# Exploring the Pharmacognostic Characteristics, Antioxidant Potential, and Anticholinesterase Activity of *Piliostigmathonningii* (Schum.) Milne-Redh.: Implications for Neuroprotection and Cognitive Health


## Abstract

**Background**: *Piliostigmathonningii* is a plant commonly known as 'camel's foot tree', which has been used in traditional medicine for various conditions such as ulcers, wounds, arthritis, malaria fever, toothache, sore throat, dysentery, cough, chills, and gingivitis. Biological activities such as anti-inflammatory and analgesic activities have been reported. These activities may contribute to its protection against neurodegenerative disorders, such as Alzheimer's disease (AD). Therefore, it is worth investigating the anti-oxidant and anti-acetylcholinesterase activities as a novel approach for the management of AD and exploring the pharmacognostic characteristics of the leaves of the plant.

**Methodology**: Physicochemical studies, including total Ash value and moisture content, were assessed using the powdered leaf sample. Leaf microscopy was conducted to examine the epidermal layer of the leaves. Anti-oxidant assays (Total phenol, total flavonoid and DPPH free radical scavenging activity), as well as acetylcholinesterase (AchE) inhibitionassay was carried out on the methanolic leaf extract of the plant.

**Results:**The powdered leaves of *P. thonningii*containeda total ash of 5.00 ± 0.00 and 7.74 ± 0.01moisture. The alcohol- and water-soluble extractive values were 8.51 ± 0.24 and 9.77 ± 0.31, respectively. Leaf microscopic analysis showed the presenceof polygonal cell walls, unicellular multiseriate trichomes, trichome base, xylem vessels, rosette-type calcium oxalate crystals, parenchyma, and collenchyma cells. The methanolic leaf extract exhibited antioxidant capacity, inhibiting DPPH in a dose-dependent manner, with total phenolics and flavonoids of 53.74 ± 1.59 and 42.51 ± 0.38, respectively. The methanolic extract of *P. thonningii* in this study showed an AchE inhibition of between 5.89 % at 10 mg/ml to 16.56 % at 20 mg/ml

**Conclusion:** findings fromthisstudy could enrichthe existing of knowledge on *P.thonningii* and its pharmacological properties, potentially unveiling novel therapeutic applications.


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## Introduction

Since ancient times, herbal medicines have been utilized to treat a variety of disorders across the world (Mohanta *et al*., 2003). There has recently been a surge in interest in phytomedicines, which are thought to be safer and more friendly to the human body than expensive synthetic pharmaceuticals that are inevitably linked with side effects (Patnia and Saha, 2012). According to the World Health Organization's data, approximately 80% of the global population relies on traditional medicines for fundamental healthcare needs (Kamboj, 2000). Medicinal plants are very effective against variety of ailments because of their pharmacological activity which is based on their elemental constituents. Phytochemicals, such as primary and secondary metabolites, are made up of diverse combinations of major, minor, and trace elements, and they have a role in the treatment and prevention of many illnesses (Pawar and Kamble, 2016).

Alzheimer's disease (AD), a significant public health concern, is a gradually advancing degenerative condition characterized by the gradual demise of brain cells, resulting in the decline of both memory and cognitive abilities. Besides, from the buildup of protein in some areas of the brain, cholinergic deficiency, neuroinflammation, and oxidative stress have all been linked to the pathophysiology of neurodegenerative disorders. The use of acetylcholinesterase enzyme inhibitors (AChEIs) has been considered as a promising treatment option for AD.

Current medications for the treatment of AD have been associated with several side effects and numerous attempts have been made to identify natural AChEIs from plants with fewer side effects. The primary oxygen free radicals produced by activated neutrophils and macrophages, such as superoxide anion, hydroxyl and peroxyl radicals, can cause significant illnesses such as neurological disorders, cancer, and atherosclerosis. Cellular damage, particularly in organs such as the brain, is caused by oxidative stress. According to certain research, an Alzheimer's patient's brain is subjected to oxidative stress due to an imbalance of calcium ions inside their neurons and mitochondria (Emilien *et al*., 2000; Tabet, 2006). Currently, several plants with anti-oxidant properties have been identified as having AChE inhibitory bioactivity. Polyphenols are rich sources of natural antioxidants that protect our bodies from disease by inhibiting the growth of free radicals and preventing lipid oxidative rancidity. As a result, many studies are being conducted on these ethnomedicinal plant resources in search of potent natural antioxidants that can replace synthetic medications.

