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Original Research Article

Anti-inflammatory and Antioxidant Activities of Selected Fractions of *Parinari kerstingii* Leaf Extract

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ABSTRACT

In Nigeria, *Parinari kerstingii*, a member of the Chrysobalanaceae family, is frequently used as a medicinal plant and is thought to have several biological properties. This study aimed to evaluate the in vitro antioxidant and anti-inflammatory properties of selected GC-MS bioactive constituent fractions of *Parinari kerstingii* leaf extract. Total antioxidant capacity (TAC) and nitric oxide radical scavenging assays (NORSA) were used to measure the extract's antioxidant activity. In contrast, the fractions' membrane stabilization potential and platelet aggregation inhibition properties were used to measure the extract's in vitro anti-inflammatory activity. GC-MS characterizations of the crude extract identified bioactive constituents responsible for these activities. The results of the GC-MS analysis revealed that bioactive compounds with known anti-inflammatory and antioxidant properties, such as n-Hexadecanoic acid, phenol, 2,4-bis(1,1-dimethylethyl)-e.t.c. were found in the fractions. The extract significantly ($p < 0.05$) lowered radical-scavenging activities compared to the fractions at 100, 400, and 800 mg/ml concentrations. Avian (Chicken) erythrocyte membrane was significantly ($p< 0.05$) protected by various doses (100–800 µg/ml) of extracts against rupture initiated by heat and hypotonic solution (water). Also, platelet aggregation inhibition was exhibited when compared with fractions. In the entire test, the fractions provided no similar order in strengths, indicating the likely presence of various bioactive constituents for each evaluated biological activity. This preliminary in vitro investigation determined the antioxidant and anti-inflammatory characteristics of *Parinari kerstingii* leaf, which might be explored further in vivo for the potential use of the plant's isolates as sources of naturally derived anti-inflammatory and antioxidant molecules.

Keywords: Antioxidant, Anti-inflammation, *Parinari kerstengii*, Bioactivity, Phytochemicals

Introduction

The study of plants and herbs has been found to enhance food flavor or usefulness in cosmetology and dying of fabrics and health restoration[1]. They form the major constituents of herbal medicine in traditional healing and have inspired numerous prominent pharmaceutical breakthroughs,[2] either as a single regimen or in combination therapy. According to Newman and Cragg, only 36% of the new chemical entities recognized between 1981 and 2010 that belong to the class of small molecules were wholly synthesized; more than half were derived from natural sources.[3]

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An exceptional advantage of medicinal plants is that they possess high efficacy and fewer adverse effects compared with synthetic drugs that are used for the treatment of myriads of diseases,[4] and as such, research on their potential utilization for pharmaceutical purposes has gained considerable attention in the last decade and still on the rising trend across the globe. More than 80% of the human population globally relies on plant-derived medication to treat various diseases,[5] and recently, their usage has expanded.[6] Leaves and roots of *Parinari kerstingii* have been used in folk medicine in West African sub-regions. Even though *Parinari kerstingii* has been used in folk medicine, little is known about its therapeutic potential. The pharmacological response and therapeutic capabilities of *Parinari kerstingii* are attributed to the secondary metabolites and several other chemical constituents present in the extract. These phytochemicals confer plant extract with various therapeutic potentials such as antibacterial, antioxidant, anti-inflammatory, and anti-hemorrhagic properties. *Parinari kerstingii*, according to Burkill is widely distributed in tropical West Africa, such as Nigeria (Bauchi, Plateau, Nsukka, Okomu), Ghana, Ivory Coast, Togo, and Sokode-Basari[7]. The phytochemical compounds of *Parinari kerstingii* leaf, as revealed by Odu *et al*.,[8] showed the existence of alkaloids, flavonoids, steroids, terpenoids, phenols, glycosides, carbohydrates, reducing sugars, and saponins, and they have demonstrated efficacy as antioxidant and anti-inflammatory agents.[9]

The antioxidant properties of *Parinari kerstingii* leaf extract are due to its ability to neutralize the reactive oxygen species and ultimately avert possible cellular damage. The goal of this study was to assess the anti-inflammatory and radical-scavenging effects of fractions of the extract in vitro as well as the identification of phytochemical components from the crude extract of *Parinar kerstingii* leaf that elicits the observed pharmacological effects using GC-MS with a view of justifying its therapeutic potentials.

