Trop J Nat Prod Res, October 2023; 7(10):4152-4159 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

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

Original Research Article

A Comparative Study on Polyphenol, Flavonoid Content, Antioxidant and Anti-Inflammatory Capacity of Different Solvent Extract from *Portulaca oleracea* in Carrageenan-Induced Paw Edema in Mice

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ARTICLE INFO

Article history:
Received 01 June 2023
Revised 16 September 2023
Accepted 06 October 2023
Published online 01 November 2023

ABSTRACT

Portulaca oleracea L., belonging to the Portulacaceae family, is a succulent annual plant, that has been used as vegetables and folk medicine in many Asian countries. There is no systematic and comprehensive research on the comparison of phytochemical quantification and biological activities from various extracts of this plant. This study examined the total polyphenol and flavonoid content of various *Portulaca oleracea* extracts and assessed their antioxidant and anti-inflammatory properties. Comparing four extracts (methanol, aqueous, ethyl acetate, n-hexane) from *P. oleracea*, methanolic extract (ME) showed the greatest total polyphenol (60.52±1.88, mg GAE/g DW) and flavonoid content (44.78±1.17 mg QE/g DW). Thus, across all activities examined, ME had the strongest *in vitro* antioxidant (IC_{50}=43.52 µg/mL for DPPH, IC_{50}=9.71 µg/mL for FRAP), and anti-inflammatory effects (80% protein denaturation and 74.81% protection of HRBC membrane). Remarkably, after treatment of ME for 5 h, ME has significantly reduced paw volume (0.098 mL) and thickness (0.62 mm) on carrageenan-induced inflammation (hind paw edema model), compared to the inflamed model (0.19 mL and 1.52 mm). Moreover, ME helps protect the inflamed site in mice based on its ability to reduce malondialdehyde (MDA) and increase glutathione (GSH). The applications of ME in the treatment of inflammatory illnesses and oxidative damage are pharmacologically supported by our results. Also, 60 minutes after ingestion of ME, it dramatically raised the plasma levels of polyphenols and flavonoids as well as the antiradical capacity in healthy mice. Our results showed that ME consumption can support *in vivo* diet-related health advantages.

***Keywords*:** antioxidant, anti-inflammatory, carrageenan-induced inflammation in mice, *Portulaca oleracea*, oxidative index, polyphenol absorption

Introduction

An imperative self-defense response to injured tissue is inflammation. The incendiary handle is supported by the resistant framework as an entire, which incorporates macrophages. These distinctive cells trigger immunological reactions by discharging cytokines, chemokines, and provocative go-betweens.[1] As a powerful incendiary arbiter, nitric oxide (NO•) contributes to vasodilation, generalized host defense, and a few organ frameworks.[2] In any case, determined irritation is associated with a few unremitting infections, such as cancer, diabetes mellitus, cardiovascular illness, and rheumatoid joint pain. [3-6]

Numerous biomolecules can be hurt by free radicals, such as reactive oxygen species (ROS) and responsive nitrogen species (RNS), which can lead to aggravation, cancers, atherosclerosis, and other inveterate clutters.[7-8] Antioxidants inhibit this pathogenesis and protect against these harmful effects.

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Citation: Thao TTP, Tu PTC, Men TT. A Comparative Study on Polyphenol, Flavonoid Content, Antioxidant and Anti-Inflammatory Capacity of Different Solvent Extract from *Portulaca oleracea* in Carrageenan-Induced Paw Edema in Mice. Trop J Nat Prod Res. 2023; 7(10):4152-4159. http://www.doi.org/10.26538/tjnpr/v7i10.6

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

It's vital to note that effective bioactive compounds like phenolic acids and flavonoids are the most important components of the numerous natural antioxidant extricates from plants that are known to display antioxidant action. [9-10]

The World Health Organization records the herb *Portulaca oleracea* L. (*P. oleracea*), known as purslane, as one of the foremost therapeutic herbs. It belongs to the family Portulacaceae and the genus *Portulaca.*[11-12] In the Middle East, Mediterranean, US, Central European, and Asian countries, *P. oleracea* is utilized by people and conventional pharmaceuticals as a treatment for a run of afflictions. [13-14] The pharmacological properties of *P. oleracea*, such as its anti-oxidant and anti-microbial properties, have been reported, [12-15] skeletal muscle relaxant, [13] wound-healing, [13] as well as a conventional pharmaceutical for diuretic, febrifuge, sepsis, fits, cerebral pain, scurvy, asthma, and fever.

Polyphenols, which contain phenolic acids and flavonoids, are copious in purslane. There have been various reports on the overall phenolic, add-up-to-flavonoid, and phenolic composition of *P. oleracea.* Already distinguished substances incorporate caffeic corrosive, ferulic corrosive, kaempferol, quercetin, and rutin. Compared to some natural antioxidants like vitamin C and vitamin E, these substances display more or comparable capable antioxidant properties.[16-17] P. *oleracea* has antioxidant, and antiproliferative properties on numerous cancer cell lines and anti-inflammatory impacts on human fringe blood mononuclear cells (PBMCs).[18-20] Although the anti-inflammatory impacts of *P. oleracea* have been documented in a few considerations, however, there is no information concerning its anti-inflammatory impacts on the *in vivo* inflame model. Also, the oral absorption of *its main constituents* remains unclear.

