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Quantitative and qualitative phytochemical analysis of ethanol leaf extract of *Pleiocarpa mutica*

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Abstract

This research project examined *Pleiocarpa mutica* leaf extract, extracted from ethanol using both quantitative and qualitative phytochemical analysis. Sourced is at Ugbene-Ajima in the Uzo-Uwani Local Government Area of Enugu State, Nigeria, where fresh *Pleiocarpa mutica* leaves were collected. After been gathered, the newly sprouted *P. mutica* leaves were cleaned. We then shade-dried the leaves until they were crispy, rotating them frequently to prevent them from rotting. A mechanical grinder was utilized to reduce the dried leaves to a powdered state while a maceration flask was used to macerate 1.5 kg of the known weight of the ground leaves in 10 liters of 100% ethanol. In using a muslin cloth, the mixture was filtered into a flask with a flat bottom after being left for 72 hours with irregular stirring. Quantitative phytochemical analysis procedures identified the specific concentration of a certain chemical constituent present in a plant sample using methods such as mass spectrometry, chromatography, and spectrophotometry. This makes it possible to determine the concentration of a component in the plant material. Qualitative phytochemical analysis techniques focus on identifying the presence or absence of several chemical groups in a plant sample. Discovering the existence of substances such as alkaloids, phenols, terpenoids, and flavonoids often requires a range of chemical analyses or the use of specific reagents. Thus, the standard quantitative and qualitative phytochemical analysis technique were used to investigate the chemical composition of plants and identify bioactive compounds that may offer nutritional, medicinal, or pharmacological advantages. Hence, it has been shown that the ethanol leaves extract of *P. mutica* contains various concentrations of phytochemical constituents, which might be responsible for its biological activities.

Keywords: Qualitative; Quantitative; Phytochemistry; Analysis; *Pleiocarpa mutica*; Ethanol

1. Introduction

Inflammation is believed to be a component of the intricate biological reaction of vascular tissues, brought on by pathogens, injured cells, or irritants [1]. Inflammation has also been associated with pathogens, injured cells, or irritants that are detrimental stimuli to bodily tissues [2]. Nevertheless, the first enzyme in the arachidonic acid cascade, phospholipase A_2 (PLA_2), is essential for inflammatory reactions. It is therefore, a class of enzymes that selectively identify the sn-2 acyl bond of phospholipids attached to membranes and hydrolyze the link to release lysophospholipids and arachidonic acid. The regulatory functions of PLA_2 are phosphorylation, and calcium concentrations. The venoms of snakes, bees, and bacteria, as well as the cells and secretions of mammals, all include the enzyme. Currently, PLA_2 active enzymes have been found in at least 19 different mammalian organs.

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Based on their enzymatic and structural characteristics, PLA_2s in mammals are classified into three groups: secretory ($sPLA_2$), cytosolic ($cPLA_2$), and $Ca2^+$ independent [3]. Conventional anti-inflammatory drugs such as aspirin and indomethacin are used to curtail inflammation. However, the majority of these drugs are associated with adverse drug reactions, which include gastrointestinal disorders, hepatotoxicity, and renal toxicity. Hence, plant-based drugs are preferably considered to be the major point of focus because they are easily available, less expensive, have multiple efficacy, and have minimal side effects [4]. Both primary and secondary metabolites are found in plants; the primary metabolites consist of certain amino acids, lactic acid, and ethanol. Due to their function in fundamental cell metabolism, these chemicals are frequently concentrated in seeds and vegetative storage organs in higher plants, where they are essential for physiological development. Microorganisms need basic metabolites in order to grow properly. Many therapeutic herbs have been found to have anti-inflammatory properties throughout time, often with negligible or no negative effects. The foundation of the alcohol business is ethanol fermentation, which is closely related to alcoholic beverages. The "hot spot" of bioenergy and a key component of the answer to the energy dilemma is the generation of ethanol from lignocellulosic biomass [5].

2. Materials and methods

2.1. Materials

2.1.1. Authentication of Plant Material

Pleiocarpa mutica fresh leaves were gathered 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.

2.1.2. Equipment

The equipments used in this study include the following:

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

2.1.3. Chemicals and reagents

The analytical-grade chemicals employed in this investigation were all products of 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. Extraction Procedure

Fresh *P. mutica* leaves were collected, cleaned to remove dirt, and then shade-dried until crispy, turning them occasionally to prevent them from decomposing. The dried leaves were then ground into a powder using a mechanical grinder, and a known weight of the ground leaves (1.5 kg) was macerated in 10 L of absolute ethanol using a maceration

flask. The mixture was left for 72 hours with periodic stirring and then filtered again using a muslin cloth to remove fine residues. The filtrate was then concentrated using a rotary evaporator at 45°C to yield the crude ethanol extract, which was then stored in a labeled, sterile screw-capped bottle.

