benzie and Strain[19], involved combining 1 mL of either the sample or Trolox standard solution with 3 mL of FRAP reagent (Fe (III)-2,4,6-Tri(2-pyridyl)-s-triazine). After shaking for 60 seconds, the mixture was allowed to incubate for 40 minutes at room temperature. Subsequently, the solution's absorbance was measured at 593 nm using a UV-vis spectrophotometer. A calibration curve was established using ascorbic acid (0-25 µg/mL; R^2 = 0.9846), and the results were reported as milligrams of ascorbic acid (Vitamin C) equivalent per 100 grams of dry weight (mg vitCE/100g).

$$FRAP\left(\frac{mg\ vitCE}{100\ g}\right)DE = \frac{OD * df * V}{TP * \alpha}$$

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay was performed according to the procedure described by Sun et al.[20] with slight modifications. 1 mL of either the sample or standard solution (Trolox) was combined with 3 mL of 1 mM DPPH. The mixture was agitated for 2 minutes and subsequently incubated for 40 minutes in darkness at room temperature. Absorbance at 517 nm was determined using a UV-vis spectrophotometer. We constructed a calibration curve using Trolox (0-200 µM; R^2 = 0.9844), and the results were expressed in millimoles of Trolox equivalents per 100 grams of dry weight (mmol TE/100g).

$$DPPH\left(\frac{\text{mmol TE}}{100\text{ g}}\right)\text{DE} = \frac{\text{OD} * \text{df} * \text{V}}{\text{TP} * \alpha}$$

ABTS radical scavenging activity was conducted following the methodology outlined by Re *et al*.[21]. In brief, 1 mL of each sample or standard (Trolox) was combined with 3 mL of 7 mM ABTS+ (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)). Following mixing, the solution was agitated for 2 minutes and subsequently allowed to incubate in darkness at room temperature for 40 minutes before measuring absorbance at 734 nm. We created a calibration curve using Trolox (0-200 µM; R^2 = 0.9883), and the outcomes were reported as Trolox millimoles equivalent per 100 grams of dry weight (mmol TE/100 g).

$$ABTS\left(\frac{mmol\ TE}{100\ g}\right)DE = \frac{OD * df * V}{TP * \alpha}$$

Assessment of the in vitro anti-inflammatory potential of MREGK

We assessed the *in vitro* anti-inflammatory activity of MREGK using the protein denaturation inhibition assay[22]. The procedure begins with the preparation of three solutions:

The experimental solution (0.5 mL) consisted of 0.45 mL of bovine serum albumin (BSA) aqueous solution and 0.05 mL of MREGK at different concentrations (10, 50, 100, 300, 500, and 1000 µg/mL)

The control solution (0.5 mL) consisted of 0.45 mL of an aqueous solution containing 5% bovine serum albumin (BSA) and 0.05 mL of distilled water.

The reference solution (0.5 mL) included 0.45 mL of 5% BSA aqueous solution and 0.05 mL of sodium diclofenac solution at different concentrations (10, 50, 100, 300, 500, and 1000 µg/mL).

The pH of all solutions was adjusted to 6.3 using 1N HCl solution. Subsequently, the samples were incubated at 37°C for 20 minutes, after which they were heated to 57°C to maintain this temperature for 3 minutes. Following cooling, 2.5 mL of phosphate-buffered saline solution (0.1M; pH 6.3) was introduced to each solution, and absorbance readings were taken at 416 nm using a UV-visible spectrophotometer. The extent of inhibition of protein denaturation was determined as follows:

$$\% \text{ Inh } = \frac{OD(contrl) - OD(test)}{DO(control)}\ x\ 100$$

OD = optical density

The control represents 100% denatured proteins.

Assessment of the *in vivo* anti-inflammatory activity: carrageenan-induced paw edema

Male mice weighing 25 and 30 g were distributed into 6 groups of 5 animals each and treated as follows:

Three groups were treated with MREGK (25, 50, and 100 mg/kg, *p.o.*);

A positive control group received diclofenac (5 mg/kg, *p.o.*);

Two control groups (normal and negative) received 10 mL/kg, *p.o.* of distilled water each.