*Piliostigmathonningii*Schum is a perennial plant that grows widely across most sub-Saharan African countries.It is commonly called 'camel's foot tree' and locally known as 'Okpoatu', 'Abefe' and 'Kalgo'(in Igbo, Yoruba and Hausa languages of Nigeria respectively). The infusion prepared from the leaves and bark of the *P.thonningii* has historically been employed in traditional medicine for the management of conditions such as ulcers, wounds, arthritis, malaria fever, toothache, sore throat, dysentery, cough, chills and gingivitis. Conversely, roots and twigs find application in alleviating dysentery, wound infections, respiratory afflictions, fever, skin-related maladies and incidents of snake bites in therapeutic contexts. Analgesic, anti-lipidemic, antibacterial, anthelminthic, and anti-inflammatory activities have all been documented for the crude extract of *P. thonningii* (Igbe*et al*., 2012; Ighodaro *et al*., 2012;Akinpelu and Obuotor, 2000; Asuzu*et al*., 1998). These biological activities may

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contribute to its protection against neurodegenerative disorders; therefore, it is thought to be worthy to investigate the anti-oxidant and anti-acetylcholinesterase activities as a novel approach for the management and explore the pharmacognostic characteristics of the leaves of the plant. Pharmacognostic studies play a crucial role in the authentication and establishment of the quality benchmarks for raw botanical materials, facilitating the discernment of impurities within botanical blends.

**Materials and method**

The leaves of *P. thonningii*were collected from the wild at Idu industrial Area, close to National Institute for Pharmaceutical Research and Development. It was subsequently air-dried and later pulverized.

**Sample extraction**

A mechanical blender was employed to grind the dried leaves into fine powder, followed by extraction of a 100g sample by maceration. The extract was dried using a rotary evaporator and were stored in the refrigerator for further use.

**Physicochemical studies**

Physicochemical attributes including total Ash value and moisture content were assessed in the desiccated and powdered leaf sample (Chandel *et al*., 2011; Rajani and Kanaki, 2008) also water-soluble extractive values were determined (Mukharjee, 2002) and (WHO, 1998).

**Leaf microscopy**

***Leaf epidermis***: In this scientific investigation, we conducted microscopic examination of the leaf's epidermal layer. The leaves from the plant were precisely sectioned at their midpoint. Subsequently, they were immersed in concentrated nitric acid for approximately 24 hours. The detection of air bubbles signified the preparedness for epidermis separation. Using delicate forceps and a dissecting needle, each leaf fragment was transferred to a sterile petri dish filled with distilled water. Here, the upper and lower epidermal layers were meticulously separated, stained using safranin, and eventually affixed onto a glass slide with glycerol.

***Powdered leaf sample***: The powdered leaf sample was cleared in hypochlorite solution (to remove the chlorophyll) for 24hrs. the prepared specimen was positioned onto a glass slide, overlaid with a coverslip, and subsequently observed through a microscope at a magnification of X40. Qualitative features such as Trichomes, Parenchyma cells, epidermal cells were observed.

**Antioxidant assays**

***Total phenolic content***

The quantification of the overall phenolic content within the methanol extract of *P. thonningii* was conducted in accordance with the procedure outlined by (Singleton and Rossi, 1965). 1 mL of methanol leaf extract was combined with equal volumes of Folin-Ciocalteu reagent and 35% saturated sodium carbonate, resulting in a final mixture. The solution was diluted to a factor of 10 using distilled water and then subjected to a 90-minute incubation at room temperature in the absence of light. Absorbance measurements were recorded at a wavelength of 725nm using a blank reference. Gallic acid was employed as the calibration standard. The

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quantification of the overall phenolic content within the methanol extract was presented in milligrams of Gallic acid equivalents (GAE).

***Estimation of total flavonoid content***

The total flavonoid content of the methanol extract of *P. thonningii* was analyzed using the method described by (Zhishen*et al.*, 1999). A 0.25 ml aliquot of the methanol extract underwent dilution with 1.25 mLof distilled water. Subsequently, 75 µL of a 5% sodium nitrite solution was introduced, and the mixture was allowed to incubate for 6 minutes. Following this incubation period, 150 µL of a 10% $AlCl_3.H_2O$ solution was added. The absorbance of the resulting solution was promptly measured at 510 nm against a blank. The quantification of flavonoid content in the methanol extract was expressed in milligrams of quercetin equivalents (QE).