Materials and Methods

Materials

Parinar kerstingii leaf, voucher specimen (Interceded 0615) was sourced from Ihu Otobo, Umu Agada in Ohom, Unadu Town (with GPS coordinate 7°00′30.672″N 7°26′28.2″E) in Igbo-Eze South Local Government Area, Enugu State, Nigeria on 2nd January, 2022. Miss Njokwu, E. U, a specialist in plant taxonomy, identified and authenticated the leaf at The Department of Plants Science and Biotechnology, University of Nigeria Nsukka, using a dichotomous key. A mechanical grinder was used to grind the leaf after it was air-dried. A total of 5000 kg of the crude plant in powdery form was obtained for the extraction. This crude was macerated for 24 hours in 2000ml methanol and subsequently filtered. After that, the filtrate was diluted at an optimum temperature of 50 ºC with a rotary evaporator (IKA Model, Germany).

Methods

Extraction and Fractionation of Parinari Kerstingii Leaves

Parinari kerstingii leaf was air-dried, ground into powder, and cold-macerated with methanol for 48 hours. Whatman No.4 filter paper was employed to filter the solution, and with the help of a rotary evaporator, the filtrate was concentrated into a semi-solid phase. A slurry-like substance of dark green color was obtained and kept in the refrigerator for further studies. With the aid of silica gel column chromatography, a quantity of the crude extract was separated into its component fractions. The mobile phase was varying solvent combinations (hexane, ethylacetate, and methanol) of increasing polarity, while the stationary phase was 70-200 mesh size silica gel. With the aid of a glass rod, column chromatography was set up in a manner that the basement of the glass column was stocked with wool. A slurry composed of silica gel and hexane was prepared in a ratio of 3:7. Mixture of 150 g of silica gel, and 350 ml of hexane was carefully spouted into the column. The top of the glass column was left on to ensure unhindered drift of solvent into an Erlenmeyer flask. The arrangement was confirmed to be in the best operational orientation when the solvent drained unhindered and without moving either the silica gel or glass wool down the column. A glass column was successfully packed. After successful packing with silica gel in hexane, the tap was appropriately locked. The period for column stabilization was 24 hours, after which the mixture-less solvent above the silica gel was allowed to go down. In preparing the silica gel column, a wet packing method was adopted. The sample preparation was done in a ceramic mortar by adsorbing 104 g of the extract in methanol to 200 g of silica gel 70-200 mesh size, after which it was evaporated to dryness. The dry powder was gently stratified above the column. The top of the column was on to permit the eluting agent to flow at the pace of 40 percolations per minute. Extract elution was done in such a way that there was a gradual increment in the polarity of eluting solvents (hexane, ethylacetate, and lastly, methanol)

Phytochemical Analysis

A method described by Harborne was adopted for the phytochemical qualitative and quantitative analysis of the sample for various groups of organic constituents.[10]

GC-MS Analysis of Parinar kerstingii Extract

The characterization of the phytochemical content of Parinar kerstingii was done at the National Research Institute for Chemical Technology (NARICT), Zaria, Kaduna State, Nigeria. GC-MS analysis of *Parinari kerstingii* was carried out using the QP 2010 series Model, a product of Shimadzu (Japan) with features: a 60 m long, 0.25 mm wide, and 0.25 mm thick VF-5ms fused silica capillary column. The oven's temperature was raised automatically from 80 to 200°C at 10°C/min, maintained at 200°C for four minutes, and then increased to 280°C at 10°C/min (isothermal for 5 minutes). A diluted sample of a known volume of 2 µl of methanol extract was carefully administered in the splitless mode with a split ratio of 1:40 and a mass scan of 40-600 M/Z. The interpretation of the mass spectrum from the GC-MS was done using the National Institute of Standards and Technology (NIST) database.