One hour post-treatment administration, mice received a 50 µL injection of a freshly prepared carrageenan suspension (1%) in 0.9% saline solution into the sub-plantar tissue of the right hind paw. The normal control group received an injection of an equal volume of physiological saline[23]. Paw thickness was measured hourly for 6 hours using a Vernier caliper[24]. The percentage inhibition of edema for each group was standardized against the negative control group. This inhibition percentage was computed using the formula provided below.

$$\% \text{ Inh } = \frac{D\ (negative\ \ control\ ) - D\ (test\ \ group\ )}{D(negative\ \ control\ )} \text{ x } 100$$

D= diameter of the paw

Acute toxicity test

Two groups (n = 3) of female rats (Wistar) weighing 150-200 g were utilized. One group served as a control and received distilled water (10 mL/kg), while another group received a high dose (2000 mg/kg) of MREGK. The rats were closely monitored at 30 minutes, 1 hour, 2 hours, 4 hours, 24 hours, 48 hours, and 14 days for any abnormal behavior or signs of toxicity. Monitored parameters included physical appearance, grooming behavior, salivation, trembling, lethargy, convulsions, diarrhea, coma, startle response, stool characteristics, changes in body weight, and mortality.

Statistical analysis

The results of the total phenolic content, antioxidant, and *in vitro* anti-inflammatory activities assays were presented as means ± SD of three replicates (n = 3). Statistical analysis was conducted using one-way analysis of variance (ANOVA) with Dunnett's post hoc tests for multiple comparisons. Statistical significance was considered at $p < 0.05$. GraphPad Prism 8.01 software was employed for data analysis.

Results

Phytochemical characteristics of MREGK

Extraction yield, total phenolic compounds, flavonoids, and tannins contents

The MREGK extraction yield was 22.33%. Various MREGK phytoconstituents including total phenolic compounds, flavonoids, and tannins are depicted in Table 1.

Antioxidant activities of MREGK

The result displayed in Table 2 revealed that MREGK exhibits a strong ability to reduce iron, with a reducing power of 75.05 mg.vitCE/100 gDE. Furthermore, the extract analyzed in this study demonstrates significant antiradical activities, with high Trolox equivalent concentrations of 426.36

Table 1 — Ferric reducing potential and scavenging activity of methanolic root extract of *G. kraussiana*

	FRAP (mg.vitC.Eq/100 g DE)	DPPH (mM.T.Eq/100 g DE)	ABTS (mM.T.Eq/100 g DE)
MREGK	75.05 ± 3.87	426.36 ± 10.17	349.14 ± 5.72

The values were expressed as means ± SEM, (n = 3) for a total of three samples. MREGK: methanolic root extract of *G. kraussiana*; ABTS: 2,2'-azinobis-(3-ethylbenzothiazoline)-6-sulfonic acid; DPPH: 2,2-diphenylpicrylhydrazyl; FRAP: ferric reducing antioxidant potential; mg.vitC.Eq : milligram equivalent of Vitamin C; mM.T.Eq : millimol of Trolox equivalent ; DE: dry extract.

Table 2 — Percentage of inhibition of protein denaturation as a function of methanolic root extract of *G. kraussiana* (MREGK) and diclofenac concentrations.

Concentration (µg/mL)	10	50	100	300	500	1000
Diclofenac (%)	57.08 ± 2.12[a]	68.46 ± 3.02[a]	71.62 ± 3.08[a]	72.88 ± 3.13[a]	87.13 ± 4.03[a]	93.35 ± 5.24[a]
MREGK (%)	50.27 ± 1.92[b]	61.67 ± 2.67[a]	65.09 ± 1.82[a]	71.74 ± 2.89[a]	80.66 ± 3.60[a]	91.82 ± 3.73[a]

The values were represented as means ± SEM, (n = 3). MREGK: methanolic root extract of *G. kraussiana*
Mean values with different superscript letters in the same column are significantly different ($p < 0.05$).