In this study, the potential anti-inflammatory properties were evaluated using different solvent extricats from *P. oleracea* on Carrageenan-Induced Paw Edema in mice. Besides, the investigation also examined whether intense *P. oleracea* admissions can cause an increment in plasma polyphenol, flavonoid levels, and antiradical capacity in mice.

Materials and Methods

Plant preparation and extraction

P. oleracea was brought in July 2021 from the Vietnamese province of Soc Trang, where the aerial portion was dried in the shade. The plant was botanically identified by Dr. Nguyen Thi Kim Hue (Can Tho University) and a voucher specimen (PO-ST610) was deposited at the Department of Biology, Can Tho University. Using blade crushers, the aerial parts were finely crushed and stored in glass flasks at 4°C until used for the study.

Four solvents of increasing polarity, including hexane (polarity = 0.0), ethyl acetate (polarity = 4.4), methanol (polarity = 5.1), and water (polarity = 1), were used to extract the aerial portions of *P. oleracea*. 30 g of fine purslane powder was combined with 300 mL of solvent. The extract was recovered after each extraction of 24 hours and repeated 3 times. The solvent from the extract was removed by using a rotary vacuum evaporator, and the obtained extracts were kept at 4°C in darkness.

Quantitative the total polyphenol, and flavonoid content

Using the Folin-Ciocalteu (FC) reagent, the total phenolic content was calculated.[21] The plant extract (0.5 mL) and FC reagent (0.5 mL) were combined and incubated for 5 min at 22°C before the addition of 2 mL of 20% Na_2CO_3. The mixture was then incubated for a further 90 min at 22°C, during which the absorbance at 650 nm was determined. Using gallic acid as the reference, the total phenolic content (mg/mL) was determined.

$AlCl_3$ was used to calculate the total flavonoid content (mg/mL).[21] The test solution, which included 0.5 mL of plant extract, 0.5 mL of distilled water, and 0.3 mL of 5% $NaNO_2$, was incubated for 5 min at 25°C. 0.3 mL of 10% $AlCl_3$ was then added just after that. The reaction mixture was mixed with two milliliters of 1 M NaOH, and the absorbance was measured at 510 nm. Quercetin was used as a standard.

In vitro antioxidant activities of the extracts

Ferric-reducing antioxidant power assay (FRAP)

The procedure outlined by Okolie *et al*. (2014) was modified to determine the extracts' reduction power.[22] Briefly, 2 mL of phosphate buffer (0.2 M, pH 6.6) and 2 mL of 1% potassium ferricyanide ($K_3Fe(CN)_6$) were combined with five different concentrations of methanolic extracts (6.25, 12.5, 25, 50, and 100 µg/mL) and L-ascorbic acid at the same quantities. The mixture was incubated at 50°C for 20 min. The mixture was then centrifuged at 1000 rpm for 10 min after 2 mL of 10% trichloroacetic acid (TCA) was added. The supernatant (2 mL) was aspirated and combined with 1 mL of 0.1% ferric chloride ($FeCl_3$) and 2 mL of distilled water. Each experiment was carried out in triplicate. The absorbances were measured at 700 nm using a UV-vis spectrophotometer. On the graph of absorbance at 700 nm versus extract concentrations, the concentrations of each extract that could produce an absorbance value of 0.5 were identified and taken as the median effective concentration (EC_{50}).

1,1, diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities

The DPPH radical scavenging assay was carried out using the method reported with a few modifications.[23] The extracts were produced in DMSO at five different concentrations (18.75, 37.5, 75, 150, and 300 µg/mL). L-ascorbic acid, a common antioxidant, was made in the same concentrations. 0.5 mL of 0.3 mM DPPH in DMSO was added to a clean test tube after 1 mL of each extract was added. The mixture was shaken and left for 15 min to stand at room temperature in the dark. The blank solutions containing the extract solutions (2.5 mL) and 1 mL of DMSO were utilized.

The absorbance readings were measured at 517 nm using a spectrophotometer after incubation in the dark. The tests were carried out three times. The Brand-Williams et al., (1995) equation was used to determine the DPPH radical scavenging activity.

From a plot of the proportion of DPPH free radical inhibition vs. extract concentration, the half-maximum inhibitory concentration (IC_{50}) of the extracts was calculated.

% Radical scavenging activity = $\frac{A_c - A_s}{A_c}$ x 100

where A_s is the absorbance of the sample, and A_c is the absorbance of the control

In vitro anti-inflammatory activity of the extracts

Protein denaturation assay

The protein denaturation assay was carried out as previously described by Gambhire *et al*., (2009), with a few adjustments made following Gunathilake *et al*., (2018).[24-25] The reaction mixture (5 mL) contained 0.02 mL of extract, 4.78 mL of phosphate-buffered saline (PBS, pH 6.4), 0.2 mL of 1% bovine albumin, and 4.78 mL of the mixture. The mixture was mixed, incubated in a water bath (37°C) for 15 min, and then heated at 70°C for 5 minutes. Using a UV/VIS spectrometer after cooling, the turbidity was measured at 660 nm. The control was a phosphate buffer solution. Using the following formula, the % inhibition of protein denaturation was determined:

% inhibition of denaturation = $100 \times (1 - A_2/A_1)$

Where A_1 = absorption of the control sample, and A_2 = absorption of the test sample

HRBC membrane stabilization method

The *in vitro* anti-inflammatory efficacy of four extracts was evaluated using the HRBC membrane stabilization method. [26] 10 µL of freshly drawn human blood was placed in heparinized centrifuge tubes. The mixture was then centrifuged with 0.85% saline after combining with an equivalent volume of Alsever's solution (dextrose 2%, sodium citrate 0.8%, citric acid 0.05%, sodium chloride 0.42%, and distilled water 100 mL). Equal volumes of extracts in four different concentrations (10, 20, 50, and 100 µg/mL) were added to 1 mL of HRBC solution. All of the assay solutions were centrifuged after 30 minutes of incubation at 37°C. A spectrophotometer set at 560 nm was used to quantify the amount of hemoglobin in the supernatant solution. The percentage of hemolysis was calculated using the following formula:

Percent of hemolysis = $\frac{OD\ of\ test\ solution}{OD\ of\ control} \times 100$

Anti-inflammatory activity was calculated using the formula:

Percent of protection = 100 - Hemolytic percentage.

OD of test = Optical density of test sample's absorbance.

OD of control = Optical density of the negative control.

Negative control used = Alsever's solution with blood.

Anti-inflammatory activity by Carrageenan-induced paw edema

Animals were randomly divided into seven groups (n=6) and kept for one week to acclimate to the laboratory environment. Forty-two male mice (Slc:ddY, 6-week-olds) were divided into seven groups (n = 6) and treated as follows. Group 1: normal mice, oral administration of distilled water (N), Group 2: carrageenan-induced inflamed mice (IM), oral administration of distilled water. [27] This group served as positive and non-treated control. Group 3, 4, 5, 6: IM_ME, IM_AE, IM_EtOAc, and IM_HE; mice were treated consecutively for one week by four different solvent extracts from *P.oleraceae* (MeOH, aqueous, ethyl acetate, n-hexane, respectively) at the same dose of 100 mg/kg BW, then they have received CAR injection on the 8th day. Group 7: IM_IN: mice received an intramuscular injection of indomethacin (IN) (100 mg/kg BW) after CAR injection (5 mg/kg of body weight). This group served as a positive and treated control. Paw thickness and paw volume were measured at various periods before and after 1, 3, and 5 h injection of carrageenan using a vernier caliper. Results were expressed as mean±SEM compared with the control group at each time interval. The anti-inflammatory effect of the extract was determined by the ability to reduce paw thickness and volumes in the treated mice, compared to the control group (untreated).

In vivo antioxidant

Blood samples in all the above mice groups were collected in EDTA tubes and were immediately used for the determination of

hematological parameters. The sediment containing erythrocytes was suspended in a phosphate-buffered saline solution and centrifuged as reported by Sinha *et al*., (2007).[28] Skin tissues, close to the inflammation foci, were homogenized (10%, W/V) with phosphate buffer saline (pH = 7.4) and centrifuged at 9000 rpm for 20 min. Both homogenized skin and erythrocytes were collected and used for oxidative stress testing.
The effect of *P. oleracea* extract on the production of endogenous oxidants and antioxidants in erythrocytes and inflamed areas of mice was determined by the analysis of malondialdehyde (MDA)[29] and glutathione (GSH) level was determined as modified by Jollow *et al*., (1974).[30] The concentration (nmol/mg protein) of MDA and GSH was calculated by the equation of linear regression of MDA and GSH standards, respectively.

Polyphenol bioavailability and antiradical capacity test
Thirty normal male mice weighing 38±2 g were used. The animals were fasted for 20–24 hours. The animals were housed in an animal breeding system (Laboratory Animal, College of Natural Sciences, Can Tho University, Vietnam) at 24±1°C and 50 % humidity with a 12 h light/dark cycle. For the polyphenol bioavailability and antiradical capacity test, all mice were randomized into five groups (n=6). Mice were orally administered four extracts (ME, AE, EtOAc, HE) at the dose of 100 mg/kg BW and distilled water for the control group. Blood samples were taken from the tail vein before (0 min) and at 30, 60, 90, 120, 240, and 300 min after oral gavage, centrifuged for 3 min at 8000 rpm (Centrifuge MCD-2000, AS ONE, Japan), and serum samples were taken. The total polyphenol, flavonoid index, and antioxidant capacity (DPPH and FRAP) in plasma were determined by the colorimetric assays, which were described in sections 2.2 and 2.3. The readings were carried out in triplicate for each extract. Data of total polyphenol and flavonoid contents were expressed in µg gallic acid and quercetin mL^{-1} of plasma, respectively. The data of antioxidants index (DPPH and ABTS) were expressed as µM ascorbic acid mL^{-1} of plasma.

Data analysis
The data are presented as the Mean ± SD. One-way Analysis of variance (ANOVA) was used to assess the significant differences among multiple groups under various treatments, followed by Tukey's posthoc test using Minitab 16 software. In all the groups, differences were considered statistically significant at $p<0.05$. Excel 2016 was used to obtain the graphs.