Nitric Oxide Radical Scavenging Assay

Based on the technique described by Odu *et al*., the effects of the fraction against nitric oxide radicals were determined spectrophotometrically.[11] The method was based on the ability of Greiss reagent to estimate the nitrite ions produced when oxygen reacts with nitric oxide. When sodium nitroprusside and aqueous solution combine at a physiological pH, nitric oxide is instantly produced. The competition between nitric oxide and oxygen led to reduced production of nitrite ions. Greiss's assay method enabled us to determine the potential of the extract to eliminate the harmful nitric oxide radicals. An aliquot of a sodium nitroprusside solution diluted in distilled water (1.0 ml, 10 mM) was added to 1 ml of plant extract in methanol at various concentrations (0.6–1.0 mg/ml), and after that, 150 minutes were spent letting the mixture stand at room temperature. After the new mixture had successfully undergone a 30-minute incubation period, 2 ml of Greiss reagent (3% phosphoric acid, 1% sulphanilamide, and 0.1% -naphthyl-ethylene diamine dihydrochloride) was added. The optical density of the pink chromophore produced by the diazotization of nitrite with α-naphthyl-ethylenediamine dihydrochloride was measured using spectrophotometry at 540 nm. Ascorbic acid served as the experiment's positive control. The percentage of nitric oxide inhibition was used to calculate the results. Triplicates of each experiment were run throughout. The percentage inhibition was determined using the following equation:

$$\% \text{ inhibition} = 100\times(AC-AT)/AC \quad \text{---------- 1}$$

Total Antioxidant Capacity

As Saeed *et al*. reported, the phosphomolybdate technique was used to determine the extract overall antioxidant capacity.[12] The principle underlying the reaction is the extract reduction of Mo (VI) to Mo (V), which led to the development of a greenish phosphate/Mo (V) complex at acidic pH. The reagent solution consisted of 0.6 M sulfuric acid, 4 mM ammonium molybdate, and 28 mM sodium phosphate. 0.1 ml of extract and 1 ml of reagent solution were mixed. A spectrophotometer (Jenway 6025) was used to measure the reaction mixture absorbance at 695 nm in comparison to a blank (0.1 ml of methanol) after allowing the mixture to cool to room temperature. At 695nm, the change in optical density of the reactants was measured against blank. The blank was made up of 0.1 ml of methanol in place of extract. The antioxidant activity of the extract is equal to the amount of ascorbic acid consumed in grams. Ascorbic acid (1000, 500, 250, 125, and 62.5 µg/ml) mixed with methanol was used in the preparation of the calibration curve.

Assay of Anti-Platelet Aggregatory Activity

Method of Born and Cross (1963), as modified, was used for the assay.[13] Fresh blood sample was sourced from domesticated chicken and emptied into plastic tubes. The fresh whole blood taken from the chicken and emptied into EDTA containers were centrifuged at 3000 rpm for 10 min. Normal saline and supernatant of equal volumes were used for the dissolution of red blood pellets. Red blood pellets dissolved above were reconstituted with isotonic 10 mM sodium phosphate buffer of pH 7.4, which adopted 40 % v/v suspension and then measured thereafter. The buffer solution was prepared by dissolving 9g, 0.2g, and 1.15g of NaCl, NaH_2PO4, and Na_2HPO4, respectively in 1 liter of distilled water.

Preparation of Erythrocyte Suspension

At 3000 rpm for 10 min, fresh whole blood taken from the chicken and emptied into EDTA containers was centrifuged. Normal saline and supernatant of equal volumes were used for the dissolution of red blood

pellets. Red blood pellets dissolved above were reconstituted with isotonic 10mM sodium phosphate buffer of pH 7.4, which adopted 40 % v/v suspension and then measured thereafter. The buffer solution was prepared by dissolving 9 g, 0.2 g and 1.15 g of NaCl, NaH_2PO_4 and Na_2HPO_4, respectively in 1 liter of distilled water.