Table 3 — Effect of the methanolic root extract of *G. kraussiana* (MREGK) on the percentage inhibition of edema induced by Carrageenan compared to diclofenac.

Time (hours)		1	2	3	4	5	6
Diclofenac (5 mg)		21.67%**	22.72%**	23.93%**	26.75%**	27.46%**	29.25%**
MREGK	25 mg/kg	0.1166	18.68%*	15.69%*	17.98%*	20.98%**	23.61%**
	50 mg/kg	26.11%**	29.79%**	25.38%**	28.07%**	34.46%***	36.52%***
	100 mg/kg	23.33%**	27.27%**	25.87%**	35.08%***	40.41%***	43.14%***

Mean values, (n = 5), * p <0.05; ** p <0.01; and *** p<0.001 compared to the Negative Control.

mmol.TE/100 gDE and 349.14 mmol.TE/100 gDE respectively, against the DPPH and ABTS radicals.

Effect of MREGK on protein denaturation inhibition

According to Table 3, the inhibition rate of protein denaturation increased with higher concentrations of both MREGK and Diclofenac. At a concentration of 10 µg/mL, an antithermodenaturing activity was noted with MREGK (50.27%) compared to that of diclofenac (57.08%). At the concentration of 100 µg/mL, an antithermodenaturing activity noted with MREGK and diclofenac was 65.09% and 71.62% respectively. At the concentration of 1000 µg/mL, a strong antithermodenaturing activity was noted with MREGK (91.82%) compared to that of diclofenac (93.35%)

Effect of MREGK on carrageenan-induced paw edema

The results obtained from this study on the anti-inflammatory effect of the MREGK, conducted on the acute paw edema model induced by carrageenan in mice are depicted in Figure 1. According to these results, paw edema significantly progressed ($p < 0.001$) in group of negative control, reaching a maximum value of 3.7 mm at the 5th hour, compared to the normal control group which measured an average diameter of 1.7 mm. In the groups treated with diclofenac (5 mg/kg) and MREGK (25, 50, and 100 mg/kg), a notable reduction in paw edema was observed compared to the negative control group from the 1st hour through the 6th hour (Figure 1), with statistical significance noted ($p < 0.001$; $p < 0.01$).

In order to highlight the anti-inflammatory effect of these extracts, the calculated inhibition percentages (Table 4) were reported relative to the negative

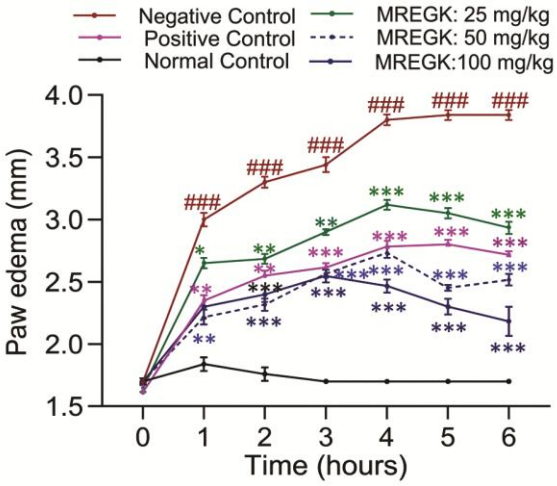


Fig. 1 — Effect of MREGK on the evolution of carrageenan-induced edema in mice The values are presented as means ± SD, (n = 5). Significant differences compared to the control groups are presented as follows: ### $p < 0.001$ compared to the normal control; * $p < 0.05$; ** $p < 0.01$; and *** $p < 0.001$ compared to the negative control. MREGK: methanolic root extract of *Gnidia kraussiana*

control, and compared to that of the standard. As early as the 1st hour, MREGK (50 and 100 mg/kg) showed paw edema inhibition percentages of 26.11% and 23.33%, similar to that observed with diclofenac (21.67%). From the 4th hour onwards, MREGK (50 and 100 mg/kg) exhibited a higher paw edema inhibition rate compared to diclofenac (5 mg/kg).

