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Effect of flavonoid-rich fraction of *Pleiocarpa mutica* leaves on phospholipase A_2 activity

Christabel Ebube Festus [1, *], Ugochukwu ThankGod Nweke [2], Uchechukwu Christiana Chukwuekwe [1] and *Gideon* Ebubechi Onunwa [1]

[1] *Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria.*
[2] *Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria.*

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Abstract

Pleiocarpa mutica (Apocynaceae) is a pharmaceutical plant investigated to identify the usefulness and efficacy of the shrubs in the treatment of various ailments. Also, flavonoids are polypenolic compounds frequently found in nature and are divided into flavones, anthocyanidins, isoflavones, catechins, flavonols, chalcones, and flavonones based on the structure of their chemicals. Hence, fresh leaves of *Pleiocarpa mutica* were collected from Ugbene-Ajima, Uzo-Uwani Local Government Area, Enugu State, Nigeria. The extraction of a flavonoid-rich fraction of *P. mutica* leaves was conducted by dissolving a certain amount of crude extract in 20 mL of 10% H_2SO_4 in a tiny flask and then heating the mixture in a water bath for 30 minutes at 100 °C to initiate hydrolysis. However, quantitative phytochemical analysis of the *P. mutica* flavonoid-rich fraction was carried out by utilizing standard conventional protocols. Newly drawn human blood samples were centrifuged for 10 minutes at 3,000 rpm, with the supernatant (plasma) being disposed of. After being measured and reconstituted as a 40% (v/v) suspension with phosphate-buffered saline, the red blood cells were rinsed three times with an equivalent volume of normal saline. The fraction substantially ($p < 0.05$) reduced the activity of phospholipase A_2 in a concentration-dependent approach when compared to the standard drug (prednisolone), with a range of 0.2 mL to 1.0 mL reducing the amount of the enzyme by 47.58% to 63.61%. These results showed that the flavonoid-rich fraction of *Pleiocarpa mutica* leaves is potent in curtailing the inflammatory response through inhibition of phospholipase A_2 activity.

Keywords: Phospholipase A_2; Activity; Flavonoids; *Pleiocarpa mutica* leaves

1. Introduction

Nutrient antioxidants like vitamin C, vitamin E, and β-carotene are inferior to flavonoids like luteolin and cathechins. Traditional medicine has demonstrated that the anti-inflammatory properties of the wide variety of medicinal plants found throughout the African continent are efficacious in treating inflammatory ailments. Due to their accessibility, effectiveness, and low side effects, a variety of natural compounds derived from medicinal plants have served as the foundation for many traditional medical systems worldwide [1,2]. Due to the benefits of herbal therapy over pharmaceuticals, there is an increasing interest in finding novel anti-inflammatory compounds derived from plant. *Pleiocarpa mutica* (Apocynaceae) is a medicinal plant that is used to treat a variety of illnesses in several parts of the world. *Pleiocarpa mutica* is a tiny tree or shrub with a maximum height of 7.5 meters. When it is found as a climbing shrub, the trunk has a diameter of 1.5–5 cm and the stem is approximately 9 m long. The shrub produces clusters of fragrant, thin tubular blooms. It is beautiful and deserving of development. The following conditions could be treated with fever, convulsions, oedema, kidney disorders, malaria, jaundice, and stomach problems [3]. High amounts of alkaloids and flavonoids, moderate concentrations of tannins and terpenoids, and low concentrations of saponins were found in the leaves according to prior phytochemical investigation [4,5].

* Corresponding author: Christabel Ebube Festus

In addition, flavonoids are polypenolic substances widely found in nature. They are divided into flavones, chalcones, anthocyanidins, isoflavones, catechins, and flavonols based on their chemical structures [6]. Also, tea, coffee, and fruit drinks are among the beverages that contain them the most, along with fruits and vegetables. Not only do flavonoids have a wide range of pharmacological and biological activities, including antimicrobial, cytotoxic, anti-inflammatory, and antitumor properties, but they are also highly effective antioxidants that can shield the human body from reactive oxygen species and free radicals. This ability to function as an antioxidant is the best characteristic shared by nearly all flavonoid groups. Because of their molecular makeup, flavonoids have the ability to function as antioxidants. Thus, the anti-inflammatory and free radical-scavenging properties of flavonoids rely on the location of hydroxyl groups and other structural components. Nevertheless, there is little proof of the plant's flavonoid-rich fraction's ability to reduce inflammation. Thus, the purpose of this study was to assess the plant's potential for treating anti-inflammatory diseases by examining how it affects phospholipase A_2.

