

Original Research Article

Antioxidant and Anti-inflammatory Studies of Aqueous and Methanol Extracts of *Pterocarpus erinaceus* poir Leaves

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Abstract

Purpose: Increasing ethno-medicinal adoption of plant based management option for chronic diseases is well documented. Phytochemistry studies have shown presence of metabolites in plants with diverse pharmacological actions. This study evaluates antioxidant and anti-inflammatory potentials in *Pterocarpus erinaceus* leaf extracts.

Method: The 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) radical reduction and ferric-reducing antioxidant power (FRAP) were assayed for antioxidant evaluation. Human red blood cell membrane stabilization and the egg albumin (protein) denaturation inhibition were determined for the anti-inflammatory.

Results: Significant concentrations of phenols, flavonoids, tannins, saponins, vitamins A, C and E were detected in the extract. The extract demonstrated a high total antioxidant capacity (TAC), DPPH radical was inhibited, IC₅₀ 0.70 µg/ ml (methanol) and 3.65 µg/ ml (aqueous), ferric pyridyltriazine (Fe³⁺-TPTZ) complex was also reduced to the ferrous ion (Fe²⁺) complex. Additionally, both extract inhibited protein denaturation and erythrocyte haemolysis. Aqueous extract however, produced more potency on protein, possessing a higher % protection with a lower IC₅₀ of 0.24 µg/ ml, not significantly different from aspirin.

Conclusion: Aqueous and methanol leaf extracts of *P. erinaceus* in this study demonstrated antioxidant and anti-inflammatory potentials. Vitamins and polyphenols in the extracts might have enhance the antioxidant and anti-inflammatory activities, therefore possible therapeutic effect of the plant could include antioxidant and anti-inflammation mechanisms.

Keywords: Polyphenol, Anti-inflammatory, Metabolite, Erythrocytes haemolysis, metabolic syndrome.

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INTRODUCTION

Phytochemistry studies have revealed myriad of natural bioactive compounds in plants, which have shown pharmacological actions and therapeutic effects. Since the medicinal uses of herbal preparations by the Chinese in 4000-5000BC. AD, globally, the continuing ethno-medicinal adoption of plant based management option for several ailments is well documented. About 64% of the world population has been reported to use medicinal herbs for the health care needs and at least, 25% estimate of pharmaceutical products is shown to often contain synthetically isolated compound from floral constituents.¹ A WHO survey, reported that about 80%, 85% and 90% of patients in India, Burma, and Bangladesh respectively were using traditional medicine. In Nigeria, 85% prevalence for herbal medicine were reported.² Additionally, studies have revealed antioxidant and anti-inflammatory applications of phytoconstituents in inflammatory diseases.^{3,4} Hence, more biological activity studies are required to substantiate the anti-inflammatory potentials in medicinal plants. This may enhance identification and isolation of pharmacologically active compounds which could be used in targeted antioxidants and anti-inflammatory drug discovery and production.

Inflammation, an aspect of immune defense mechanisms in response to injury, infections or diseases, toxin and irradiation can be acute or chronic.^{5,6} Acute inflammation minimizes injury and restored tissue damage via cellular and molecular events thus, serving a protective mechanism to the body. In chronic inflammatory state which results from continuous, uncontrolled and prolonged inflammation, destructive catabolic mechanisms accompanied by excessive free radicals generation are enhanced. These has been implicated in pathogenesis of chronic disease states. It has also been shown to exacerbate metabolic syndromes (obesity, diabetes or coronary heart diseases), cancer and the neuro-degenerative conditions such as dementia, Alzheimer's disease and Parkinson's syndrome.^{7,8,9} Anti-inflammatory and antioxidant drugs are therefore essential to mitigate the pathophysiological response. However, the commonly used phenolic synthetic antioxidants such as butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT), Tert-Butyl hydroquinone (TBHQ) and propyl gallate.¹⁰ as well as steroidal and Non-steroidal anti-inflammatory drugs (NSAIDs) may cause adverse side effects including gastro- intestinal tract (GIT) irritation, alteration in kidney functions and cardiovascular effects.^{11,12} It is therefore necessary to explore new management option from medicinal herbs with reported antioxidant and anti-inflammatory potency, minimal side effects and easy accessibility.