2.2.2. Preparation of Ethanol Leaf extract of Pleiocarpa mutica leaves

The process of extracting *P. mutica* leaf extract using ethanol was done in accordance with Chu *et al.* (2002) [6]. In a tiny flask, a determined amount of the crude extract was dissolved in 20 milliliters of 10% H2SO4, and it was then hydrolyzed by heating it to 100°C for 30 minutes in a water bath. To enable the flavonoid aglycones to precipitate, the mixture was chilled for fifteen minutes. After cooling the mixture, it was filtered, and the filtrate, a mixture of flavonoids and aglycones, was dissolved in 50 milliliters of warm 95% ethanol (50°C). The final mixture was once again filtered into a 100 mL volumetric flask that had been filled to the brim with 95% ethanol. The obtained filtrate was dried out by using a rotary evaporator.

2.2.3. Phytochemical analysis of Pleiocarpa mutica leaves fraction

As demonstrated by (Harborne, 1998; Evans and Trease, 2002) [7, 8], a standard conventional technique was used to perform a quantitative phytochemical analysis of *P. mutica* ethanol leaf extract. This is how the concentrations of the different phytochemical ingredients were calculated:

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

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

2.3. Quantitative phytochemical analysis of the ethanol leaf extract of *Pleiocarpa mutica*

Quantitative phytochemical analysis measures the concentration of particular substances in plant samples. This was accomplished using a variety of techniques, including mass spectrometry, spectrophotometric approaches, and chromatographic procedures (such as gas chromatography and high-performance liquid chromatography).

High Performance Liquid Chromatography (HPLC): High Performance Liquid Chromatography is one of the most widely used methods for the analysis of phytochemicals, as it provides high sensitivity and specificity. This involves the separation of compounds in a liquid chromatography column based on their chemical properties. Detection can be achieved with UV, fluorescence, or mass spectrometry [9].

Gas Chromatography (GC): Gas chromatography is a chromatographic technique that can be used for the analysis of volatile phytochemicals. It involves the separation of compounds in a gas chromatography column based on their volatility. Detection can be achieved with a flame ionization detector (FID) or mass spectrometry [10].

Spectrophotometric Methods: Spectrophotometric methods involve the measurement of the absorbance or emission of light by phytochemicals at specific wavelengths. These methods are often used for the quantification of compounds with characteristic UV-Vis spectra, such as flavonoids and polyphenols [11].

Mass Spectrometry: Mass spectrometry is a technique for the identification and quantification of phytochemicals based on their mass-to-charge ratio. It can provide high sensitivity and specificity, making it suitable for the analysis of complex mixtures [12].

2.3.1. Quantitative Determination of Flavonoids Content

The 0.5 g sample was filtered after being macerated in 20 cc of ethyl acetate. 5 ml of 28% diluted ammonia was added to 5 ml of the filtrate. After shaking the mixture, the top layer was separated, and its absorbance at 490 nm was measured.

2.3.2. Quantitative Determination of Terpenoids Content

The 0.5 g sample was filtered after being macerated in 20 ml of ethanol. A 1 milliliter solution of 5% phosphomolybdic acid was added to 1 milliliter of the filtrate. One milliliter of concentrated sulfuric acid was added gradually and combined. After allowing the mixture to stand for thirty minutes, two milliliters of ethanol were added. At 700 nm, the absorbance was measured.

2.3.3. Quantitative Determination of Steroids Content

The 0.5 g sample was filtered after being macerated in 20 ml of ethanol. After adding 2 ml of color reagent to 2 ml of the filtrate, the mixture was allowed to stand for 30 minutes. At 550 nm, the absorbance was measured.

2.3.4. Quantitative Determination of Saponins Content

After being macerated in 20 ml of petroleum ether, the sample (0.5 g) was decanted into a beaker. Using 10 milliliters of petroleum ether, the extract was once again cleaned. Following their combination, the filtrates were dried by evaporation. After adding two milliliters of color reagent to a test tube and transferring two milliliters of the residue there, six milliliters of ethanol were used to dissolve it. At 550 nm, the absorbance was measured after being let stand for 30 minutes.

2.3.5. Quantitative Determination of Alkaloids Content

The sample (0.5 g) was filtered after being macerated in 20 ml of ethanol and 20% sulphuric acid (1:1). 5 milliliters of 60% sulfuric acid were applied to one milliliter of the filtrate. 5 cc of 0.5% formaldehyde in 60% sulphuric acid was added and properly mixed after 5 minutes. The absorbance at 565 nm was then measured after letting this stand for three hours.

2.3.6. Quantitative Determination of Tannins Content

20 milliliters of distilled water were used to macerate the 0.5-gram sample before it was filtered. Five milliliters of the filtrate were combined with three milliliters of 0.1 N ferric chloride in 0.1 N hydrochloric acid and three milliliters of 0.0008 M potassium ferricyanide. We measured the absorbance at 720 nm.

2.3.7. Quantitative Determination of Phenols Content

A 20 ml 80% ethanol maceration was performed on the 0.5g sample before it was filtered. A solution of Folin-Ciocalteau's reagent (0.5 ml) was added to 5 ml of the filtrate and left to stand for 24 hours. 2 cc of 20% sodium carbonate was added after that. A 650 nm measurement was made of the absorbance.