Acute toxicity of the MREGK

According to Table 4, rats administered 2000 mg/kg of MREGK exhibited moderate signs of toxicity four hours after administration, such as drowsiness, isolation, and hypoactivity. Doughy stools were

Table 4 — Results of acute toxicity tests of methanolic root extract of *G. Kraussiana* in rats.

Clinical Sign	Period Dose	D1				D2	D4	D6	D8	D10	D12	D14
		1H	2H	3H	4H							
Grooming	MEGK (2 g/kg)	N	N	N	N	N	N	N	N	N	N	N
	Distilled water	N	N	N	N	N	N	N	N	N	N	N
Fur appearance	MEGK (2 g/kg)	N	N	N	N	N	N	N	N	N	N	N
	Distilled water	N	N	N	N	N	N	N	N	N	N	N
Trembling	MEGK (2 g/kg)	N	N	N	N	N	N	N	N	N	N	N
	Distilled water	N	N	N	N	N	N	N	N	N	N	N
Coma	MEGK (2 g/kg)	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
	Distilled water	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
Salivation	MEGK (2 g/kg)	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
	Distilled water	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
Convulsion	MEGK (2 g/kg)	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
	Distilled water	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
Startle response	MEGK (2 g/kg)	R	R	R	N	N	N	N	N	N	N	N
	Distilled water	N	N	N	N	N	N	N	N	N	N	N
Stool appearance	MEGK (2 g/kg)	N	N	N	P	P	N	N	N	N	N	N
	Distilled water	N	N	N	N	N	N	N	N	N	N	N
Mortality	Distilled water and MEGK(2 g/kg)	0	0	0	0	0	0	0	0	0	0	0

N = Normal; P = Pasty; R = Reduced; Abs = Absent.

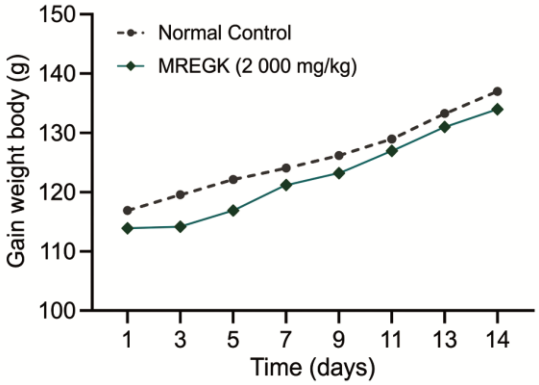


Fig. 2 — Effect of MREGK on the body weight gain of rats MREGK: methanolic root extract of *Gnidia Kraussiana*.

observed on the second day. From the 3rd until the 14th day of the experiment, all animals behaved like to the normal control group.

Moreover, there were no notable changes observed in the weight progression of the animals studied during the 14-day observation period. This is depicted in Figure 2.

Discussion

The objective of the current study was to assess the anti-inflammatory effects of MREGK. This was assessed both *in vitro* on protein denaturation inhibition and *in vivo* using the carrageenan-induced paw inflammation model.

Phytochemical analyses of medicinal plants are important for further recognizing the therapeutic efficacy of the plant. Analysis of MREGK's phytochemical composition revealed the presence of phenolic compounds, tannins, and flavonoids, known for their robust antioxidant properties that could support various therapeutic benefits[25]. These compounds provide protection against disorders caused by free radicals while inhibiting the action of enzymes producing these free radicals such as xanthine oxidase[25,26]. Protection is better ensured when the anti-radical activity in ABTS and DPPH assays is high, and when antioxidant activity in the FRAP test is significant. The present study showed that MREGK exhibited high antioxidant potential *in vitro*, particularly in terms of radical scavenging activity (DPPH and ABTS) and iron-reducing power. Compounds with antioxidant properties are capable of preventing protein denaturation, which is one of the causes of inflammatory diseases. The automatic generation of antigens in this disorder could be due to protein denaturation. Protein denaturation involves modifications of electrostatic, hydrophobic, and disulfide bonding[22,27].