Pterocarpus erinaceus poir. in the family Fabacea is commonly called African rosewood, African Kino or African teak.^{13,14} In Nigerian, it is locally referred to as, "Marke / Mabobiya / Shajini, Agbelosun / Apepo, and Ase egu" in the North, Southwest, and East respectively.^{13,15} The *P. erinaceus*, a deciduous tree species is widely distributed in West Africa including Nigeria. It can grow up to 12-15m in height and measure up to 1.2m diameter. It has a finely scaly grey bark with brown-blackish inner. Leaves are arranged alternately ovate lanceolate in shape and 30cm long. Flowers are bisexual, with golden yellow colour, clawed petals, and campanulate calyx. The *Pterocarpus* is coined from a Greek words "pteran" meaning a wing and, "karpos" meaning 'fruit'.^{3,16}

Ethno pharmacological studies revealed that leaves of *Pterocarpus erinaceus* is used in abortifacient mixture. Bark is used for ringworm on the scalp, for dressing chronic ulcers, and gonococcal conjunctivitis. It is also gargle in tooth and mouth problems. The bark and resin are used to manage urethral discharge and as an astringent for dysentery and diarrhea. The root has also been used as remedy for coughing. Anti-inflammatory, analgesic, antimalarial, antifungal and the antioxidant activities of *Pterocarpus erinaceus* roots, stem bark and leave extracts have been studied elsewhere.^{16, 17, 18, 19} These studied reported diverse composition of pharmacologically active compounds such as polyphenols, glycosides and micronutrients which are found in the plant. It is expected that further phytochemical profile of extracts from the different parts of *Pterocarpus* plant in different locations will provide insights to identification and authentication of phytochemical(s), which may be responsible for the specific intended therapeutic effects. Therefore, this study aims to evaluate the antioxidant and anti-inflammatory potentials in aqueous and methanol leaf extracts of the *Pterocarpus erinaceus* collected in Kano, North- west Nigeria, using selected *in vitro* assay.

MATERIALS AND METHODS

Plant Collection, Identification and Authentication

Stem branches of *Pterocarpus erinaceus* Fabaceae - Papilionoideae Lam.^{13,14} were collected from Rano area of Kano State, Nigeria. The plant was authenticated in Department of Biological Sciences Laboratory Unit Northwest University, Kano. Voucher specimen accession number YUMSUKHAN 23/040 was deposited in the herbarium. Kano lies between latitude 12°0'0" in the North, 11°0'0" in the South and longitudinally at 8°0'0"W (west) and 9°0'0 in the East.²⁰

Preparation of Leaf Extract

The leaves were picked and cleaned using running tap water, air dried and then grind to powder using grinding machine. Each hundred gram (100g) of the pulverized

sample was weighed using digital Weighing Balance (OHAUS PA413) and were separately macerated in 500 ml of distilled water and the 500 ml of methanol. After 48 hours, each solvents was evaporated in water bath maintained at 40°C to obtain the respective crude aqueous and methanol extracts which were used for the biochemical analysis.

Phytochemical Analysis

Standard laboratory procedures and methods were used to screen for phenols, flavonoids, tannins and the saponins. The total phenols, flavonoids, tannin and saponins were then quantitatively determined as previously described by.^{21, 22}

In vitro Antioxidant Assay

Rutkowski *et al*²³ and Dias *et al*.²⁴ methods and the procedures, slightly modified, were adopted to measure the vitamins A, C, E concentrations and the DPPH radical reduction activity of the extract as previously described in Oseni *et al*.²⁵