***DPPH free radical scavenging activity***

The method of Patel Rajesh and Patel Natvar (2011) was used to determine the DPPH free radical scavenging activity of methanol extract of *P. thonningii*. A quantity of 4.3 mg of DPPH was dissolved in 3.3 mL of methanol under conditions that prevented exposure to light. Solutions for testing were prepared using concentrations of 15.75, 31.125, 62.5, 125, 250, and 500 µg/mL from the methanol extract. Each test solution received an addition of 150 µL of DPPH at the respective concentration, followed by dilution with methanol to reach a final volume of 3 mL. In order to establish a control, 150 µL of DPPH solution was combined with 3 mL of methanol. Following a 30-minute incubation period at room temperature in darkness, the absorbance was measured at 517 nm. As a reference standard, ascorbic acid was employed, while methanol served as the blank solution.

The inhibitory percentage value was calculated using the following formula.

$$\frac{\text{Percentage inhibition} = \text{absorbance of control} - \text{absorbance of test sample}}{\text{absorbance of control}}$$

**Animals**

In this study, albino rats sourced from the Animal Facility Centre at the National Institute for Pharmaceutical Research and Development (NIPRD) in Abuja, Nigeria, were employed. The rats were provided with a conventional laboratory diet and had unrestricted access to water. They were housed in controlled laboratory environments with a temperature maintained at 22 ± 1°C, relative humidity at 14 ± 1%, and a light-dark cycle of 12 hours each. All procedures related to the care and management of the experimental animals strictly adhered to the guidelines outlined in the 'NIH Guide for the Care and Use of Laboratory Animals'.

**Acetylcholinesterase inhibition**

The study employed the modified electrometric technique, as outlined by Mohammad *et al.*, (2007) to assess erythrocyte cholinesterase activities in Wister rats. In a standard assay, a 10 mL beaker was used, containing 3 mL of distilled water, 0.2 mL of whole blood, and 3 mL of pH 8.1 buffer solution (for drug tests, 2.9 mL of buffer solution and 0.1 mL of the test drug were employed). The initial pH of the mixture, denoted as pH1, was determined using a pH meter. Subsequently, 0.1 mL of a 7.5% aqueous solution of acetylthiocholine iodide (BDH, UK) was introduced into the mixture. The reaction mixture was then incubated at 37°C for a

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duration of 30 minutes. Following the incubation, the pH of the reaction mixture (referred to as pH2) was measured.

And the percentage inhibition was calculated as:

$$\% \text{ ChE inhibition} = \frac{[\text{ChE blank} - \text{ChE test}]}{\text{ChE blank}} \times 100$$

The blank was without acetylthiocholine iodide. The pH 8.1 buffer solution was prepared by dissolving 1.237 grams of sodium barbital (BDH, UK), 0.63 grams of potassium dihydrogen phosphate (Merck, Germany), and 35.07 grams of sodium chloride (BDH, UK) in one litre of distilled water.

**Statistical analysis**

The analysis of the data was conducted utilizing Microsoft excel 2021 version (% DPPH inhibition) and GraphPad Prism version 6.0, with the data being presented in terms of cholinesterase activities and the percentage of cholinesterase inhibition. A graph of percentage activity was plotted against test concentration and the $IC_{50}$ was calculated.

**Results**

**Table 1.:** Physicochemical evaluation of *P. thonningii* leaf powder

| SN | Physicochemical Parameter | Mean | SD |
| --- | --- | --- | --- |
| 1. | Total Ash | 5 | ±0.00 |
| 2. | Moisture content | 7.736 | ±0.01 |
| 3. | Alcohol soluble extractives | 8.505 | ±0.24 |
| 4. | Water-soluble extractives | 9.7733 | ±0.31 |

**Table 2.**: Chemomicroscopy of *P. thonningii* leaf powder

| Tests | Inferences |
| --- | --- |
| Lignin | - |
| Cellulose | + |
| Tannin | + |
| Starch | + |
| Oxalate crystals | + |
| Oil | + |
| Mucilage | + |