Membrane Stabilization Activity Test

Taking advantage of the hypotonicity and red blood cell hemolysis caused by heat, the extract ability to stabilize membranes was evaluated according to the methodology described by Shinde *et al.*[14]

Effects of Extract on Heat-Induced Haemolysis

Extract samples were dissolved in an isosmotic phosphate buffer solution. In fourfold (4 sets for each dose), a set of five centrifuge tubes containing 5 ml of different doses of extracts (0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml) and indomethacin (100, 200, 400, 800, and 1000 g/ml) were prepared. A total of 5 ml of the vehicle was contained in two sets of control tubes. In each of the tubes was added 0.1 ml suspended in chicken red blood cells and carefully blended. One tube of extract and the vehicle were incubated for 120 seconds at 54°C in a water bath under regulation. The remaining sets of tubes were kept in a freezer at −10°C for 120 seconds. Subsequently, for 3 minutes at 1300 rpm, the tubes were centrifuged. Next was the spectrophotometric estimation of the hemoglobin content of the supernatant at 540 nm. The hemolysis % inhibition of the extract was calculated using the formula below:

% inhibition of hemolysis = $1-(OD_2 - OD_1) \times 100/OD_3 - OD_1$

Where OD_1= Unheated test sample absorbency

OD_2 = Heated test sample absorbency

OD_3 = Heated control sample absorbency

Statistical Analysis

The results, which were shown as mean standard deviation, were analyzed using a one-way analysis of variance (ANOVA). The data were examined with SPSS version 18, which stands for Statistical Product and Service Solutions. Significant results were defined as $P < 0.05$.

Results and Discussion

Qualitative Phytochemical Compositions of the Fractions of Parinari kerstingii Leaf Extract

The qualitative phytochemical compositions of the fractions of *Parinari kerstingii* leaf extract, as shown in Table 1, revealed the absence of saponins, reducing sugar, carbohydrate, and glycoside in the n-hexane fraction; Reducing sugar, tannins, oil, and glycoside in the ethylacetate fraction. Methanol extract revealed the various components analyzed.

Comparative Quantitative Phytochemical Content of the Crude Extract and Fractions of Parinari kerstingii Leaf Extract

The quantitative phytochemical content of the fractions of *Parinari kerstingii* leaf, as depicted in Table 2, shows the absence of saponins, reducing sugar, carbohydrate, and glycoside in the n-hexane fraction; Reducing sugar, tannins, and glycoside in the ethylacetate fraction. Methanol extract and fraction revealed the various components analyzed.

GC-MS Analysis of Methanolic Extract of Parinari kerstingii Leaf

A total of ten peaks were detected from the GC–MS analysis and identified mainly as fatty acid esters, phenols, alkanes, alkynes, and aromatic compounds. The maximum peak area was shown by 9-Octadecenoic acid (42.21 %) followed by n-hexadecanoic acid (23.92 %), Z-17-Nonadecen-1-ol acetate (8.65 %), and Heptadecane, 4-propyl (5.60 %). The remaining peaks account for an area of less than 3 %. Table 3 lists these chemicals in order of their retention time. The compounds' peak areas, molecular formulas, weights, and structures were also indicated.

Table 1: Qualitative phytochemical compositions of the fractions of *Parinari kerstingii* leaf extract

Phytochemical	MEOH fraction	n-Hexane fraction	Ethyl-acetate fraction
Phenolics	+	+	+
Tannins	+	+	ND
Glycosides	+	ND	ND
Flavonoids	+	+	+
Steroids	+	+	+
Terpenoids	+	+	+
Carbohydrate	+	ND	+
Reducing sugar	+	ND	ND
Saponins	+	ND	+
Alkaloids	+	+	+
Oil	+	+	ND

ND = Not detected+ = Present

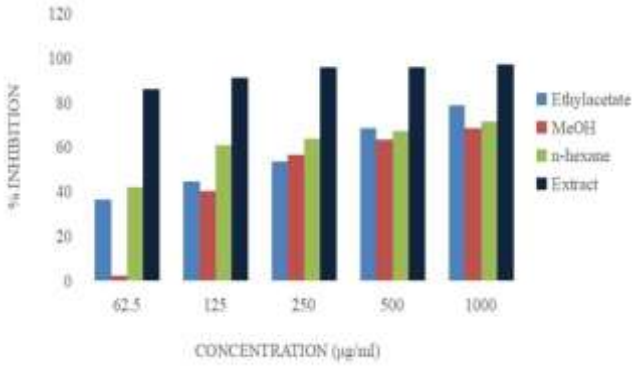


Figure 1: Comparative effect of crude extract and fraction of *Parinari kerstingii* leaf extract on nitric oxide scavenging activity.