Ferric Reducing Antioxidant Power

The ferric reducing antioxidant power (FRAP) was determined as described by Senguttuvan *et al*. 2014²¹ with slight modifications. The principle of the method is based on transfer of hydrogen atom (HAT) from antioxidants and the reduction of colourless ferric-2, 4, 6-tri-2-pyridyl-s-triazine (Fe³⁺-TPTZ) to blue ferrous ion complex (Fe²⁺-TPTZ) in acidic medium. A 75 µl of each extract in varying low and high concentrations (7.81-1000 µg/ml) was added to 2 ml of FRAP reagent; 500 ml of acetate buffer, (300 mM pH 3.6), 50 ml of TPTZ (10 mM) and 50 ml of FeCl₃.6H₂O (50 mM). The decrease in absorbance taken at 593nm against reagent blank after 2 minutes (Model 6305 UV, Bibby Scientific Ltd, England) was used to calculate the percentage reduction of the ferric ion complex.

Ethical Approval

The departmental Research Committee, Biochemistry Department, Northwest University gave approval for the study dated 20th September, 2023. The human volunteer also agreed to participate and signed the consent form.

In vitro Anti-inflammatory Assay

Red blood cell membrane stabilization

Red blood cell membrane stabilization activity was carried out.²⁶ After an oral informed consent, five milliliter (5 ml) of blood sample was collected from an apparently healthy human volunteer using standard procedure. The blood was carefully transferred into a plastic test tube filled with normal saline and then centrifuged at 1000rpm for 5 minutes until the RBC were packed. The supernatant serum was pipetted. The blood cells were re-suspended in 2 ml of normal saline and this process of washing was repeated twice. The last wash had a clear supernatant with no signs of haemolysis. It

was labeled as Erythrocyte Suspension (ERS) and was kept at room temperature (28°C) ready for analysis. Two milliliter (2 ml) each of a known concentration (125 µg/ml) for each exact were placed in a well labeled tube in triplicate, to each test tube, 3 drops of the ERS, 2 ml of saline and 1 ml of 0.14M phosphate buffer were added. The mixture was incubated at 37°C for 30 minutes and then centrifuge at 2000rpm for 10 minutes. The haemoglobin content of the supernatant was determined using spectrophotometer (Model 6305 UV, Bibby Scientific Ltd, England) at 560nm. Aspirin was used as standard and the protection on the red cell membrane was determined (equation 1)

$$Protection = 100 \times \frac{[OD_1 - OD_2]}{OD_1}$$

Where; OD₁ = Optical density of extract treated

OD₂ = Optical density of the control

Inhibition of protein denaturation

The inhibition of protein denaturation activity was determined using varying concentrations of plant extract (0.0625-1 mg/dl). A 0.2 ml of 1% egg albumin and 4.8 ml of phosphate buffer were added to the reaction mixture to a final volume of 5 ml each. 5 ml of distilled water was used as control. The test tube were incubated at 37°C in a water bath for 15 minutes. The test tube were then cooled and the absorbance was measured at 660nm in a spectrophotometer. Aspirin (0.0625-1mg/dl) was used as standard and was treated similarly at different concentration and the absorbance percentage inhibition of the protein denaturation was calculated (equation 2).²⁶

$$\% Inhibition = 100 \times \frac{[A_{sample} - 1]}{[A_{control}]} \text{ ----- Equation 2}$$

Where A= absorbance for sample and the control

Statistical Analysis

Data were analyzed by descriptive statistics using statistical package for social science (SPSS), version 21.0, Inc., Chicago, USA. The results are presented as mean ± standard deviation of triplicate values.

RESULT AND DISCUSSION

In this study, we determined quantities of selected polyphenols and vitamins and evaluated antioxidant and anti-inflammatory activities, using *in vitro* study models.^{6, 27} The DPPH and FRAP reducing activities as well as erythrocyte membrane stabilization and the inhibition of protein denaturation were evaluated in the aqueous, a similitude of the commonly adopted ethno-medicinal method for various decoctions and methanol, a widely used polar organic solvent. The result showed that the aqueous extract has significantly higher concentrations of flavonoids, tannins and saponins

compared with the methanol extract (Table 1). However the methanol extract was significantly more in phenol, as well as vitamin C and the total antioxidant capacity (Table 2). The

detected flavonoid agrees with the earlier reported flavonoids found also in roots and the stem bark of a *pterocarpus*.^{16,18}.