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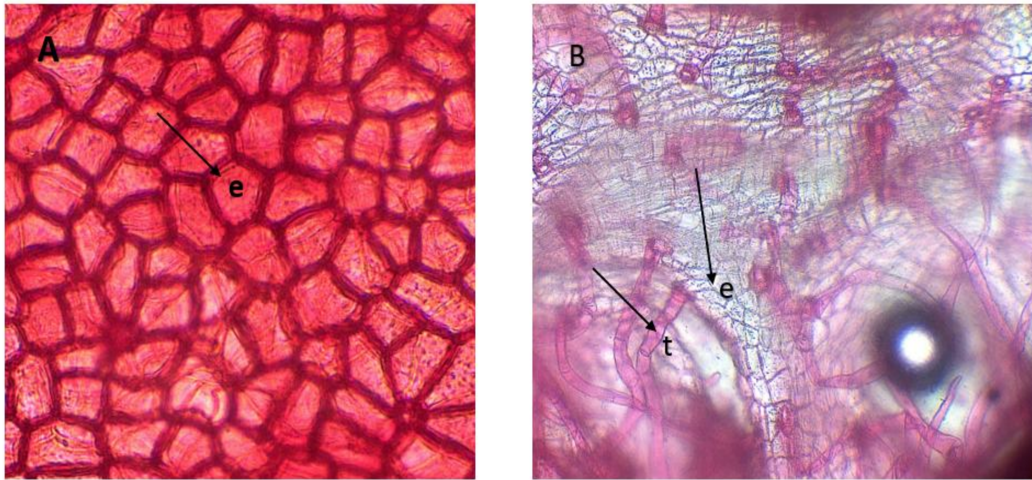


**Figure 1.**: Microscopic features of leaf epidermis of *P. thonningii*showing A – lower epidermal surface; polygonal/regular epidermal cells (e), B- upper epidermal surface; unicellular multiseriate trichome (t) and epidermal cell (e). Magnification x 400

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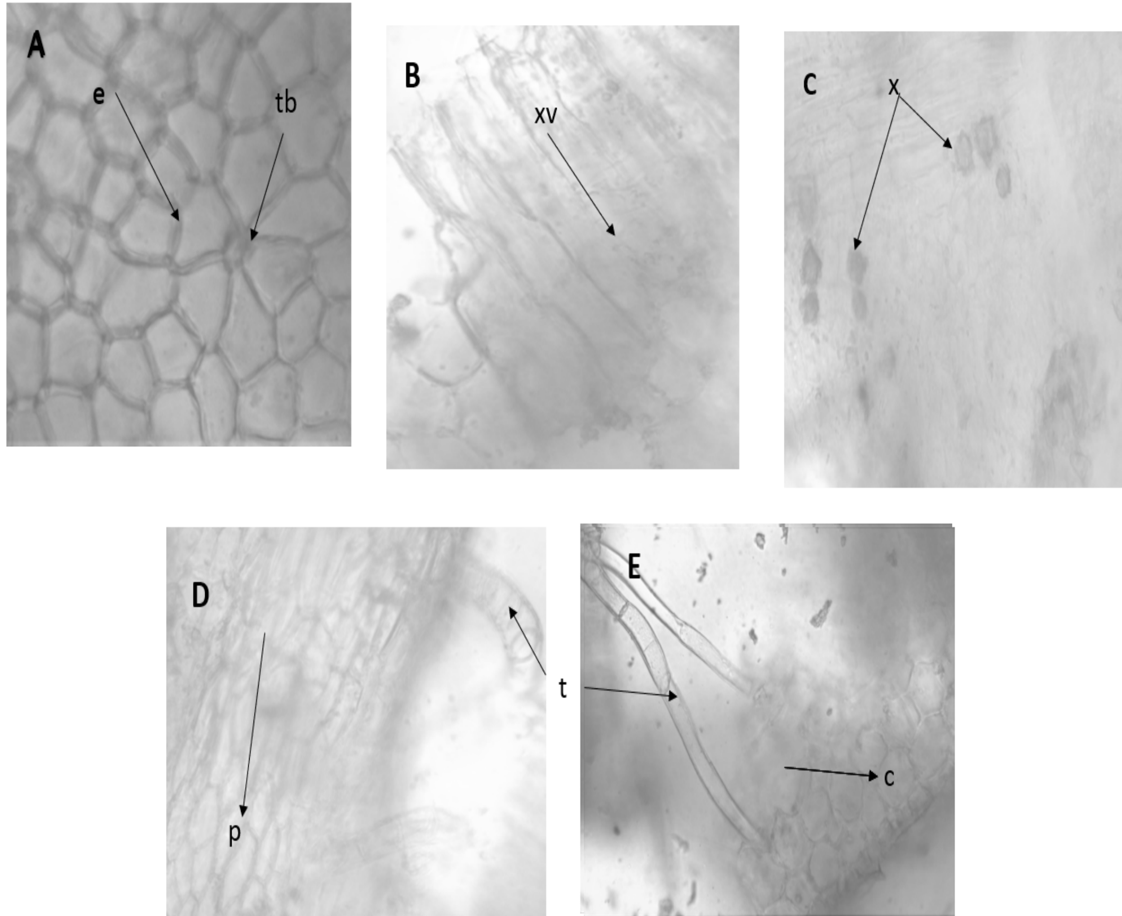