Results and Discussion

Total polyphenol and flavonoid content
Phenolic compounds are important phytochemicals with redox capacities responsible for antioxidant activity.[31] The phenolic compounds in four extracts (n-hexane, ethyl acetate, aqueous, and methanol extract) ranged are 4.89 to 60.62 mg gallic acid equivalents (GAE) per gram dry extract weight (GAE/g DW), representing an approximate 15-fold variation (Table 1). The methanol extract had the greatest phenolic contents (60.52±1.88 mg GAE/g DW), while the smallest phenolic contents were found in n-hexane, ethyl acetate, and aqueous extract (4.89 ±0.66, 7.41±0.56 and 15.96±1.08 mg GAE/g DW, respectively).
Extracts' flavonoid concentrations ranged from 0.97 to 44.7 mg QE (quercetin equivalents)/g DW, or approximately a 45-fold variance (Table 1). Similarly, the highest concentration of flavonoids was detected in the methanol extract (44.78±1.17 mg QE/g DW), while the lowest concentrations were discovered in n-hexane, ethyl acetate, and aqueous extract (0.97± 0.05, 2.93±0.17 and 21.65± 1.17 mg QE/g respectively).

In vitro antioxidant activities
The extracts of *P.oleraceae* also showed impressive *in vitro* DPPH radical scavenging capabilities in a dose-dependent manner (Table 2). According to their concentration to scavenge 50% of the DPPH radicals, the various extracts' activities were assessed (IC_{50}). The IC_{50} values for methanol, aqueous, ethyl acetate, and n-hexane were 43.52 µg/mL, 78.45 µg/mL, 185.95 µg/mL, and >300 µg/mL, respectively. The IC_{50} value of the standard (L-ascorbic acid) was 40.96 µg/ml. All of the extracts fell far short of the standard's (L-ascorbic acid) substantially better DPPH radical scavenging abilities. The methanol extract of *P. oleraceae* produced considerably stronger DPPH radical scavenging capabilities than those seen for the other extracts at all the investigated doses.
The FRAP approach revealed striking concentration-dependent increases in the absorbance values of the four *P. oleraceae* extracts at a wavelength of 700 nm (Table 3). Methanol extract had significantly greater absorbance values than those reported for aqueous, ethyl acetate, and n-hexane extracts at all of the tested doses.

Table 1: The total polyphenol, and flavonoid content in different extracts from *P.oleraceae*

Content	Methanol	Aqueous	Ethyl acetate	n-hexane
Total polyphenol (mg GAE/g DW)	60.62 ± 1.88^{a}	15.96 ± 1.08^{b}	7.41 ± 0.56^{c}	4.89 ± 0.66^{d}
Total flavonoid (mg QE/ g DW)	44.78 ± 1.17^{a}	21.65 ± 1.17^{b}	2.93 ± 0.17^{c}	0.97 ± 0.05^{d}

Note: TPC, total phenolic content; mgGAE/g, milligrams gallic acid equivalent per gram of sample. TFC, total flavonoid content; mg QE/g, milligrams of quercetin equivalent per gram of sample Values are expressed as mean ± SEM. Means with different superscript letters within the same row are significantly different by one-way ANOVA followed by Tukey's test.

Table 2: *In vitro* DPPH scavenging activities of different extracts from *P.oleraceae*

Concentration (µg/mL)	DPPH (%)				
	Methanol	Aqueous	Ethyl acetate	n-hexane	L-ascorbic acid
18.75	$36.86 \pm 0.83_{e}^{B}$	$29.97 \pm 0.25_{d}^{C}$	$19.88 \pm 0.09_{d}^{D}$	$10.02 \pm 0.20_{c}^{E}$	$38.49 \pm 0.68_{e}^{A}$
37.5	$44.47 \pm 0.58_{d}^{B}$	$33.14 \pm 0.14_{d}^{C}$	$23.31 \pm 0.25_{d}^{D}$	$17.40 \pm 0.56_{c}^{E}$	$45.45 \pm 0.58_{d}^{A}$
75	$58.31 \pm 0.96_{c}^{A}$	$47.73 \pm 0.37_{c}^{C}$	$37.94 \pm 0.37_{c}^{D}$	$27.91 \pm 0.19_{c}^{E}$	$58.96 \pm 0.19_{c}^{A}$
150	$74.27 \pm 0.28_{b}^{A}$	$59.16 \pm 0.5_{b}^{C}$	$47.99 \pm 0.85_{b}^{D}$	$39.50 \pm 0.29_{b}^{E}$	$75.25 \pm 0.28_{b}^{A}$
300	$87.76 \pm 0.35_{a}^{A}$	$76.17 \pm 0.62_{a}^{C}$	$56.61 \pm 0.59_{a}^{D}$	$47.96 \pm 0.53_{a}^{E}$	$88.41 \pm 0.24_{a}^{A}$
IC_{50}	43.52	78.45	185.95	>300	40.96

Note: The values are expressed as mean ± SEM. Values with the same uppercase superscript letter within the same row and those with the same lowercase subscript letter within the same column are not significantly different (P>0.05, one-way ANOVA followed by Tukey's test).

Also, the study calculated the half-effective concentrations (EC_{50}) of the plant extracts under study necessary to provide an absorbance value of 0.5. It was shown that L-ascorbic acid's EC_{50} value was lower than those of aqueous, ethyl acetate, and n-hexane. Importantly, methanol extract exhibited essentially equal absorbance values to the standard (L-ascorbic acid) at all the tested doses.