Table 1: Selected secondary metabolites composition of *Pterocarpus erinaceus* leaf extracts

| <i>P. erinaceus</i> | Total Concentration (mg/l) | | | |
|---------------------|----------------------------|-----------------------------|----------------------------|---------------------------|
| | Flavonoids | Phenols | Tannins | Saponins |
| Aqueous extract | 34.13 ± 32 ^b | 494.01 ± 1.78 ^a | 262.92 ± 5.82 ^e | 23.10 ± 0.28 ^c |
| Methanol extract | 25.03 ± 49 ^a | 736.48 ± 11.24 ^b | 226.40 ± 6.36 ^c | 8.19 ± 3.58 ^d |

Values are mean ± SD of three replicates. Values in the same column with different superscript letters are significantly different (p<0.05)

Table 2: Vitamins A, C E and the total antioxidant capacity composition of *Pterocarpus erinaceus* leaf Extracts

| <i>P. erinaceus</i> | Vitamins concentration | | | |
|---------------------|---------------------------|---------------------------|---------------------------|----------------------------|
| | A (µmol/l) | C (mg/dl) | E (mg/dl) | TAC (µg/ml) |
| Aqueous extract | 19.84 ± 0.16 ^a | 5.930 ± 0.14 ^c | 41.33 ± 1.78 ^b | 437 ± 5.85 ^a |
| Methanol extract | 19.92 ± 0.30 ^a | 6.11 ± 0.02 ^d | 42.85 ± 1.67 ^b | 560.80 ± 4.99 ^b |

Values are mean ± SD of three determinations. Values in the same column with different superscript letters are significantly different (p<0.05). TAC = total antioxidant capacity

The studies however, did not report presence of phenols in the root and stem bark extracts. In the double dilution assay, using low and high concentrations, the DPPH radical was inhibited with a low IC₅₀ value of 0.70 µg/ml and 3.65 µg/ml for the methanol and aqueous extracts (Table 3). The Fe³⁺-TPTZ complex was also reduced to Fe²⁺-TPTZ in a concentration dependent manner (Table 4). These reductions are an indication of antioxidant activity. The polyphenols, vitamins C, E and A, which were determined and found in the extract are potent antioxidants. They contain varying pharmacophoric substituents like poly hydroxyl (OH) group, hydroxylated ketone lactone, chromanol rings and the isoprenyl chain respectively^{28,29} (Figures 1), which by hydrogen atom transfer (HAT) and/or electron donor (ET) actions to reduce pro-oxidants can confer the antioxidant activities producing the reduced DPPH-H and Fe²⁺ complex.

Furthermore, the aqueous and methanol extracts demonstrated similar erythrocytes membrane stabilization activity which were not different statistically (Figure 1). This suggest that the extract can reduce red blood cell haemolysis by stabilizing the cell membrane. Stabilization of membrane and the inhibition of heamolysis is an anti-inflammatory effect, as agreed with the study of Yesmin *et al.*²⁷ Free radicals can cause lipid peroxidation in polyunsaturated lipids of membranes and lead to loss of fluidity and cell lysis. Consequently, susceptible to the release of lysosomal constituents including inflammatory mediators.

Table 3: The 2, 2-Diphenyl-1-picryl-hydrazyl radicals scavenging activities of *Pterocarpus erinaceus* leaf extracts

| Concentration (µg/ml) | DPPH radical inhibition (%) | | |
|-----------------------|-----------------------------|---------------------------|--------------------------|
| | Aqueous extract | Methanol extract | Ascorbic acid |
| 7.81 | 47.46 ± 0.45 | 69.46 ± 0.22 | 69.88 ± 0.2 |
| 15.63 | 61.29 ± 0.78 | 73.84 ± 0.54 | 80.37 ± 0.63 |
| 31.25 | 73.04 ± 3.11 | 79.14 ± 0.43 | 88.22 ± 4.32 |
| 62.25 | 77.7 ± 1.02 | 81.43 ± 0.90 | 94.37 ± 0.35 |
| 125 | 79.5 ± 0.86 | 82.65 ± 0.25 | 94.93 ± 0.75 |
| 250 | 80.07 ± 0.99 | 83.01 ± 0.81 | 95.11 ± 0.76 |
| 500 | 82.94 ± 0.87 | 84.66 ± 0.33 | 95.46 ± 0.31 |
| 1000 | 83.44 ± 0.43 | 86.17 ± 0.33 | 95.88 ± 0.31 |
| IC ₅₀ | 3.65 ± 0.47 ^c | 0.706 ± 0.15 ^b | 0.05 ± 0.02 ^a |

