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

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this research is to investigate the pharmacognostic characteristics, antioxidant potential, and anticholinesterase activity of *Piliostigma thonningii* (Schum.) Milne-Redh. This study seeks to evaluate its potential applications for neuroprotection and cognitive health enhancement.

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Place and Duration of Study: This study was carried out in Nigeria by the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development, Idu – Abuja, Nigeria between January 2024 and March 2024.
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 2,2- diphenyl-1-picrylhydrazyl [DPPH] free radical scavenging activity), as well as acetylcholinesterase (AchE) inhibition assay was carried out on the methanolic leaf extract of the plant.
Results: The powdered leaves of *P. thonningii* contained a total ash of 5.00% ± 0.00 and 7.74% ± 0.01 moisture. The alcohol- and water-soluble extractive values were 8.51% ± 0.24 and 9.77% ± 0.31, respectively. Leaf microscopic analysis showed the presence of 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(GAE) ± 1.59 and 42.51mg QE/g ± 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: The study reveals significant pharmacognostic characteristics, robust antioxidant potential, and notable anticholinesterase activity of *P. thonniingii*, suggesting its promising role in neuroprotection. These findings highlight its potential application in enhancing cognitive health and preventing neurodegenerative diseases.

Keywords: Analgesic; pharmacognostic characteristics; antioxidant potential; anticholinesterase activity.

1. 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 [1].

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 [2]. 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.

Piliostigma thonningii 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) [3]. 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., [4], Ighodaro et al., [5], Akinpelu and Obuotor, [6], Asuzu et al., [7].

Pharmacognostic characteristics of plants are crucial for several reasons. They help in the accurate identification and authentication of medicinal plants, ensuring the use of the correct species in herbal preparations [8]. These characteristics, including macroscopic and microscopic features, physicochemical parameters, and phytochemical profiles, are essential for quality control and standardization of plant materials. By providing detailed information about a plant's morphological and anatomical traits, pharmacognostic studies support the detection of adulteration and contamination, enhancing the safety and efficacy of herbal medicines. Ultimately, understanding these characteristics is fundamental for developing reliable and effective plant-based therapeutic agents.

Due to the importance of setting pharmacognostic standards for herbal crude drugs and the array of biological activities *P. thonningii* possess which may contribute to its protection against neurodegenerative disorders, it is therefore, thought to be worthy to investigate the anti-oxidant and anti-acetylcholinesterase activities as a novel approach for the management of AD 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.

The objective of this research is to investigate the pharmacognostic characteristics, antioxidant potential, and anticholinesterase activity of *P. thonningii* leaves to assess their potential role in neuroprotection and cognitive health.

2. MATERIALS AND METHODS

The leaves of *P. thonningii* were collected from the wild at Idu industrial Area, close to National Institute for Pharmaceutical Research and Development (NIPRD), Abuja Nigeria, on the 5th of January, 2024. The plant was identified and authenticated by a taxonomist at the herbarium in NIPRD. It was subsequently air-dried and pulverized.

2.1 Physicochemical Studies

Physicochemical attributes including total Ash value and moisture content were assessed in the desiccated and powdered leaf sample [9,10] also water-soluble extractive values were determined [11,12].

2.2 Sample Extraction

A mechanical blender was employed to grind the dried leaves into fine powder, followed by methanol extraction of a 100g sample by maceration. The extract was dried using a rotary evaporator and were stored in the refrigerator at 4ºC to prevent degradation and inhibition of microbial growth.

2.3 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 and subsequently observed through a microscope at a magnification of X40 [11].

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 [11].

2.4 Antioxidant Assays

2.4.1 Determination of total phenolic content

The quantification of the overall phenolic content within the methanolic extract of *P. thonningii* was conducted in accordance with the procedure outlined by Singleton and Rossi [13]. 1 mL of methanolic 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 quantification of the overall phenolic content within the methanolic extract was presented in milligrams of Gallic acid equivalents (GAE).