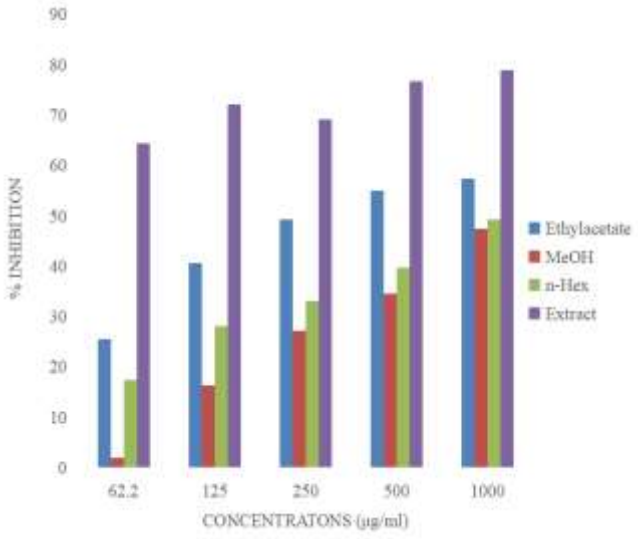


Figure 2: Comparative effect of the crude extract and fraction of *Parinari kerstingii* leaves on total antioxidant capacity

Table 2: Comparative quantitative phytochemical composition of fractions of *Parinari kerstingii* leaf extract

Phytochemical (mg/100g)	MeOH extract	MeOH fraction	n-hexane fraction	Ethyl-acetate fraction
Phenolics	8731.100 ± 0.120[a]	1542.000 ± 0.007[c]	223.600 ± 0.600[d]	2045.700 ± 0.030[b]
Tannins	952.000 ± 0.004[a]	38.5 ± 0.05[b]	8.3 ± 0.7[c]	ND
Glycosides	146.000 ± 0.005[a]	42.600 ± 0.900[b]	ND	ND
Flavonoids	28365.100 ± 0.004[a]	1048.400 ± 0.400[d]	2166.700 ± 0.020[b]	1174.000 ± 0.006[c]
Steroids	208.000 ± 0.003[a]	16.310 ± 0.030[d]	147.400 ± 0.200[b]	76.400 ± 0.100[c]
Terpenoids	3276.000 ± 0.004[a]	997.800 ± 0.190[d]	1527.700 ± 0.080[c]	1895.500 ± 0.600[b]
Carbohydrate	5.200 ± 0.002[c]	4187.700± 0.700[b]	ND	4215.200 ± 0.050[a]
Reducing sugar	1608.000± 0.002[a]	1491.100± 0.100[b]	ND	ND
Saponins	68.500 ± 0.004[b]	45.600 ± 0.400[c]	ND	637.200 ± 0.500[a]
Alkaloids	1001.000 ± 0.006[b]	835.400 ± 0.130[c]	270.100 ± 0.030[d]	2165.9 ± 0.700[a]

n=3 Values represent mean ± standard deviation ($p < 0.05$).
Mean values, across the row, with different letters as superscripts are considered significant at $p < 0.05$
ND = Not Detected

Comparative Effect of Crude Extract and Fraction of Parinari kerstingii Leaf Extract on Radical Scavenging Activity of Nitric Oxide (NO)

Figure 1 shows the result of the effect of *Parinari kerstingii* fractions on the scavenging activity of nitric oxide. Percentage nitric oxide inhibitions of the samples were: crude extract (97.2 %, 96.2 %, 96 %, 91.2 %, and 86.1 %), ethylacetate fraction (36.5 %, 44.8 %, 53.7 %, 68.7%, and 78.9 %), methanol fraction (2.1%, 40.5 %, 56.5 %, 63.5 % and 68.4 %) and n-hexane fraction (42.2 %, 61.0 %, 63.8 %, 67.2 % and 71.4 %) at the concentrations of 1000, 500, 250, 135 and 62 µg/ml, respectively. Percentage nitric oxide inhibitions were concentration-dependent. The plant extract showed a higher percntage of inhibition than the fractions followed by ethyl acetate, n-hexane, and methanolic fractions.