Values are mean ± SD of three determination

The half maximum inhibitory concentrations (IC₅₀) values in the same row with different superscript letters are significantly different (p<0.05).

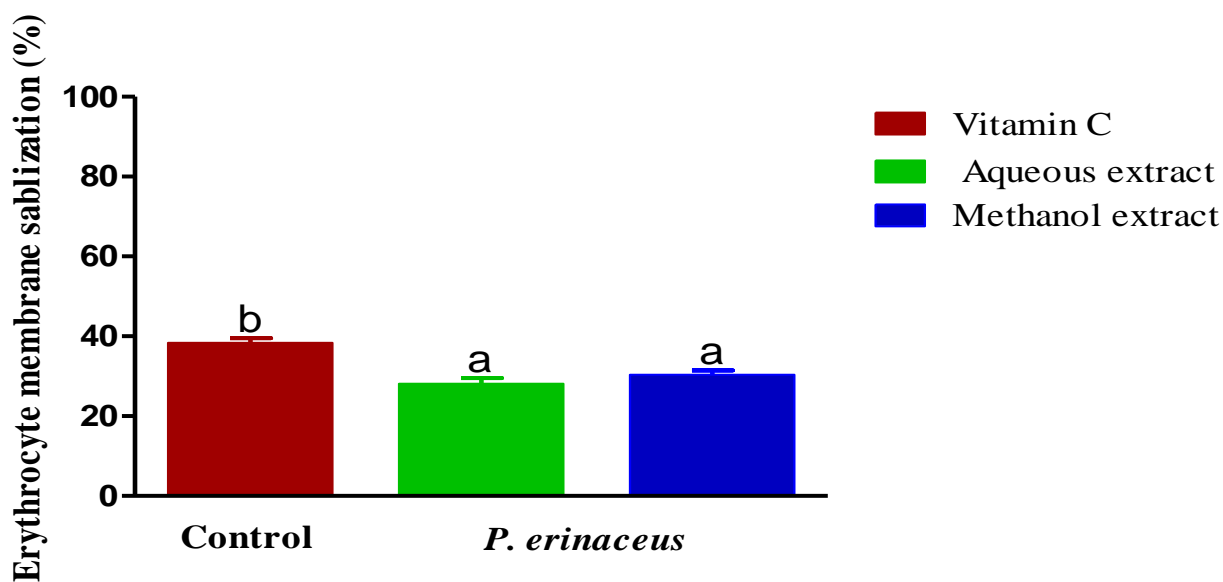


Figure 1: Erythrocyte membrane stabilization activity of *Pterocarpus erinaceus* leaf extracts

Bars are mean \pm SD for three determinations. Bars with different letters are significantly different ($p < 0.05$). The stabilized erythrocyte membrane observed in this study implies that the extract possesses anti-inflammatory constituents that might limit free radical-induced inflammatory responses leading to the release of activated inflammatory mediators. Anti-inflammatory activity of flavonoids is attributed to inhibiting release of histamine from mast cells and other inflammatory mediators by its stabilization of the cell membrane. Saponins from certain flora has showed anti-inflammatory activity against several experimental model.^{4,30} Thus, in this study, the inhibition of erythrocyte membrane haemolysis observed can justify probable antioxidant and anti-inflammatory activities due to phenols, flavonoids and saponins in the extracts. Additionally, both extracts also inhibited denaturation of albumin. However, the aqueous extract produced a higher % inhibitory protection with a lower IC_{50} value which was not significantly different from the standard control (Table 5). The result of the present study agrees with the anti-inflammatory activity of the stem bark extract earlier reported which were related to the flavonoids and phenol contents.³¹