2.4.2 Estimation of total flavonoid content

The total flavonoid content of the methanolic extract of *P. thonningii* was analysed using the method described by Zhishen et al. [14]. A 0.25 ml aliquot of the methanolic extract underwent dilution with 1.25 mL of 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 methanolic extract was expressed in milligrams of quercetin equivalents (QE).

2.4.3 DPPH free radical scavenging activity

The method of Rajesh and Natvar (2011) was used to determine the DPPH free radical scavenging activity of methanolic 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 methanolic 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 of methanolic extract of *P. thonningii* was calculated using the following formula.

> Percentage inhibition = absorbance of control - absorbance of test sample / absorbance of control

2.4.4 Study Animals for acetylcholinesterase assay

In this study, albino rats sourced from the Animal Facility Centre at NIPRD 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 'National Institutes of Health Guide for the Care and Use of Laboratory Animals'.

2.4.5 Acetylcholinesterase inhibition

The study employed the modified electrometric technique, as outlined by Mohammad et al., [15] 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 duration of 30 minutes. Following the incubation, the pH of the reaction mixture (referred to as pH2) was measured.

And the percentage inhibition of methanolic extract of *P. thonningii*

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. The test being the methanolic leaf extract of *P. thonningii*

2.5 Statistical Analysis

The analysis of the data was conducted utilizing Microsoft excel 2021 version 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. A graph of % DPPH inhibition was also plotted against concentration for *P. thonningii* antioxidant activity.

3. RESULTS

3.1 Physicochemical Studies

The total ash value of *Piliostigma thonningii* was found to be 5± 0.00, indicating the plant's inorganic content and purity, essential for ensuring authenticity and absence of adulteration. The moisture content was 7.736± 0.01, reflecting the plant's quality and stability, which are critical for its shelf life and susceptibility to microbial contamination. The alcohol-soluble and water-soluble extractive values were 8.505± 0.24 and 9.773± 0.31, respectively, highlighting the presence of bioactive compounds that determine the plant's therapeutic potential and efficacy in herbal formulations as seen in Table 1 below. Table 2 also shows the presence of important components of the plant that contribute to its structure, defence mechanisms, and therapeutic properties.

3.2 Leaf Microscopy

The microscopic examination of the leaf structure of the medicinal plant revealed distinct anatomical features crucial for its identification and authentication. Detailed observations of the epidermal layer and internal tissues provided insights into the plant’s pharmacognostic properties, which are fundamental for ensuring quality and efficacy in medicinal applications as seen in Figs. 1 and 2.

3.3 Antioxidant Assays

The antioxidant potential of the methanolic extract of *P. thonningii* was evaluated through a series of assays, including DPPH free radical scavenging activity, total phenolic content, and total flavonoid content. These assays provide a comprehensive assessment of the extract's capacity to neutralize free radicals and its overall antioxidant capacity as seen in Table 3 and Fig. 3.