The methanol extract (ME) of *P. oleracea* was demonstrated the highest antioxidant as compared to other extracts in all tested assays (Table 2 and Table 3). The total polyphenols and flavonoids in ME may be responsible for these activities. ME had the highest content of polyphenols and flavonoids as compared to other extracts (aqueous, ethyl acetate, n-hexane) (Table 1). The polyphenols and flavonoids in plant extracts are well-known for their strong antioxidant and free radical scavenging activities. Due to their capacity to contribute hydrogen atoms to free radicals, phenolic and flavonoid molecules are crucial antioxidant components for the deactivation of free radicals. They possess the appropriate structural qualities for scavenging free radicals. [32] DPPH and FRAP assays were used in this study to investigate the antioxidant activities of the extracts from different parts of *P. oleracea*. In the human body, there is a variety of oxidants, including reactive oxygen species (ROS) and reactive nitrogen species (RNS). Each method exhibits a certain type of oxidant. The plant extract possessing strong activities against a range of oxidants would have great potential for application. [33]

In vitro anti-inflammatory activities

Anti-inflammatory studies-HRBC membrane stabilization method

The anti-inflammatory activity of *P. oleracea* extracts was determined by using an inhibition assay of bovine albumin denaturation and HRBC membrane stabilization method. Stabilization of the lysosomal membrane had a crucial role in reducing inflammatory responses and preventing the extracellular release of its contents and damage to tissues. [34] The *in vitro* anti-inflammatory efficacy of four extracts from *P. oleraceae* was investigated at the same concentration range (10-100 µg/mL). Four *P. oleraceae* extracts responded favorably to the tests that used the HRBC membrane stabilization method to measure the anti-inflammatory activity (Fig. 1A and B). The n-hexane had the least activity, whereas the other sample extracts all had fruitful anti-inflammatory.

At a concentration of 100 µg/mL, the methanol extract showed maximum protection and minimal hemolysis of the HRBC (74.81% protection and 24.18% hemolysis, respectively). The results were comparable to those of normal diclofenac, which demonstrated an 80.54% level of protection and a 19.45% hemolysis. The aqueous extract demonstrated maximal hemolysis and minimal protection of 67.42 and 41.64%, respectively, at 100 µg/mL. Compared to methanol and aqueous extract, the results were very low for n-hexane and ethyl acetate, and reduced anti-inflammatory properties.

According to the results of the two-way ANOVA, there is a statistically significant difference between the interaction effect of plant extract and concentration and the percentage of protection in the anti-inflammatory experiment, with $p = 0.0001$. Analysis of correlations revealed a very strong relationship between each extract's concentration in anti-inflammatory activity ($P<0.0001$).

An anti-arthritic study by protein denaturation method

The protein denaturation method was used to obtain each plant extract from the substantial anti-arthritic effects. A process by which proteins lose their secondary and tertiary structure through the alteration of electrostatic hydrogen, hydrophobic, and disulfide bonding is called protein denaturation. [35] This process results in the production of autoantigens in certain inflammatory diseases, such as arthritis. Protein denaturation is considered a marker for inflammatory diseases.[36] The inhibition % of protein denaturation of these plant extracts ranged from 12.6% to 80.0% at the concentration range of 25–100 µg/mL (Fig. 1C). In comparison with other extracts, *P. oleraceae*'s methanol extract showed a substantially higher level of inhibition, whereas hexane showed the lowest levels of inhibition ($p<0.0001$). The order of inhibition was methanol, aqueous, ethyl acetate, and n-hexane respectively. ME had the highest anti-inflammatory as compared to other extracts in all tested assays (Figure 1 A, B, and C). It may be owing to its highest content of polyphenols and flavonoids (Table 1). Polyphenols and flavonoids possess strong inhibitory activity against albumin denaturation.

The correlation between the total phenolic, and flavonoid content, the antioxidant, and anti-inflammatory activities

Phenolic and flavonoid molecules are important antioxidant components that are for deactivating free radicals based on their ability to donate hydrogen atoms to free radicals. [32] Table 4 illustrates the relationship between total phenolic and flavonoid concentration and antioxidant and anti-inflammatory activity. At a 95% confidence level, there were significant associations between total flavonoids, total polyphenols (DPPH, $R^2=0.96$; FRAP, $R^2=0.84$; HMBC, $R^2=0.87$; Protein denaturation, $R^2=0.92$) as well as antioxidant and anti-inflammatory potential. The results were in agreement with previous studies that antioxidants, [37,38] and anti-inflammatory activities [39] were positively correlated to polyphenols and flavonoids of plant extracts.