**Figure 2.:** Microscopic features of leaf powder of *P. thonningii*showing A – polygonal/regular epidermal cells (e) with trichome base (tb), B -xylem vessels (xv), C – rosette type calcium oxalate crystal (x), D – parenchyma cells (p), E – unicellular multiseriate trichome (t) and collenchyma cells(c). Magnification x 400

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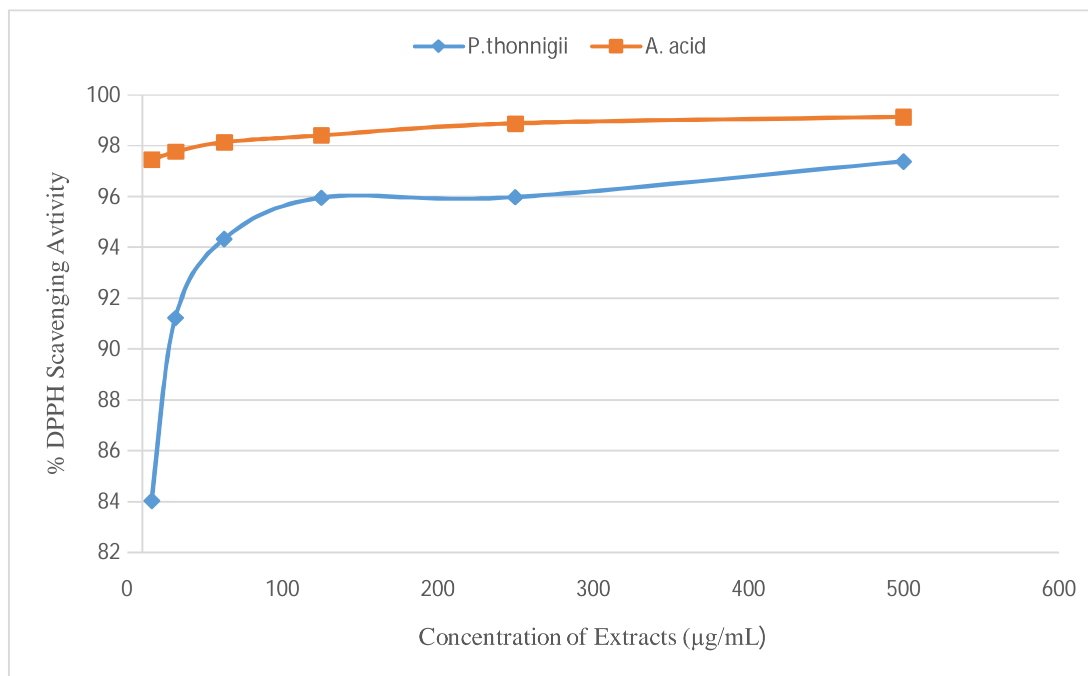


**Figure 3.**. Inhibition (%) of DPPH Radical Scavenging in the presence of different Concentrations of Methanol extract of *P. thonningii* against the Ascorbic acid standard