In the present study, MREGK significantly inhibited protein thermal denaturation in the *in vitro* assay. Consequently, it is suggested that MREGK's prevention of protein denaturation and auto-antigen production may be responsible for its anti-arthritic effect[28]. Thus, it has been reported that a compound with a percentage inhibition of protein denaturation greater than 20 % is expected to possess anti-

inflammatory properties[29]. These effects are likely attributed to certain phenolic compounds (flavonoids and tannins) present in MREGK. Phenolic compounds have the ability to interact with the aliphatic region around lysine or threonine residues on the albumin protein, thus preventing protein denaturation[28,30].

The results from *in vitro* studies suggest that MREGK could potentially provide protective effects in live organisms against oxidative damage and free radicals implicated in various health conditions such as tumors, cancers, diabetes, sickle cell disease, and rheumatoid arthritis[31,32]. Articular cartilage is primarily composed of proteins whose denaturation leads to the development of inflammation[33]. Several agents, including substances such as carrageenan, can cause harm to articular cartilage[33,34].

Carrageenan induces local edema through tissue damage, reaching its peak after 4 hours. Injection of carrageenan subcutaneously induces a localized inflammatory response characterized by five well-known signs: swelling, redness, heat, loss of function, and local hypersensitivity[35,36].

The development of edema following carrageenan injection is a biphasic process. The initial phase occurs immediately after injection, resulting in the release of histamine and serotonin by mast cells. These factors induce vascular changes that lead to plasma exudation[23], followed by a second phase of swelling, caused by increased release of arachidonic acid metabolites such as prostaglandins and leukotrienes in the inflammatory area[37]. This stage is responsive to prostaglandin synthesis antagonists and natural or synthetic anti-inflammatories such as glucocorticoids[38]. Non-steroidal anti-inflammatory drugs also inhibit cyclooxygenase enzymes involved in prostaglandin synthesis[23].

In this study, the validity of the method was first assessed by testing diclofenac, a non-steroidal anti-inflammatory drug known to be effective against carrageenan-induced oedema[39]. The increase in paw diameters observed in the edematous control group up to the 6th hour suggests that any reduction in edema during the 6-hour period would be attributed to the effects of the administered treatments. Throughout the study, diclofenac significantly decreased ($p < 0.01$) edema formation starting from the first hour and continuing for the duration of the experiment. MREGK (25, 50, and 100 mg/kg) significantly exhibited inhibitory effects on edema formation at all evaluation time points, similar to that observed in the diclofenac-treated group. The maximal activity of MREGK observed from the 3rd hour of experimentation, coinciding with the release of prostaglandins at the inflammatory site, which could be attributed to the presence of cyclooxygenase inhibitors in these extracts that may lead to the inhibition of prostaglandin synthesis. This finding suggests that MREGK possesses an anti-inflammatory mechanism of action similar to that of nonsteroidal anti-inflammatory drugs, possibly due to the presence of bioactive compounds such as total phenolic compounds, flavonoids, and tannins[39]. Many studies have shown that polyphenols and their metabolites inhibit enzymatic activities involved in arachidonic acid metabolism and reduce the production of inflammation mediators such as prostaglandins and leukotrienes[31,40]. The outcomes of our investigation are in agreement with previous studies that validate the anti-inflammatory activity and lysosomal enzymes release inhibition potential of *Gnidia. Kraussiana* release of strongly implicated in the inflammatory response[14].

The acute toxicity study of MREGK focused on observing physiological changes in female rats. At the end of the 14-day observation period, the parameters studied showed no signs of toxicity. No mortality was recorded, resulting in an LD_{50} greater than 2000 mg/kg. Therefore, following Hodge and Stenner's[41] toxicity scale, MREGK falls into the non-toxic classification. This result corroborates those of other researchers[42] who found similar results in their work.