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	+

Table 3. Total phenolics and total flavonoid content of *P. thonningii*

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

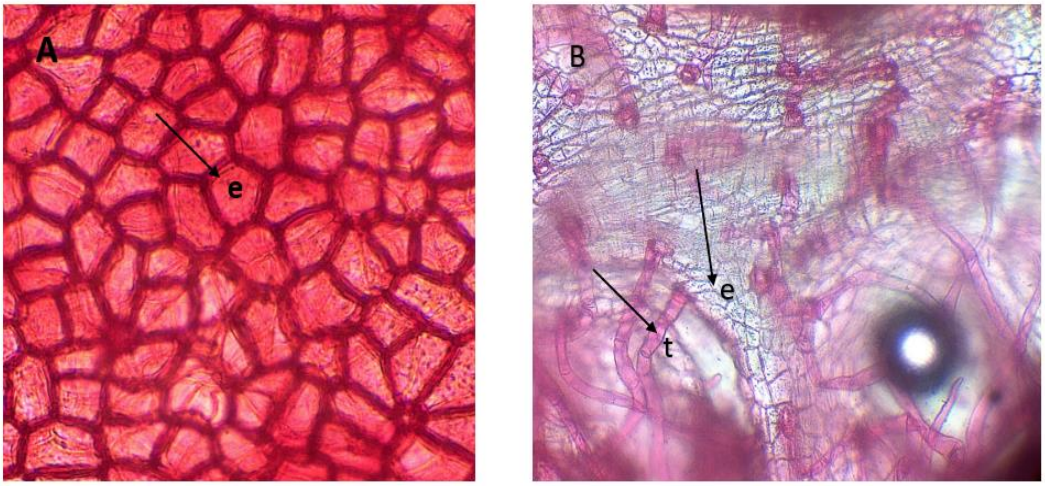

Fig. 1. Microscopic features of leaf epidermis of *P. thonningii* showing A – lower epidermal surface; polygonal/regular epidermal cells (e), B- upper epidermal surface; unicellular multiseriate trichome (t) and epidermal cell (e). Magnification x 400

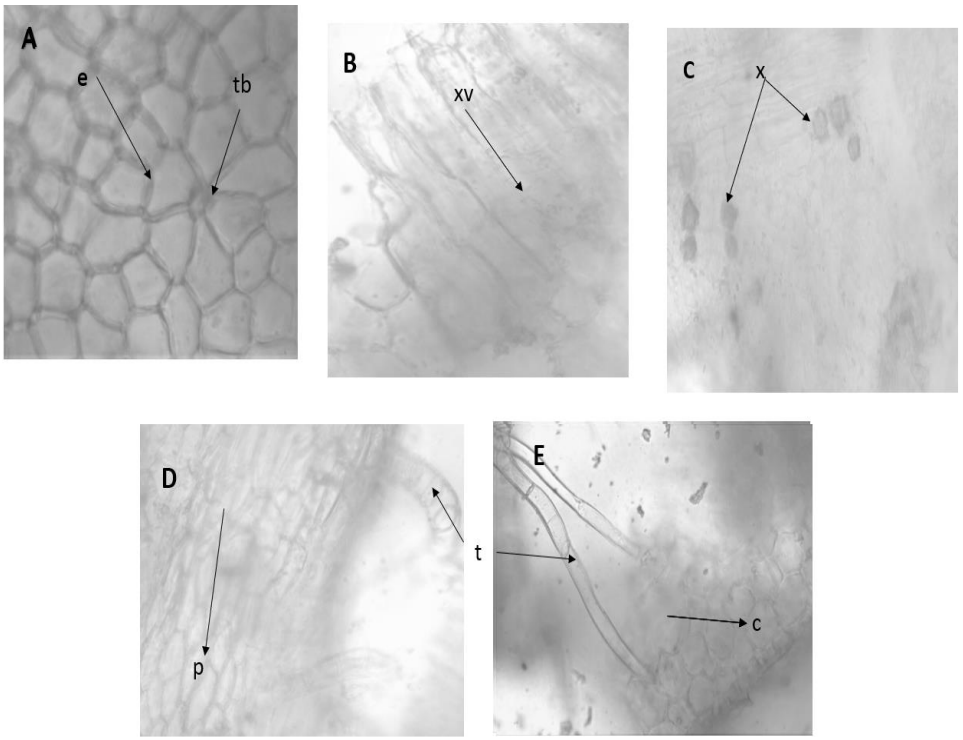

Fig. 2. Microscopic features of leaf powder of *P. thonningii* showing A – polygonal/regular epidermal cells (e) with trichome base (tb), B -xylem vessels (xv), C – rosette type calcium oxalate crystal (x), D – parenchyma cells (p), E – unicellular multiseriate trichome (t) and collenchyma cells(c).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