2. Materials and methods

2.1. Plant material authentication

Pleiocarpa mutica fresh leaves were obtained in Enugu State, Nigeria's Ugbene-Ajima, Uzo-Uwani Local Government Area. Mr. Alfred Ozioko of the Bio-Resources Development and Conservation Programme (BDCP) Research Centre in Nsukka, Enugu State, identified and verified the leaves. For reference, a voucher specimen with the number Intercedd/301 was placed at the herbarium.

2.1.1. Equipments

The equipments used in this research are as follows:

Equipment	Manufacturer
Measuring Cylinder	Pyrex, England
Weighing Balance	Vickas Ltd, England
Centrifuge	Vickas Ltd, England
Spectrophotometer (E312 Model)	Jenway, UK
Refrigerator	Thermocool, England
Water bath	Gallenkamp, England
Rotary evaporator	Hujin, China
Beaker	Ningbo, China
Stop clock	Crown, India
Test tubes	Pyrex, Germany

2.1.2. Reagents and Chemicals

The analytical-grade chemicals utilized in this study were all obtained from Sigma Aldrich, USA; British Drug House (BDH), England; Qualikems, India; Fluka, Germany; May and Baker, England; and Burgoyne, India. The assays employed commercial kits and products from Teco (TC), USA, and Randox, USA, as reagents.

2.2. Methods

2.2.1. Procedure for Extraction

The *P. mutica* fresh leaves were gathered and cleaned to get rid of any dirt. The leaves were then shade-dried until crispy, rotating them frequently to prevent decomposition. A mechanical grinder was used to grind the dried leaves into a powder. Using a maceration flask, a known weight of the ground leaves (1.5 kg) was macerated in 10 liters of pure ethanol. The mixture was stirred occasionally for 72 hours, and then a cotton cloth was used to filter it into a flask with a flat bottom. To get rid of tiny residues, Whatman No. 4 filter paper was used for additional filtration. To obtain the crude ethanol extract, the filtrate was concentrated using a rotary evaporator set at 45 °C. A labeled, sterile screw-capped bottle was used to hold the concentrated extract.

2.2.2. Preparation of Flavonoid-Rich Fraction of Pleiocarpa mutica leaves

Using the procedure outlined by Chu *et al.* (2002) [7], the flavonoid-rich fraction of *P. mutica* leaves was extracted. 30 minutes at 100 °C in a water bath hydrolyzed a determined amount of the crude extract that had been dissolved in 20 mL of 10% H_2SO_4. Aglycones, which are flavonoids, precipitated out of the mixture by placing it on ice for fifteen minutes. 50 mL of warm 95% ethanol (50 °C) was used to dissolve the filtrate, which was the flavonoids-aglycone combination, after the cooled solution had been filtered. The resulting solution was again filtered through a 100-mL volumetric flask that was completely filled with 95% ethanol. With the use of a rotating evaporator, the collected filtrate was dried out.

2.3. Phytochemical analysis of *Pleiocarpa mutica* leaves fraction

Utilizing standard, conventional techniques as outlined by (Harborne, 1998; Evans and Trease, 2002) [8,9], a quantitative phytochemical study of the *P. mutica* flavonoid-rich fraction was conducted. This is how the concentrations of the different phytochemical components were calculated:

$$\text{Concentration (mg/100 g or g/100 g)} = \frac{absobance\ of\ the\ sample}{absorbance\ of\ the\ standard} \times Dilution\ factor$$

$$\text{Dilution factor} = \frac{\text{total volume}}{\text{weight of extract}}$$

2.4. Drugs

Prednisolone 5 mg round pills were the medications used. The company, Unicure Pharmaceuticals Ltd., manufactured it in Lagos, Nigeria. They functioned as the assay's standard medication for phospholipase A_2 activity.