Conclusion

Based on the study's results, the methanolic root extract of *Gnidia kraussiana* shows strong scavenging activity against DPPH and ABTS radicals and effectively reduces ferric ions *in vitro*, indicating significant antioxidant activity. Moreover, the methanolic root extract of *Gnidia kraussiana* demonstrates remarkable anti-inflammatory efficacy both *in vitro* and *in vivo* due to various bioactive compounds endowed with antioxidant potentials responsible for its pharmacological effectiveness. Additionally, the studied plant extract showed no signs of acute oral toxicity in experimental rats at a high dose of 2000 mg/kg. Further research aimed at evaluating chronic toxicity and other models of chronic inflammation is necessary.

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Conflict of interest

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References

1 Kernouf N, Effet des extraits de *Capparis spinosa* sur la production des médiateurs inflammatoires des neutrophiles et des monocytes. *Thèse de doctorat*. Université Université Ferhat Abbas Sétif 1, (2019) 3.

2 Prabhu SR, Inflammation: An Overview. In: Textbook of General Pathology for Dental Students, *Springer Cham*, (2023) https://doi.org/10.1007/978-3-031-31244-1_6.

3 Foyet HS, Tsala DE, Bodo JZE, Carine AN, Heroyne LT & Oben E, Anti-infammatory and anti-arthritic activity of a methanol extract from *Vitellaria paradoxa* stem bark. *Pharmacogn Res*, 7 (2014) 367.

4 Kalpesh RP, Jadhav RB, Mahajan VK, Patil PR & Gaikwad PS, Antiarthritic activity of bartogenic acid isolated from fruits of Barringtonia racemosa Roxb. (*Lecythidaceae*), *Altern. Med*, 2011 (2011) 785245. Doi: 10.1093/ecam/nep148.

5 Kanagasanthosh K, Shanmugapriyan S & Kavirajan V, Evaluation of acute toxicity, anti-inflammatory activity and phytochemical screening of ethanolic extract of *Azadirachta indica* leaves. *International Journal of Research and Development in Pharmacy and Life Sciences*, 4 (2015) 1737. http://www.ijrdpl.com/docs/v4i5/7 .

6 Acquaviva A, Di Simone SC, Nilofar BA, Zengin G, Recinella L, Leone S, Brunetti L, Uba AI & Guler O, Screening for Chemical Characterization and Pharmacological Properties of Different Extracts from *Nepeta italica*. *Plants*, 12 (2023) 2785. https://doi.org/ 10.3390/plants12152785

7 Salehi B, Zucca P, Sharifi-Rad M, Pezzani R, Rajabi S, Setzer WN, Varoni EM, Iriti M, Kobarfard F & Sharifi-Rad J, Phytotherapeutics in cancer invasion and metastasis. *Phytother. Res*, 32 (2018) 1425. doi :10.1002/ptr.6087

8 Zengin G, Mahomoodally MF, Aktumsek A, Jek″o J, Cziáky Z, Rodrigues MJ, Custodio L, Polat R, Cakilcioglu U & Ayna A, Chemical Profiling and Biological Evaluation of *Nepeta baytopii* Extracts and Essential Oil: An Endemic Plant from Turkey. *Plants*, 10 (2021)1176. https://doi.org/ 10.3390/plants1006117

9 Montesano D, Rocchetti G, Putnik P & Lucini L, Bioactive profile of pumpkin: An overview on terpenoids and their health promoting properties. *Curr. Opin. Food Sci*, 22 (2018) 81.

10 Yagi S, Zengin G, Cetiz MV, Cziáky Z, Jeko J, Bahşi M, Çakılcıoğlu U, Turumtay H & Aktumsek A, Caractérisation chimique, activités antioxydantes et inhibitrices enzymatiques de différents extraits de trois espèces de *Phlomis*. *ChimieOpen*, 14 (2025) 202500004. doi: 10.1002/open.202500004.

11 Chaabane F, Boubaker J, Loussaif A, Neffati A, Kilani-Jaziri S, Ghedira K & Chekir Ghedira L, Antioxidant, genotoxic and antigenotoxic activities of daphne gnidium leaf extracts. *BMC Complementary and Alternative Medicine*, 12 (2012) 153.