2.4. Qualitative phytochemical analysis of the ethanol leaf extract of *Pleiocarpa mutica*

Qualitative analysis of phytochemicals involves the identification of various compounds present in plant materials. There are several methods that can be used for this purpose; they include:

Thin-layer chromatography (TLC): Thin-layer chromatography is a cost-effective method used for the separation and identification of phytochemical compounds in plant extracts. Various mobile phases and detection reagents can be used to visualize the separated compounds. Recent research by Pal *et al.* (2019) [13] demonstrated the use of TLC for analyzing phytochemicals in different medicinal plants.

Nuclear magnetic resonance (NMR) spectroscopy: Nuclear magnetic resonance spectroscopy is a non-destructive analytical technique that can be used for the structural elucidation of phytochemicals in plant extracts. It provides detailed information about the chemical structure and connectivity of compounds based on their magnetic properties. Nuclear magnetic resonance spectroscopy is particularly useful for the identification of complex molecules such as flavonoids, alkaloids, and terpenoids [14].

Fourier transform infrared spectroscopy (FTIR): Fourier transform infrared spectroscopy is a non-destructive analytical technique used for the identification of functional groups present in plant compounds. It offers valuable information about the chemical composition of plant extracts based on their unique infrared spectra. Recent research by Luthra *et al*. (2020) [15] employed Fourier transform infrared spectroscopy for characterizing phytochemicals in medicinal plants.

Gas chromatography-mass spectrometry (GC-MS): Gas chromatography-mass spectrometry is a powerful technique for the qualitative analysis of phytochemicals in plant extracts. It separates compounds based on their volatility and then identifies them using mass spectrometry. Gas chromatography-mass spectrometry is able to detect even trace amounts of phytochemicals and can provide information on their chemical structures [16].

High performance liquid chromatography (HPLC): High Performance Liquid Chromatography is another powerful analytical technique that can be used for qualitative analysis of phytochemicals. High Performance Liquid Chromatography separates and identifies compounds in a mixture based on their interactions with a liquid mobile

phase and a solid stationary phase. This method is highly sensitive and can detect a wide range of phytochemicals in plant extracts [17].

2.5. Statistical analysis

Using Statistical Product and Service Solution (SPSS) version 22.0, one-way analysis of variance (ANOVA) was used to examine the collected data, which were then reported as mean ±SD. The results were considered significant if the mean values were $p<0.05$.

3. Results

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

Table 2 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	27.6

3.2. Quantitative Phytochemistry of Ethanol leaf extract of *Pleiocarpa mutica* leaves

Table 2 Quantitative Phytochemistry of Ethanol leaf extract of *Pleiocarpa mutica* leaves

Phytochemical Constituents	Concentration (mg/100 g)
Tannins	1204.82 ± 26.49
Steroids	0.14 ± 0.01
Phenols	1183.560 ± 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)

Table 3 Qualitative Phytochemistry of Ethanol leaf extract of *Pleiocarpa mutica l*eaves

Phytochemical Constituents	Present	Absent
Tannins	+	
Steroids	+	
Phenols	+	
Alkaloids	+	
Flavonoids	+	
Saponins	+	
Terpenoids	+	

Table 3 shows the results of the quantitative phytochemical analysis of the ethanol leaf extract of *Pleiocarpa mutica* leaves. The ethanol leaf extract 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) was quantified in minimum amounts.

4. Discussion

Plants naturally produce substances called phytochemicals through their primary and secondary metabolisms. They are able to shield the plant from predators and diseases. Phytochemical analysis is useful in screening plant sources for a variety of purposes, including traditional medicinal formulations, antibacterial action, antioxidant activity, and possible medical usage.

Tables 2 and 3 demonstrate the quantitative and qualitative phytochemical analysis of *P. mutica* using an ethanolic leaf extract. The plant includes various levels of tannins, steroids, terpenoids, phenols, glycosides, flavonoids, alkaloids, and saponins. To fully assess plant components, a combination of quantitative and qualitative phytochemical examination techniques is required. While qualitative techniques like thin-layer chromatography, high-performance liquid chromatography, and mass spectrometry are useful for identifying unknown substances, quantitative techniques like spectrophotometry and chromatography offer precise measurements of particular phytochemicals. Researchers can learn more about the chemical makeup of plant extracts and their possible health advantages by combining the two types of studies. Overall, the integration of quantitative and qualitative techniques in phytochemical analysis is crucial for advancing the field of natural product research [18].

Numerous investigations have indicated that the phytochemical components found in medicinal plants facilitate the process of wound healing [19]. The potential advantages of *P. mutica* for healing could stem from a range of documented actions, including anti-inflammatory properties [20].

5. Conclusion

It has been shown that the ethanol leaf extract of *P. mutica* contains various phytochemical constituents, which might be responsible for its biological activities. The findings suggest that, if used, the herb may provide anti-inflammatory properties.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of Interest to be disclosed.

Statement of informed consent

Informed consent was obtained from all individual participants in the study.

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