Table 3: *In vitro* ferric reducing antioxidant power activities of different extracts from *P.oleraceae*

Concentration (µg/mL)	Absorbance at 700 nm				
	Methanol	**Aqueous**	**Ethyl acetate**	**n-hexane**	**L-ascorbic acid**
6.25	$0.42 \pm 0.011_e^A$	$0.26 \pm 0.001_d^B$	$0.07 \pm 0.002_d^C$	$0.04 \pm 0.001_c^D$	$0.42 \pm 0.001_e^A$
12.5	$0.58 \pm 0.003_d^A$	$0.33 \pm 0.036_d^B$	$0.15 \pm 0.001_d^C$	$0.08 \pm 0.001_c^D$	$0.59 \pm 0.001_d^A$
25	$0.86 \pm 0.002_c^A$	$0.64 \pm 0.002_c^B$	$0.32 \pm 0.005_c^C$	$0.15 \pm 0.000_c^D$	$0.87 \pm 0.000_c^A$
50	$1.26 \pm 0.003_b^A$	$1.14 \pm 0.02_b^B$	$0.63 \pm 0.005_b^C$	$0.32 \pm 0.011_b^D$	$1.29 \pm 0.011_b^A$
100	$2.41 \pm 0.014_a^A$	$1.99 \pm 0.002_a^B$	$1.08 \pm 0.06_a^C$	$0.62 \pm 0.004_a^D$	$2.37 \pm 0.004_a^A$
IC_{50}	9.71	18.76	43.20	80.04	9.12

Note: The values are expressed as mean ± SEM. Values with the same uppercase superscript letter within the same row and those with the same lowercase subscript letter within the same column are not significantly different ($P>0.05$, one-way ANOVA followed by Tukey's test).

Table 4: The correlation between the total phenolic and flavonoid content, and the antioxidant, and anti-inflammatory activities

Compounds	**Antioxidant activity**		**Anti-inflammatory activity**	
	DPPH	FRAP	Protein denaturation	HMBC assay
Polyphenols	0.86^*	0.82^*	0.84^*	0.80^*
Flavonoids	0.96^*	0.84^*	0.87^*	0.92^*

Note: * indicates a significantly different at the level of 0.05 (2-tailed).

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

Fig. 1

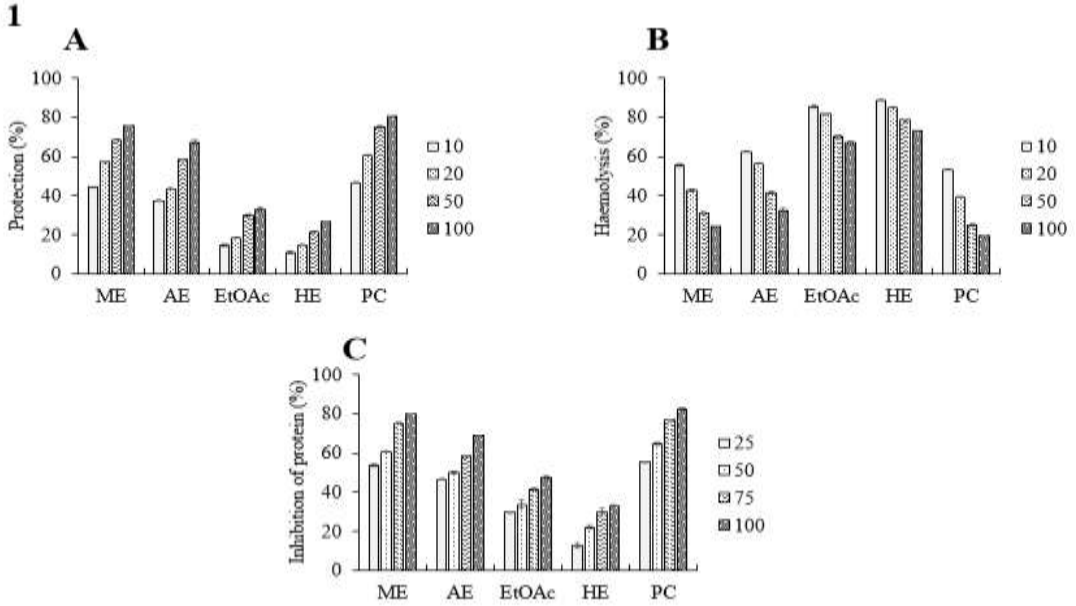

Figure 1: Anti-inflammatory studies-HRBC membrane stabilization (percent of protection (A) and percent of hemolysis (B)), and anti-arthritic studies by protein denaturation (C) of different extracts from *P.oleraceae*. ME: methanolic extract, AE: aqueous extract, EtOAC: ethyl acetate extract, HE: hexane extract, PC: positive control.