12 Vidal V, Potterat O & Louvel S, Library-based discovery and characterization of daphnane diterpenes as potent and selective HIV inhibitors in daphne gnidium. *Journal of Natural Products*,75 (2012) 414.

13 Botineau M. Fruit rouge à maturité, Garou. Guide des plantes à fruits charnus comestibles et toxiques, *Lavoisier*. Paris, 2015.

14 Koubala BB, Elize D, Dieudonne N, Stephane Z, Alphonse L & Gisèle A, Hydromethanolic Root Extract of *Gnidia Kraussiana* Demonstrates Anti-Inflammatory Effect Through Anti-Oxidant Activity Enhancement in a Rodent Model of Gout. *Dose-Response: An International Journal*, 2023 (2023) 1.

15 Asefa M, Teshome N & Degu A. Anti-Inflammatory and Analgesic Activity of Methanolic Root Extract of *Verbascum sinaiticum* Benth. *J Inflamm Res*, 15 (2022) 6381.

16 Singleton VL, Orthofer R & Lamuela-Raventos RM, Analysis of total phenols and other oxidant substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, 219 (1999) 152.

17 Mimica-Dukić N, Bugarin D, Grbovic S, Mitic-Culafic D, Vukovic-Gacic B & Orcic D, Essential Oil of *Myrtus communis L*. As a Potential Antioxidant and Antimutagenic Agents. *molecules*, 15 (2010) 2759.

18 Brainbridge Z, Tomlins K, Wellings K & Westby A, Methods to assessing quality characteristics of non-grain start (Part 3. Laboratory Methods). Chatham, *UK: Natural Resources Institute*, 83 (1996) 185.

19 Benzie IFF & Strain JJ, The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry*, 239 (1996) 70.

20 Sun T, Tang J & Powers JR, Effect of pectolytic enzyme preparations on the phenolic composition and antioxidant activity of Asparagus juice. *Journal of Agricultural and Food Chemistry*,113 (2005) 964.

21 Re R, Pellegrini N, Proteggente A, Pannala A, Yang M & Rice-Evans C, Antioxidant Activity Applying an Improved ABTS Radical Cation Decolorization Assay. *Free Radical and Biological Medicine*, 26 (1999) 1231.

22 Alamgeer NSG, Uttra AM, Qaiser MN & Ahsan H, Appraisal of anti-arthritic and nephroprotective potential of *Cuscuta refexa*. *Pharmaceutical Biology*, 55 (2017) 792.

23 Shabbir A, Batool SA, Basheer MI, Shahzad M, Sultana K, Tareen RB & Iqbal J, *Ziziphora clinopodioides* ameliorated rheumatoid arthritis and infammatory paw edema in diferent models of acute and chronic infammation. *Biomed Pharmacother*, 97 (2018) 1710.

24 Bamgbose SO & Noamesi BK, Studies on cryptolepine. II: Inhibition of carrageenan induced oedema by cryptolepine. *Planta Medica*, 41 (1981) 392.

25 Gohil P, Patel V, Deshpande S, Chorawala M & Shah G, Anti-arthritic activity of cell wall content of *Lactobacillus plantarum* in freund's adjuvant-induced arthritic rats: involvement of cellular inflammatory mediators and other biomarkers. *Inflammopharmacology*, 26 (2018) 171.

26 Halliwell B. Understanding mechanisms of antioxidant action in health and disease, *Nat Rev Mol Cell Biol*, 25 (2024) 13.

27 Masson P & Sofya L, Conformational Stability and Denaturation Processes of Proteins Investigated by Electrophoresis under Extreme Conditions. *Molecules*, 27 (2022) 6861.

28 Falcão S, Baptista P, Freire C, Vilas-Boas M & Ferreira ICFR. Antioxidant, Antioxidant activity of *Agaricus sp.* mushrooms by chemical, biochemical and electrochemical assays. *Food Chemistry*, 11 (2016) 61.