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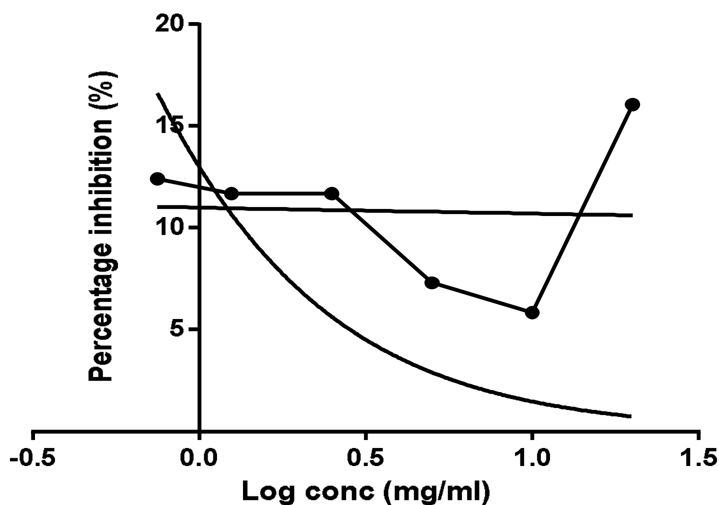
**Table 3.:** Total Phenolics and Total Flavonoid content of *P. thonningii*

| SN | Experiment | Value |
|---|---|---|
| **1.** | Total Phenolic Content (mg/GAE/g) | 53.74 ±1.59 |
| **2.** | Total Flavonoid Content (mg/QE/g) | 42.51 ± 0.38 |

**Table 4.**: Determination of Percentage cholinesterase Inhibition of *P.thonningii*

| Concentration of *P.thonnigii* | pH 1 | pH 2 | pH 1 – pH 2 | Average | % Inhibition |
|---|---|---|---|---|---|
| **20 mg/ml** | 7.42 | 7.03 | 0.39 | 0.42 | 16.05839 |
| | 7.43 | 6.98 | 0.45 | | |
| **10 mg/ml** | 7.6 | 7.11 | 0.49 | 0.49 | 5.839416 |
| | 7.55 | 7.06 | 0.49 | | |
| **5 mg/ml** | 7.65 | 7.18 | 0.47 | 0.48 | 7.29927 |
| | 7.65 | 7.16 | 0.49 | | |
| **2.5 mg/ml** | 7.67 | 7.39 | 0.28 | 0.45 | 11.67883 |
| | 7.8 | 7.18 | 0.62 | | |
| **1.25 mg/ml** | 7.5 | 7.08 | 0.42 | 0.455 | 11.67883 |
| | 7.66 | 7.17 | 0.49 | | |
| **0.75 mg/ml** | 7.79 | 7.16 | 0.63 | 0.445 | 12.40876 |
| | 7.65 | 7.39 | 0.26 | | |
| **Normal saline** | 7.86 | 7.25 | 0.61 | 0.685 | |
| | 7.94 | 7.15 | 0.76 | | |




$IC_{50} = 0.1493$

**Figure 4**: Graph of Percentage inhibition over log concentration of *P. thonningii*. $IC_{50}$ determined to be 0.1493

**Table 5.**: Determination of Percentage cholinesterase Inhibition of Eserine

| Concentration of Eserine | pH 1 | pH 2 | pH 1 – pH 2 | Average | Percentage Inhibition |
|---|---|---|---|---|---|
| 0.04 mg/ml | 7.7 | 7.57 | 0.13 | 0.15 | 55.47445 |
| | 7.72 | 7.55 | 0.17 | | |
| 0.02mg/ml | 7.75 | 7.49 | 0.26 | 0.27 | 37.9562 |
| | 7.82 | 7.54 | 0.28 | | |
| 0.01mg/ml | 7.84 | 7.54 | 0.3 | 0.285 | 35.76642 |
| | 7.9 | 7.63 | 0.27 | | |
| 0.005 mg/ml | 7.89 | 7.53 | 0.36 | 0.34 | 27.73723 |
| | 7.88 | 7.56 | 0.32 | | |
| 0.0025 mg/nl | 7.93 | 7.58 | 0.35 | 0.34 | 27.73723 |
| | 7.92 | 7.59 | 0.33 | | |
| 0.00125 mg/ml | 7.9 | 7.61 | 0.29 | 0.305 | 32.84672 |
| | 7.91 | 7.59 | 0.32 | | |
| Normal saline | 7.86 | 7.25 | 0.61 | 0.685 | |
| | 7.94 | 7.15 | 0.76 | | |