2.5. *In-vitro* anti-inflammatory study

2.5.1. Determination of the Effect of the FlavonoidRich Fraction of Pleiocarpa mutica Leaves on Phospholipase A_2 Activity:

The effect of the fraction on phospholipase A_2 activity was determined using modifications of the methods of Vane (1971) [10].

Principle

Using its effect on the erythrocyte membrane, phospholipase A_2 activity was measured. As a result of the leakage it creates, hemoglobin is able to enter the medium as free fatty acids are liberated from the membrane phospholipids. As a result, the amount of hemoglobin in the media directly correlates with the enzyme activity. Due to hemoglobin's maximum absorption at this wavelength, the measurement was made at 418 nm.

Enzyme Preparation

A preparation of fungal enzymes was obtained using a culture of Aspergillus niger. 15 g of Sabouraud dextrose agar was dissolved in 1000 ml of distilled water to create the nutrient broth, which was then homogenized in a water bath for 10 minutes and poured into 250 ml conical flasks. Cotton wool and foil paper were used to seal the conical flasks. After that, the broth was autoclaved for 15 minutes at 121 °C. Following a 72-hour incubation period at room temperature, the organisms in the Petri plates were aseptically injected into the broth after it had cooled to room temperature. After the culture was moved into test tubes with 3 milliliters of phosphate-buffered saline, the tubes were centrifuged for 10 minutes at 3000 rpm. The test tube's bottom was occupied by the fungus cells, and the crude enzyme preparation was made from the supernatant.

Substrate Preparation

New blood samples were centrifuged for 10 minutes at 3,000 rpm, and the supernatant, or plasma, was thrown away. After three equal washes in regular saline, the red blood cells were quantified and reconstituted as a 40% (v/v) suspension in phosphate-buffered saline. This functioned as phospholipase A_2's substrate.

Assay Procedure

Test tubes containing 0.2 ml of $CaCl_2$ (2 mM), 0.2 ml of human red blood cells (HRBC), 0.2 ml of the crude enzyme preparation, and various doses of normal saline were used to incubate the extract and the reference medication for one

hour. $CaCl_2$, a free enzyme, and a suspension of human red blood cells were present in the control. Separately, 0.2 cc of boiling enzyme was applied to the blanks. For ten minutes, the reaction mixtures used for incubation were centrifuged at a speed of 3000g. The absorbance of the solutions was measured at 418 nm after 1.5 ml of the supernatant sample was diluted with 10 ml of regular saline. The control medication was prednisolone, a recognized inhibitor of phospholipase A_2. The relationship shown below was used to compute the percentages of maximum enzyme activity and inhibition:

2.6. Statistical analysis

Statistical Product and Service Solution (SPSS) version 22.0 was used to analyze the data using one-way analysis of variance (ANOVA), and the results were displayed as mean ±SD. The mean values of the results with $p < 0.05$ were considered significant.

3. Results

3.1. Percentage Yield of Crude extract of *Pleiocarpa mutica* leaves

Table 1 represents the percentage yield of the crude extract of the *Pleiocarpa mutica* leaves. The dried crude sample (1500g) of *Pleiocarpa mutica* leaf powder was extracted with absolute ethanol. After extraction, the percentage yield was calculated. The percentage yield was 2.76%.

Table 1 Percentage Yield of Crude extract of *Pleiocarpa mutica* leaves

Weight of pulverized sample (g)	Weight of crude extract (g)	Percentage yield (%)
1500	41.34	2.76

3.2. Quantitative Phytochemistry of Flavonoid-Rich Fraction of *Pleiocarpa mutica* leaves

Table 2 shows the results of the quantitative phytochemical analysis of the flavonoid-rich fraction of *Pleiocarpa mutica* leaves. The flavonoid-rich fraction of *Pleiocarpa mutica* leaf constituents at high concentrations include tannins (1204.82 ± 26.49mg/100 g), phenols (1183.56 ± 7.90mg/100 g), alkaloids (528.71 ± 13.86mg/100 g), and flavonoids (1531.70 ± 36.98mg/100 g). The flavonoid-rich fraction of *Pleiocarpa mutica* leaf constituents at low concentrations includes terpenoids (398.74 ± 3.57 mg/100 g) and steroids (0.14 ± 0.01mg/100g), while saponins (0.54 ± 0.01mg/100 g) were quantified in minimum amounts.