29 Bailey-Shaw YA, Williams A, Green CE, Shawntae R & Smith AM, In-Vitro Evaluation of the Anti-Inflammatory Potential of Selected Jamaican Plant Extracts using the Bovine Serum Albumin Protein Denaturation Assay. International Journal of Pharmaceutical Sciences Review and Research, 47 (2018) 145.

30 Mutha RE, Tatiya AU & Surana SJ, Flavonoids as natural phenolic compounds and their role in therapeutics: an overview. *Futur J Pharm Sci*. 7 (2021) 25.

31 Kple TKM, Akakpo-Akue J, Golly JK, Fofié Y, Ahon MG, Kra MA, Sanogo I & Yapo-Crezoit AC), Phytochemical characterization of three plants and their antisickling activity in the management of sickle cell disease. *Journal of Bosciences and Medecine*, 8 (2020) 100.

32 Rather MA, Gupta K, Gupta AK, Mishra P, Qureshi A, Dutta TK, Joardar SN & Mandal M, Phytochemical Analysis and Demonstration of Antioxidant, Antibacterial, and Antibiofilm Activities of Ethnomedicinal Plants of North East India. *Appl Biochem Biotechnol*, 195 (2023) 3257. doi: 10.1007/s12010-022-04273-0

33 Williams LAD, O'Connar A, Latore L, Dennis O, Ringer S & Whittaker JA, The in vitro anti-denaturation effects induced by natural products and non-steroidal compounds in heat treated (immunogenic) bovine serum albumin is proposed as a screening assay for the detection of anti-inflammatory compounds, without the use of animals. *West Indian Medical Journal*, 57 (2008) 327.

34 Jalalpure SS, Mandavkar YD, Khalure PR, Shinde GS, Shelar PA & Shah AS, Antiarthritic activity of various extracts of *Mesua ferrea Linn. seed*. *Journal of Ethnopharmacology*, 138 (2011) 700.

35 Meira NA, Klein LC Jr. & Rocha LW, Anti-inflammatory and anti-hypersensitive effects of the crude extract, fractions and triterpenes obtained from *Chrysophyllum cainito* leaves in mice. *Journal of Ethnopharmacology*, 151 (2014) 975.

36 Berrueta L, Muñoz-Vergara D, Martin D, Thompson R, Sansbury B, Spite M, Badger G & Langevin H, Effect of stretching on inflammation in a subcutaneous carrageenan mouse model analyzed at single-cell resolution. *J Cell Physiol*, 238 (2023) 2778. doi: 10.1002/jcp.31133.

37 Mandal G, Chatterjee C & Chatterjee M, Evaluation of anti-inflammatory activity of methanolic extract of leaves of Bougainvillea spectabilis in experimental animal models. *Pharmacognosy Res*, 7 (2015) 18.

38 Reto M, Almeida C, Rocha J, Sepodes B & Figueira ME, Green Tea (*Camellia sinensis*): Hypocholesterolemic Effects in Humans and Anti-Inflammatory Effects in Animal. *Pharmacology & Pharmacy*, 5 (2014) 1113.

39 Adnan M, Nazim Uddin CM, Kamal ATMM, Barlow JW, Faruque MO & Yang X, Evaluation of anti-nociceptive and anti-inflammatory activities of the methanol extract of *Holigarna caustica* (Dennst.) Oken leaves. *Journal of Ethnopharmacology*, 236 (2019) 401.

40 Amari NO, Missoun F, Mansour S, Sekkal FZ & Djebli N, In vivo Anti-inflammatory Activity of Aqueous Extract of *Carthamus caeruleus L* Rhizome Against Carrageenan-Induced Inflammation in Mice. *Jorda*n *Journal of Biological Sciences*, 14 (2021) 529.

41 Hodge HC & Stenner JH, Determinations of substance acute toxicity by LD50. *American Industrial Hygiene Association*, 10 (1943) 93.

42 Etame LG, Yinyang J, Okalla EC, Makondo BV, Ngaba GP & Mpondo ME, Étude de la toxicité aigüe et subaigüe de l' extrait au vin des graines de *Carica papaya Lin*. *Journal of Applied Biosciences*, 120 (2017) 12077.