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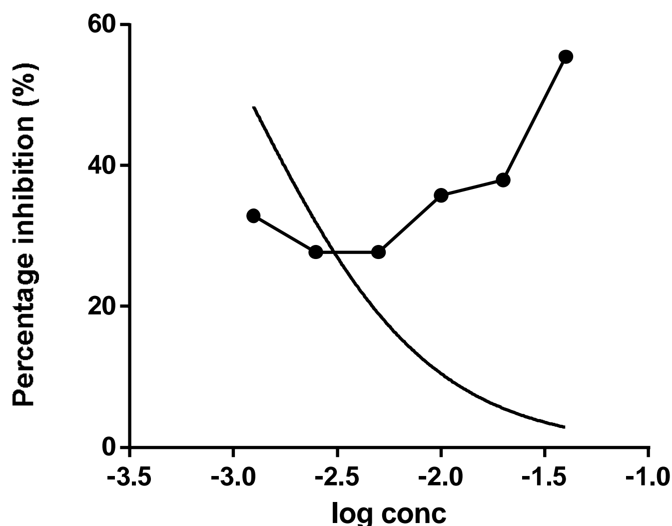


$IC_{50} = 0.001171$

**Figure 5**: Graph of % inhibition over log concentration of *P. thonningii* ($IC_{50}$ determined to be 0.001171).

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**Discussion**

Establishing the quality, identity, and purity of crude pharmaceuticals requires a thorough study of pharmacognostic and proximate characteristics. Every crude medication that is to be included in an herbal pharmacopoeia must meet certain pharmacognostic requirements. In the processing, preservation, and storage of medicinal plants, moisture content is one of the most crucial and often utilized measures (African Pharmacopoeia, 1986). Establishing the physiological and non-physiological characteristics, determining the possibility of microbial growth or contamination, and determining the presence of contaminants all depend on the analysis of physicochemical parameters like moisture content and ash value (Pandey et al., 2015). When compared to the limit for water content (8 - 14%) for vegetable medications (African Pharmacopoeia, 1986), the plant sample had a moisture content of 7.73±0.01% (**Table 1.0**), which indicates an acceptable moisture content limit. In addition to helping establish the purity of crude pharmaceuticals, ash values and extractive values are trustworthy techniques for spotting adulteration. Ash from medicinal plants is the total of the residue that remains after all moisture has been eliminated and all organic material, including fat, protein, carbs, vitamins, and organic acids, has been burned at a temperature of roughly 600°C. The total ash value of the *Piliostigmathonningii* leaf (5.00%) indicates that it includes a sizable number of carbonates, phosphates, or a combination of them (**Table 1.**). In order to identify and assess the chemical components of a crude medicine, extractive values are primarily helpful (African Pharmacopoeia, 1986). They may also be used to estimate the concentration of particular components that are soluble in a given solvent. The extractive values can be used to assess the chemical components of a crude medicine and can also be used to estimate which components are soluble in a given solvent (Ozarkar, 2005). The amount of the active ingredients in a given amount of plant material after extraction with a certain solvent is determined by measurement of extractive values. Any crude drug medication can be extracted

with a specific solvent to produce a solution with a variety of phytoconstituents. Alcohol and water extractive values of the studied plant was found to be 8.50% and 9.97% respectively (**Table 1.**). All values gotten from the pharmacognostic analysis of *P. thonningii* crude drug is within the WHO set limits (WHO, 1998). Chemo-microscopic test showed the presence of Tannin, Starch, Oxalate crystals, Oil and Mucilage as seen in **Table2.**. The presence of mucilage and tannins supports the use of the plant for wound healing, anti-oxidant and anti-inflammatory agent, as well as for its cardiovascular demulcent properties (Ahmad*et al.*, 2019).