Comparative Effect of the Crude Extract and Fraction of Parinari kerstingii Leaves on Total Antioxidant Capacity

The result of the experiment on the total antioxidant capacity of *Parinari kerstingii* fractions is presented in Figure 2. The total antioxidant capacity of the extract and fractions was concentration-dependent. The crude extract showed the highest antioxidant potential, followed by the ethylacetate fraction, n-hexane, and methanol fractions.

Comparative Effect of Parinari kerstingii Leaf Extract and Fractions on Platelet Aggregation

The comparative effects of *Parinari kerstingii* leaf extract and fractions on platelet aggregation are presented in Table 4. Across all doses, Parinari kerstingii crude extract and n-hexane fraction caused a concentration-dependent increase in inhibition of platelet aggregation activity. The percentage inhibition of the extract was more potent at concentrations 0.1, 0.4, and 0.8 mg/ml compared with the fractions. Ethylacetate fraction at different concentrations showed significantly ($p < 0.05$) lower inhibition of platelet aggregation than the control group. Inhibitory activities of the samples are in the order of crude extract > n-hexane > methanol > ethyl acetate fractions.

Comparative Effect of Parinari Kerstingii Leaf Extract and Fractions on Membrane Stabilization

The comparative effects of *Parinari kerstingii* leaf extract and fractions on membrane stabilization are shown in Table 5. All doses (0.1–1.0 mg/ml) of the extract protected the chicken erythrocyte membrane against lysis induced by heat and hypotonic solution. The fractions at concentrations ranging from (0.8–1.0 mg/ml) also inhibited both heat– and hypotonicity–induced HRBC membrane hemolysis.

The weight and percentage yield recorded from the fractions of *Parinari kerstingii* extract revealed that the methanol fraction gave the highest yield of 38.36 g (37.8 %) while the least was the n-hexane fraction with 1.96 g (1.9 %). The yields of the solvent fractions increased with an increase in solvent polarity. This agrees with the findings of Russo *et al*.[15] stating that extract yield rises with an increase in solvent polarity since extraction yield depends on the nature of the solvent used.

The phytochemical analysis of the various fractions (Tables 1 and 2) revealed that the ethylacetate fraction of methanol extract of *Parinari kerstingii* leaf exhibited the highest total phenolic content. This is validated by the outcome from the research of Gow-chi *et al*.[16] and due to the polarity of its solvent, ethyl acetate has a concentrating impact on the amount of phenolic compounds in plants.[17] The ethyl acetate fraction equally had a good amount of flavonoids, terpenoids, and alkaloids when compared with the other fractions. Considerable amounts of the phytoconstituents were also found in the other fraction. Furthermore, the crude extract possessed a greater amount of the phytoconstituents as analyzed. This suggests a synergistic effect of the constituents.

GC-MS analysis on the crude extract revealed a significant number of active compounds such as phenols (Phenol, 2,4-bis(1,1-dimethylethyl)-), aliphatic compounds (n-Hexadecanoic acid, [1,1'-bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester, etc), and aliphatic hydrocarbons (1-Octadecyne, etc) as prominent compounds (Table 5). Most of these constituents have exhibited high biological potency against diseases. Notably, phenol 2,4-bis(1,1-dimethylethyl) is reported to possess antioxidant,[17] antitumor,[18,19] antimicrobial,[20] and antifungal,[21] activities. Hexadecanoic acid ethyl ester and n-hexadecanoic acid have been reported to act as antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic, hemolytic agents, and 5- Alpha reductase inhibitor.[22] n-Hexadecanoic acid, as an agent of anti-inflammation, has demonstrated significant inhibitory activity against phospholipase A2 as reported by Aparna *et al*.[22] [1, 1-bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester possesses hemolytic, pesticide, skin irritant, hypocholesterolemic properties.[23] Oleic acid (9-octadecanoic acid) is shown to have anti-inflammatory and anticarcinogenic properties. It also possesses antibacterial activities, which helps to lower high cholesterol levels and stimulates the synthesis of antioxidant.[24] 1-octadecyne has antimicrobial and antifungal activity.[25]