Table 4: The ferric-reducing antioxidant power activity of *A.leocapus* leaf extracts

| Concentration ($\mu\text{g/ml}$) | Ferric reduction (%) | | | | | |
|---------------------------------------|----------------------|-------|---------------------|-------|------------------|-------|
| | Aqueous extract | | Methanol extract | | Ascorbic acid | |
| 7.81 | 0.12 | \pm | 0.18 | \pm | 0.16 | \pm |
| | 0.01 | | 0.01 | | 0.02 | |
| 15.63 | 0.16 | \pm | 0.19 | \pm | 0.24 | \pm |
| | 0.01 | | 0.05 | | 0.02 | |
| 31.25 | 0.17 | \pm | 0.26 | \pm | 0.34 | \pm |
| | 0.02 | | 0.02 | | 0.01 | |
| 62.25 | 0.19 | \pm | 0.34 | \pm | 0.54 | \pm |
| | 0.03 | | 0.02 | | 0.01 | |
| 125 | 0.16 | \pm | 0.47 | \pm | 0.75 | \pm |
| | 0.00 | | 0.03 | | 0.02 | |
| 250 | 0.19 | \pm | 0.59 | \pm | 0.87 | \pm |
| | 0.01 | | 0.02 | | 0.02 | |
| 500 | 0.33 | \pm | 1.24 | \pm | 1.97 | \pm |
| | 0.02 | | 0.01 | | 0.03 | |
| 1000 | 0.33 | \pm | 1.89 | \pm | 2.52 | \pm |
| | 0.01 | | 0.02 | | 0.01 | |

Values are mean \pm SD of three determination

Table 5: The inhibition of denaturation activity of *Pterocarpus erinaceus* leaf extracts

| Concentration (mg/ ml) | Inhibition of protein denaturation (%) | | |
|---------------------------|---|---------------------|-------------------|
| | Aqueous extract | Methanol extract | Ascorbic acid |
| 0.06 | 18.93 ± | 15.52 ± | 36.29 |
| | 3.26 | 0.65 | ±1.65 |
| 0.12 | 20.64 ± | 17.70 ± | 44.52 |
| | 3.02 | 2.07 | ±3.97 |
| 0.25 | 24.03 ± | 26.20 ± | 53.20 |
| | 4.40 | 2.94 | ±3.30 |
| 0.50 | 89.2 ± | 63.47 ± | 76.30 |
| | 1.06 | 4.40 | ±1.22 |
| 1.00 | 98.77 ± | 83.30 ± | 83.30 ± |
| | 1.00 | 0.40 | 0.40 |
| IC ₅₀ | 0.24 ± | 0.35 ± | 0.15 ± |
| | 0.02 ^a | 0.01 ^b | 0.01 ^a |

Values are mean ± SD of three determinations.

The half maximum inhibitory concentrations (IC₅₀) values in the same row with different superscript letters are significantly different (p<0.05).

The lower inhibitory concentration for the extract is an indicative of higher stabilization of protein denaturation activity. The denaturation of protein can lead to inflammation, through production of activated mediators.²⁶ The *P.terocarpus* extracts have considerable concentration of flavonoids, phenols, tannins and saponins. These secondary metabolites are with reported anti-inflammatory activity.³¹ which could enable them to possibly inhibit production of inflammation mediators. Therefore, the phytochemicals might contribute to the protective role of the plant on protein and suggests that *p. erinaceus* plant has anti-inflammatory potential.

CONCLUSION

In conclusion aqueous and methanol leaf extracts of *P. erinaceus* in this study demonstrated antioxidant and anti-inflammatory potentials. The pharmacologically active metabolites including vitamins C, A and E, flavonoids and phenols present in the floral extract which can reduce free radicals might have enhance the antioxidant and anti-inflammatory activities, therefore

possible therapeutic effect of the plant could include antioxidant and anti-inflammation mechanisms.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS DECLARATION

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

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