The underlying structural pattern of a leaf produces features that make it easier to identify a leaf in a powder. When the more specific anatomical characteristics are combined, they help in the identification of plants parts contained in a crude drug up to the genus and species level. Characters that are not frequently found in other plant leaves help distinguish between plants of different classes. Knowing all of the diagnostic characteristics of any leaf in depth makes it easier to spot contaminants and adulterants (Mukharjee, 2002). The abaxial epidermal surface of the leaf showed polygonal/regular epidermal cells with no stomata and absence of trichome while the adaxial surface was characterized with the abundance of unicellular multiseriate trichome (**Figure 1.**). Generally speaking, trichomes serve as barriers that guard against natural dangers such as excessive transpiration, disease attacks, etc. Stomata were absent from both surfaces of the plant's epidermal layer, which was one distinguishing feature noted. The microscopy of the powdered sample further revealed the presence of polygonal/regular epidermal cells with trichome base, xylem vessels, rosette type calcium oxalate crystal, parenchyma cells, unicellular multiseriate trichome and collenchyma cells (**Figure 2.**). This result of the anatomical features present in the powdered leaf sample is similar to the report of Ebele *et al.*, (2021) who reported anatomical feature such as Scalariform xylem vessel, rosette crystals, spiral xylem vessel and non-glandular uniseriate trichome. One striking observation from the epidermal leaf microscopy report by Ebele *et al.*, (2021) was the presence of actinocytic stomata.

DPPH; 2,2-diphenyl-1-picrylhydrazyl, is a chemical compound used in antioxidant assays to measure the ability of substances to scavenge free radicals or acts as hydrogen donors. The methanolic extract of *P. thonningii* in this study shows a significant tendency to scavenge DPPH radicals in a dose dependent manner as seen in **Figure 3.**. The extract of *P. thonningii* showed the highest activity at 97.38% at 500 µg/mL.**Table 3.** shows results of the Total phenols content (TPC) and Total flavonoids content (TFC) revealing the methanolic extract of *P. thonningii* have quite a high flavonoid (42.51 ± 0.38 mg/QE/g) and phenolic (53.74 ±1.59 mg/GAE/g) content. Flavonoids and phenolics are groups of plant chemicals found in various fruits, vegetables, grains, and beverages like tea and wine. Flavonoids give vibrant colours to fruits and flowers and are recognized for their antioxidant properties, combating free radicals in the body (Zaa*et al.*, 2023). Phenolics, widely present in plants, serve multiple roles, including defence against pathogens, UV protection, and pollinator attraction. Like flavonoids, phenolic compounds possess antioxidant capabilities, shielding cells from oxidative damage. They offer diverse health benefits, including neuroprotection, anti-inflammatory effects, cancer prevention, and antimicrobial properties (Tatipamula*et al.*, 2021). The ability of the methanol extract of *P. thonningii* to scavenge free DPPH radicals may be linked to its high flavonoid and phenolic phytoconstituents. Research has shown that plant extracts rich in phenolics and flavonoids exhibit potent antioxidant activity due to their

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ability to neutralize free radicals (Makowczyńska*et al.*, 2015). Studies have demonstrated a positive correlation between the phenolic and flavonoid contents of plant extracts and their antioxidant capacity (Silva-Beltrán *et al.*, 2015).

Confirmation of acetylcholinesterase inhibition is based on the measurement of cholinesterase activity. Cholinesterase inhibition is described as the percentage reduction in AchE activity, resulting in the increased availability of the acetylcholine to the cholinergic receptor and also a proportionate amount of acetic acid produced leading to a change in pH towards acidity with a resultant cognitive and memory impairment (Askar*et al.*,2011).The methanolic extract of *P. thonningii* in this study showed an AchE inhibition of between 5.89 % at 10 mg/ml to 16.56 % at 20 mg/ml (**Table 4.**). An agent with cholinesterase inhibition between 1 to 14% is said to possess desired therapeutic inhibition, emphasizing the need for careful dose titration. There is a narrow safety margin between the beneficial inhibitory dose and the toxic inhibition that leads to flaccid paralysis (Imbimbo 2001).This safety margin is a critical consideration for drugs with a narrow therapeutic index (NTI) (Imbimbo 2001).

It has been shown that cholinesterase inhibitors used in the treatment of Alzheimer's disease have dose-related adverse effects linked to the degree of cholinesterase inhibition. There is an increasing inhibitory effect from 10 mg/ml to 0.75 mg/ml. However, eserine inhibition increases at increasing concentration. The positive control, eserine has a lower $IC_{50}$(0.001171) (**Figure 5**) compared to *P. thonningii*(0.1493) (**Figure 4**), indicating that the eserine is more potent (**Table 5.**).
The hyper-excitatory state downstream in the site of action is expressed as increased activation of the autonomic and central nervous systems, which, depending on the extent of AchE inhibition, results in muscarinic, nicotinic and central nervous symptoms of intoxication (Grossberg 2003).

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