The fractions exhibited inhibitory effects against nitric oxide scavenging activities and total antioxidant capacity (Figs 1 and 2). Ethyl acetate fraction gave the highest inhibitory effect against nitric oxide scavenging activities and total antioxidant capacity when compared with the other fractions. This could be attributed to ethyl acetate fraction constituents, mainly phenolic and flavonoid compounds. Phenols and flavonoids from research are proven to be good scavengers of free radicals due to their hydroxyl groups.[26,27] The hexane fraction demonstrated considerable activity (Tables 4 and 5) against platelet aggregation and membrane stabilization compared to other factions. This suggests that the n-hexane fraction contains active components capable of stabilizing the membrane and inhibiting platelet aggregation, which could result from essential oils in the fraction (Table 1). Studies have shown that the concentration of arachidonic acids in cell membrane phospholipids involved in inflammation decreases with an

increase in the intake of essential fatty acids in a dose-dependent manner.[28] Methanol fraction (0.8 mg/ml) of the extract led to minute inhibition of the aggregatory activity of the platelet, even though no significant difference was seen when compared with the aggregatory (control) group. All doses of ethylacetate, methanol (0.1 and 0.4 mg/ml), and n-hexane (0.1 mg/ml) fractions showed no inhibition of platelet aggregation. In the study of the effect of the fractions on membrane stabilization (Table 5), ethyl acetate and methanol fractions demonstrated a negative response at lower concentrations, which is proof of the inefficacy of the fractions to forestall rupture or hemolysis in hypotonic and heat-induced stress condition. Moreover, all doses of the crude extract demonstrated a significant effect on both platelet aggregation and membrane stabilization of the HRBC when compared with the fractions. All the fractions of plant extract tested exhibited some degree of anti-inflammatory and antioxidant activity, but they produced a reduced activity when compared with the extract, and this suggests that the goals of anti-inflammatory and antioxidant activity of *Parinari kerstingii* are synergy-dependent.

Conclusion

The in vitro study, supported by the results of GC-MS analysis, has revealed that selected fractions of n-hexane, ethyl-acetate, and methanolic leaf extract of *Parinari kerstingii* have anti-inflammatory and antioxidant properties because they contain several phytochemicals with therapeutic values

Table 3: Compounds identified from the methanolic extract of *Parinari kerstingii* leaves

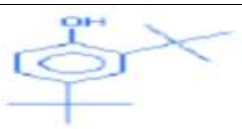
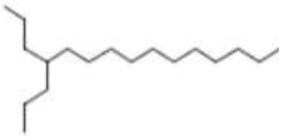
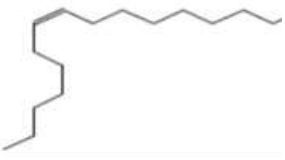
S. N	Retention Time (min)	Name of the compound	Molecular Formula	Structure	Molecular Weight	Peak Area (%)
1.	11.2	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$		206	0.80
2.	12.9	[1,1'-bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	$C_{21}H_{38}O_2$		322	1.00
3.	15.2	1-Octadecyne	$C_{18}H_{34}$		250	0.34
4.	17.9	n-Hexadecanoic acid	$C_{16}H_{32}O_2$		256	23.92
5.	18.1	Hexadecanoic acid, ethyl ester	$c_{18}h_{36}o_2$		284	2.84
6.	20.8	9-Octadecenoic acid	$C_{18}H_{34}O_2$		282	42.21
7.	21.0	n-Hexadecanoic acid	$C_{16}H_{32}O_2$		256	12.03
8.	21.8	Heptadecane, 4-propyl	$C_{20}H_{42}$		282	5.60
9.	23.7	Z-17-Nonadecen-1-ol acetate	$C_{21}H_{40}O_2$		324	8.65
10	26.0	9-Hexadecenal	$C_{16}H_{30}O$		238	2.60

Table 4: Effects of fractions of methanol extract of *Parinari kerstingii* on platelet aggregation