Fig. 2

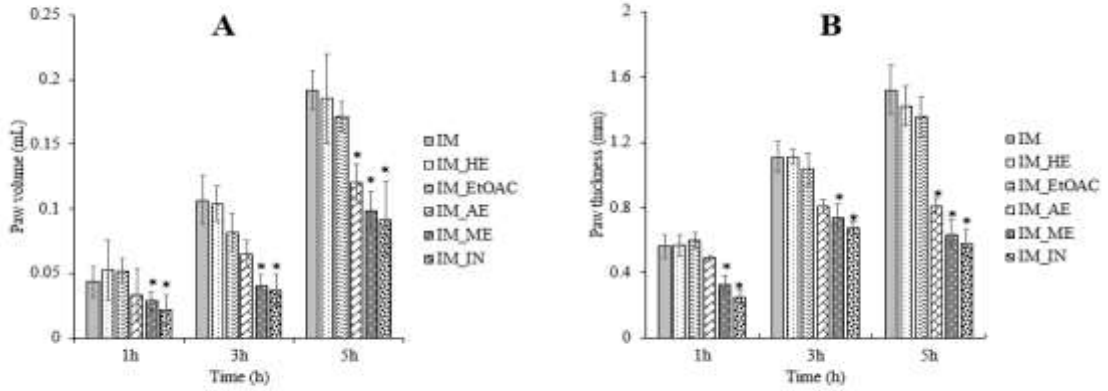

Figure 2: The edema volume (A) and thickness (B) of mice paw at 1, 3, and 5 h. *Significant difference from the inflamed group (IM) ($P<0.05$) according to one-way ANOVA with Tukey's HSD test. inflamed mice (IM), inflamed mice and treated with different plant extracts: methanol extract (IM_ME), aqueous (IM_AE), ethyl acetate (IM_EtOAC), hexane (IM_HE), and inflamed mice treated with indomethacin (IM_IN).

Carrageenan-induced paw edema in mice

The carrageenan-induced paw edema model was used to verify the *in vivo* anti-inflammatory efficacy of four extracts from *P. oleraceae*. The swelling of carrageenan-induced paw edema was reduced by oral administration of ME (methanol extract) from *P. oleraceae* at a dose of 100 mg/kg with a significant difference from inflamed mice at 1, 3, and 5 h, and it was similar to indomethacin. Ethyl acetate and hexane, however, showed no activity at any point in time (Figure 2). The results of an *in-vivo* study on anti-inflammatory action utilizing carrageenan-induced paw edema demonstrated that ME could significantly reduce edema compared to the control. Histamine, serotonin, kinins, PGs, complements, and proinflammatory cytokines are some of the inflammatory mediators that play a major role in mediating carrageenan-induced paw edema.[40] These mediators are responsible for the acute phase of inflammation. Excessive generation of reactive oxygen species (ROS) at the site of inflammation has been connected to pro-inflammatory cytokines.[41] The ME treatment demonstrated an anti-inflammatory effect by preventing the release and/or production of inflammatory mediators involved in edema formation, which was similar to the *in vitro* results.

In-vivo antioxidant activity

Carrageenan has been used to induce paw edema in animal models. The inflamed action of carrageenan is mainly due to the induction of free radical formation by a redox reaction.[42] Table 5 showed that MDA and GSH (endogenous antioxidant) level in the epidermis and erythrocytes of inflamed mice was about 2.5 times higher and about 1.7 times lower than those of normal mice, respectively. The results indicated carrageenan disrupted the balance between oxidant and antioxidant in inflamed mice in favor of increasing oxidant. Non-treated inflamed mice had a significant increase in MDA (oxidant) and reduction of GSH (antioxidant) levels in the epidermis and erythrocytes as compared to those of normal mice (Table 5). Oral administration of *P. oleracea* extract greatly decreased MDA and increased GSH levels in a dose-

dependent manner in all inflamed mice. ME treatment significantly increased GSH levels and reduced MDA levels in a dose-dependent manner in all observations of inflamed mice (Table 5). Interestingly, the treatment of ME showed superior performance as compared to indomethacin treatment in terms of increasing GSH level and decreasing MDA level in all tests. Apart from its antioxidant activities (Table 5), ME could induce the generation of endogenous antioxidant GSH as well as the suppression of endogenous oxidant MDA. In other words, ME could stimulate the antioxidant defense in carrageenan-induced paw edema.

Polyphenol bioavailability and antiradical capacity

The extracts contain polyphenols and flavonoids, which are thought to be the most effective ingredients for treating metabolic syndrome both *in vitro* and *in vivo*. Previous studies suggested that plant extract that is rich in polyphenols may synergistically contribute to a rise in plasma antioxidant activity, with possible anti-inflammatory effects.[43] However, their health benefits depend on oral bioavailability.[44]

A substantial increase in plasma total index was seen from baseline up to 300 min after each extract from *P.oleraceae* was taken orally (Fig. 3A). When compared to other extracts, the ME showed a significant rise in plasma total phenol index ($p<0.05$). The colorimetric Folin-Ciocalteu test was used to measure a peak in plasma total polyphenol levels 60 minutes after ME consumption ($p<0.05$). Similar to the total phenol index, administering the extract raised plasma total flavonoid levels for up to 60 min in comparison to baseline ($p<0.05$) (Figure 3B).

A comparison of their contributions to the total plasma antioxidant capacity is possible by measuring the plasma's total antioxidant capacity following the ingestion of foods high in polyphenols. The DPPH and FRAP radical scavenging assays in our experiment reached their highest activity 60 min after the injection of *P. oleraceae* extracts (Fig. 3C, D). In both assays, the greatest rise in antiradical capacity was observed following the consumption of ME.