Table 2 Quantitative Phytochemistry of Flavonoid-Rich Fraction of *Pleiocarpa mutica* leaves

Phytochemical Constituents	Concentration (mg/100 g)
Tannins	1204.82 ± 26.49
Steroids	0.14 ± 0.01
Phenols	1183.56 ± 7.90
Alkaloids	528.71 ± 13.86
Flavonoids	1531.70 ± 36.98
Saponins	0.54 ± 0.01
Terpenoids	398.74 ± 3.57

Results are expressed in Means ± SD (n=3)

3.3. Effect of Flavonoid-Rich Fraction of *Pleiocarpa mutica* leaves on Phospholipase A_2 Activity

Table 3 shows the effect of the flavonoid-rich fraction of *Pleiocarpa mutica* leaves on phospholipase A_2 activity. The flavonoid-rich fraction of *Pleiocarpa mutica* significantly ($p < 0.05$) inhibited PLA_2 at 1.0 mg/ml in a concentration-dependent manner when compared to the other concentration. Prednisolone, a standard anti-inflammatory drug, followed a similar trend with increasing doses of prednisolone.

Table 3 Effect of Flavonoid-Rich Fraction of *Pleiocarpa mutica* leaves on Phospholipase A_2 Activity

Concentration(mg/ml)	Percentage inhibition of enzyme (Phospholipase A_2) activity (%)	
	Flavonoid-rich fraction	**Standard drug (Prednisolone)**
0.2	47.58 ± 0.68^a	44.66 ± 0.45^a
0,4	52.95 ± 1.35^b	57.03 ± 0.39^b
0.6	56.97 ± 0.93^c	68.65 ± 0.79^c
0.8	59.66 ± 1.01^d	71.47 ± 0.72^d
1.0	63.61 ± 1.27^e	74.43 ± 0.43^e

n=3; Result expressed as mean ± S.D.; Mean values with different lowercase letters as superscripts across the groups are considered significant at $p < 0.05$.

4. Discussion

Plants continue to be a reliable source of lead compounds for the development of innovative treatments, as they have historically been for active pharmaceutical ingredients [11]. The therapeutic efficacy of flavonoids against a broad spectrum of pathological conditions and disorders has been unequivocally shown. In this work, phospholipase A_2 activity was used as an in vitro model to investigate the anti-inflammatory characteristics of the flavonoid-rich fraction of *Pleiocarpa mutica* leaves. The conclusion is that this herb possesses potent anti-inflammatory qualities.

According to the results of Enechi *et al.* (2016) [12], who utilized an ethanol extract of *Pleiocarpa mutica* leaves, the phytochemical composition of the plant's leaves was assessed; the results showed a considerable amount of tannins, alkaloids, terpenoids, and saponins in addition to a high flavonoid content. According to the 2017 investigation by Omoyemi *et al.* [13], there is a proven abundance of phytochemicals in the plant fraction.

Different dosages of the flavonoid-rich fraction of *Pleiocarpa mutica* leaves significantly ($p < 0.05$) suppressed phospholipase A_2 activity, according to an investigation of the effect on the enzyme's activity. From 0.2 to 1.0 mg/ml, the flavonoid-rich fraction of *Pleiocarpa mutica* leaves demonstrated a concentration-dependent and significant ($p < 0.05$) inhibition of PLA_2 activity. This was shown by reduced absorbance, which occurred when the inhibitor prevented PLA_2 from functioning on the erythrocyte membrane, and as a result, less hemoglobin leaked, which absorbs at 418 nm, the maximum wavelength. This inhibitory impact might be brought on by the presence of phytochemical components such as flavonoids and alkaloids in the plant fraction [14].

5. Conclusion

It has been shown that flavonoids, found in plants, are important compounds that can have a positive effect on human health when included in a healthy diet. These natural products have strong antioxidant properties, which contribute to their overall biological activity.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

Statement of Informed consent

Informed consent was obtained from all individual participants in the study

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