Groups	0 sec	30 sec	60 sec	90 sec	120 sec

Control	0.199 ± 0.04^{d}	0.192 ± 0.03^{d}	0.191 ± 0.03^{d}	0.189 ± 0.03^{d}	0.182 ± 0.02^{d}
400 mg/ml Indomethacin (Standard)	0.306 ± 0.13^{def}	0.278 ± 0.01^{def}	0.264 ± 0.02^{def}	0.259 ± 0.03^{def}	0.256 ± 0.03^{def}
800 mg/ml Methanol Extract	0.769 ± 0.35^{h}	0.763 ± 0.40^{h}	0.747 ± 0.04^{h}	0.725 ± 0.04^{h}	0.715 ± 0.05^{h}
400 mg/ml Methanol Extract	0.671 ± 0.10^{g}	0.634 ± 0.72^{g}	0.615 ± 0.07^{g}	0.617 ± 0.06^{g}	0.591 ± 0.06^{g}
100 mg/ml Methanol Extract	0.303 ± 0.42^{ef}	0.279 ± 0.05^{ef}	0.270 ± 0.05^{ef}	0.268 ± 0.05^{ef}	0.265 ± 0.05^{ef}
800 mg/ml N-Hexane Fraction	0.579 ± 0.56^{g}	0.577 ± 0.57^{g}	0.578 ± 0.06^{g}	0.578 ± 0.06^{g}	0.580 ± 0.06^{g}
400 mg/ml N-Hexane Fraction	0.302 ± 0.10^{f}	0.302 ± 0.08^{f}	0.304 ± 0.01^{f}	0.307 ± 0.01^{f}	0.308 ± 0.01^{f}
100 mg/ml N-Hexane Fraction	0.094 ± 0.08^{bc}	0.096 ± 0.08^{bc}	0.100 ± 0.01^{bc}	0.106 ± 0.01^{bc}	0.108 ± 0.01^{bc}
800 mg/ml MEOH Fraction	0.217 ± 0.02^{de}	0.220 ± 0.16^{de}	0.222 ± 0.02^{de}	0.224 ± 0.02^{de}	0.227 ± 0.02^{de}
400 mg/ml MEOH Fraction	0.115 ± 0.10^{c}	0.116 ± 0.01^{c}	0.118 ± 0.01^{c}	0.121 ± 0.01^{c}	0.122 ± 0.01^{c}
100 mg/ml MEOH Fraction	0.045 ± 0.01^{ab}	0.046 ± 0.07^{ab}	0.050 ± 0.01^{ab}	0.050 ± 0.01^{ab}	0.051 ± 0.01^{ab}
800 mg/ml Ethyl Acetate Fraction	0.181 ± 0.15^{d}	0.179 ± 0.02^{d}	0.179 ± 0.02^{d}	0.170 ± 0.001^{d}	0.181 ± 0.02^{d}
400 mg/ml Ethyl Acetate Fraction	0.097 ± 0.22^{bc}	0.094 ± 0.02^{bc}	0.095 ± 0.02^{bc}	0.097 ± 0.02^{bc}	0.097 ± 0.02^{bc}
100 mg/ml Ethyl Acetate Fraction	0.020 ± 0.03^{a}	0.019 ± 0.04^{a}	0.019 ± 0.05^{a}	0.019 ± 0.01^{a}	0.019 ± 0.01^{a}

n=3 Values represent mean ± standard deviation ($p < 0.05$).Mean values, down the column, with different letters as superscripts are considered significant at $p < 0.05$.

Table 5: Membrane stabilizing effect of *Parinari kerstingii* fraction on haemolysed HRBCs (Percentage inhibition)

Conc (mg/ml)		Heat (%)			Hypotonicity (%)			
	Crude extract	MeOH	Ethylacetate	n-Hex	Crude extract	MeOH	Ethylacetate	n-Hex
0.1	0.3	-62	-88	-478	69.22	-34.8	-76.6	2.6
0.2	1.0	-24	-38.6	-123	62.79	-18	-43.7	14.6
0.4	21.1	-22	-40.8	-6	99.30	-10	36.3	20.0
0.8	38.0	4.0	-6.59	2.99	69.28	31.2	38.2	28.1
1.0	44	19.3	4.87	32.87	63.04	35.8	35.6	50.3

n=3. Values represent mean ± standard deviation of triplicate samples. ($p< 0.05$). Percent inhibition of haemolysis was calculated relative to control.

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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