We might assume that ME's high polyphenol and flavonoid content is what causes the greatest boost in antioxidant capacity. The increase in plasma antioxidant activity is here demonstrated and may have some pathophysiological significance, as oxidative stress is involved in the etiopathogenesis of significant chronic-degenerative diseases. However, it is still necessary to conduct further research before drawing any conclusions about the direct health effects of ME in healthy mice, including inflamed models. [45,46]

Table 5: Evaluation of oxidative and antioxidant statutes in skin tissue and erythrocytes: Normal mice, inflamed mice (IM), inflamed mice and treated with different plant extracts: methanol extract (IM_ME), aqueous (IM_AE), ethyl acetate (IM_EtOAC), hexane (IM_HE) and inflamed mice treated with indomethacin (IM_IN)

	Normal	**IM**	**IM_ME**	**IM_AE**	**IM_EtOAC**	**IM_HE**	**IM_IN**
Skin tissue							
MDA (nmoles /mg protein)	0.39±0.020	$1.03\pm0.071^{***}$	$0.41\pm0.026^{\#\#\#+}$	$0.47\pm0.021^{\#\#\#+}$	$0.73\pm0.032^{\#\#}$	$0.99\pm0.09^{\#}$	$0.37\pm0.037^{**\#\#\#}$
GSH (nmoles /mg protein)	1.15±0.045	$0.67\pm0.026^{***}$	$1.31\pm0.039^{\#\#\#++}$	$0.99\pm0.138^{*\#\#\#+}$	$0.82\pm0.063^{\#}$	$0.685\pm0.06^{\#}$	$1.21\pm0.114^{**\#\#\#}$
Erythrocytes							
MDA (nmoles /mg protein)	1.85±0.062	$3.0\pm0.079^{***}$	$1.90\pm0.05^{\#\#\#++}$	$2.17\pm0.18^{\#\#\#++}$	$2.329\pm0.372^{\#\#}$	$3.0\pm0.07^{\#}$	$1.96\pm0.09^{*\#\#}$
GSH (nmoles /mg protein)	2.19±0.07	$1.35\pm0.087^{***}$	$2.42\pm0.159^{\#\#\#++}$	$1.92\pm0.074^{*\#\#\#}$	$1.56\pm0.054^{\#}$	$1.332\pm0.091^{\#}$	$1.95\pm0.029^{**\#\#\#}$

Note: Values are expressed as mean ± SEM. Symbols (*, #, and +) exhibit significant statistical differences between the groups. *p<0.05; **p <0.01 and ***p<0.001 versus normal group, #p <0.05; ##p<0.01 and ###p <0.001 versus IM group, +p <0.05; ++p <0.01 and +++p<0.001 versus IM_IN group

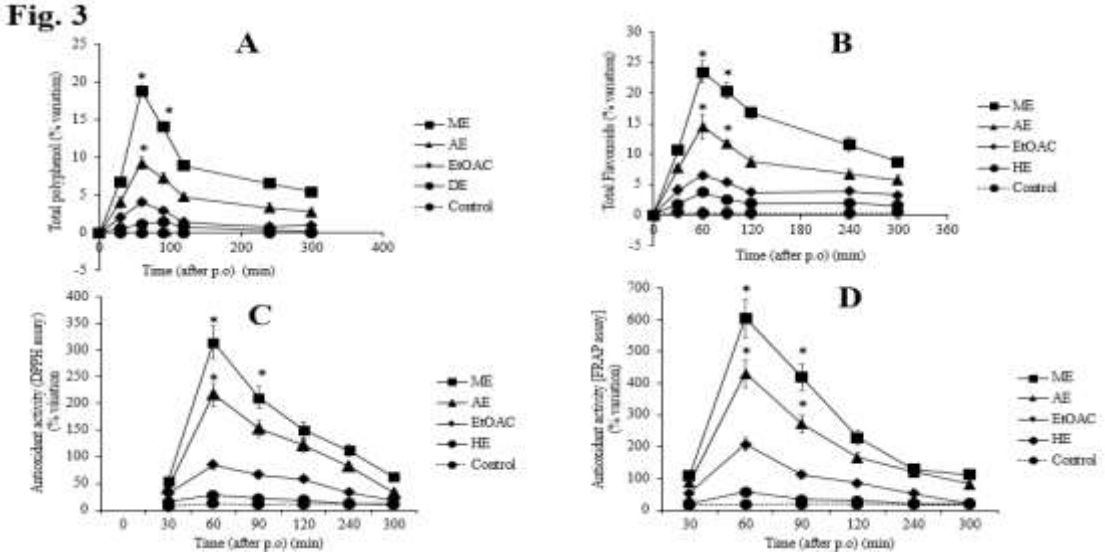

Figure 3: Percentage variation (mean ± standard error) in the plasma total polyphenol (A) and flavonoid index of mice at 30, 60, 90, 120, 180, and 300 min after oral administration of different extracts from *P.oleraceae*. *Significant difference from the control group ($P<0.05$) according to one-way ANOVA with Tukey's HSD test. ME: methanolic extract, AE: aqueous extract, EtOAC: ethyl acetate extract, HE: Hexane extract.

Conclusion

In this study, the methanol extract (ME) from *P.oleraceae* was demonstrated to have the highest *in vitro* antioxidant, and anti-inflammatory as compared to other extracts. Since ME had the highest content of total polyphenols and flavonoids. ME treatment significantly increased the antioxidant index in Carrageenan-Induced Paw Edema in mice in a dose-dependent manner. We showed that the acute intake of ME significantly increased the plasma levels of polyphenols and flavonoids and, correspondingly, the plasma antiradical power in normal mice. Consequently, ME can be a great potential source for the development of pharmaceuticals to control inflammation.

Conflict of Interest

The authors declare no conflict 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.

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