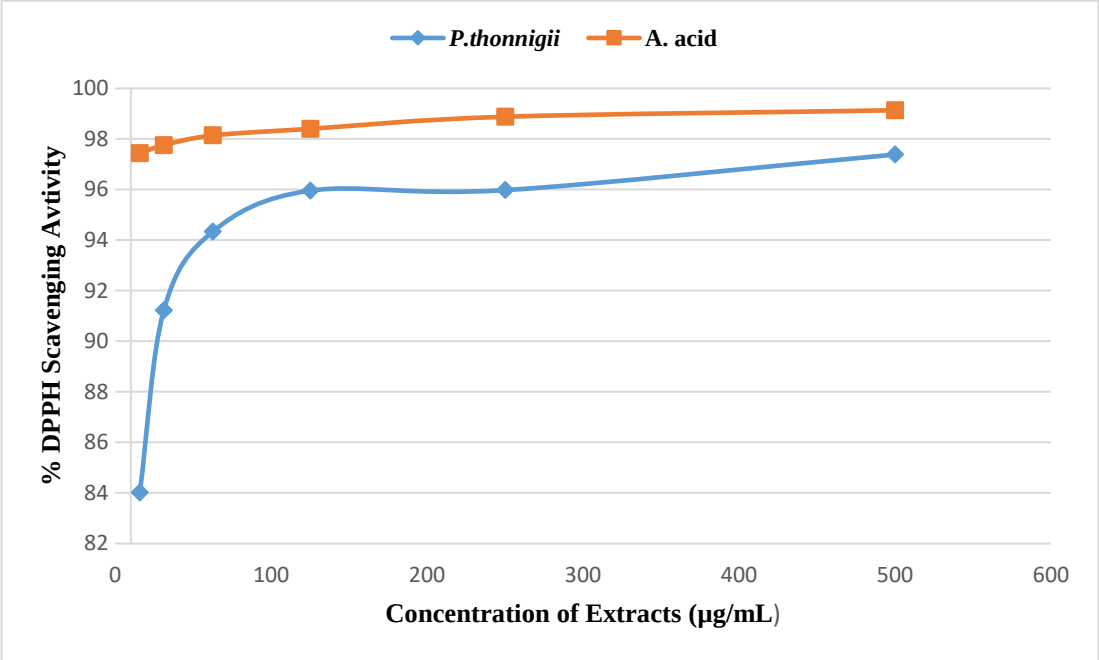

Fig. 3. Inhibition (%) of DPPH radical scavenging in the presence of different Concentrations of Methanolic extract of *P. thonningii* against the Ascorbic acid standard

3.4 Acetylcholinesterase Activity

The acetylcholinesterase (AChE) inhibitory activity of the methanolic extract of the medicinal plant was evaluated to determine its potential efficacy in managing neurodegenerative conditions such as Alzheimer's disease. The results are presented as percentages of AChE inhibition at various concentrations of the extract and eserine (standard) as seen in Table 4. The inhibitory concentration (IC_{50}) of the methanolic leaf extract of *P. thonningi* and that of eserine (standard) was also calculated to be 0.1493 and 0.001171respectively as seen in Fig. 4 and Fig. 5.

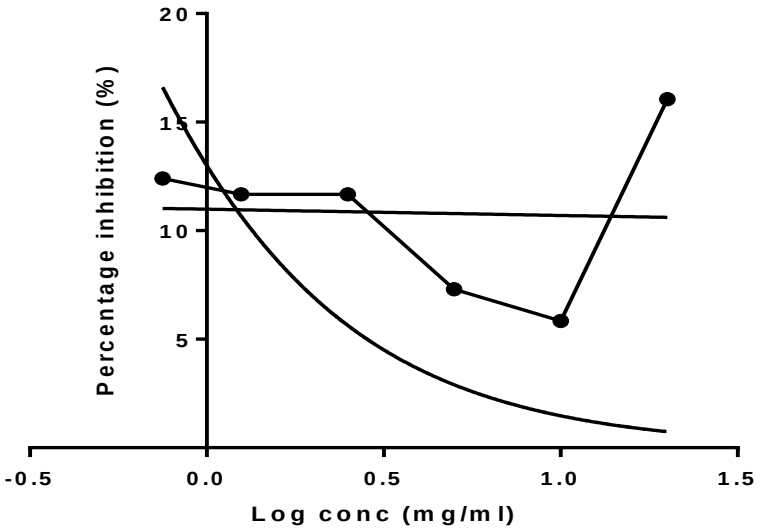

Fig. 4. Percentage inhibition over log concentration of leaf methanolic extract of *P. thonningii*
IC_{50} determined to be 0.1493

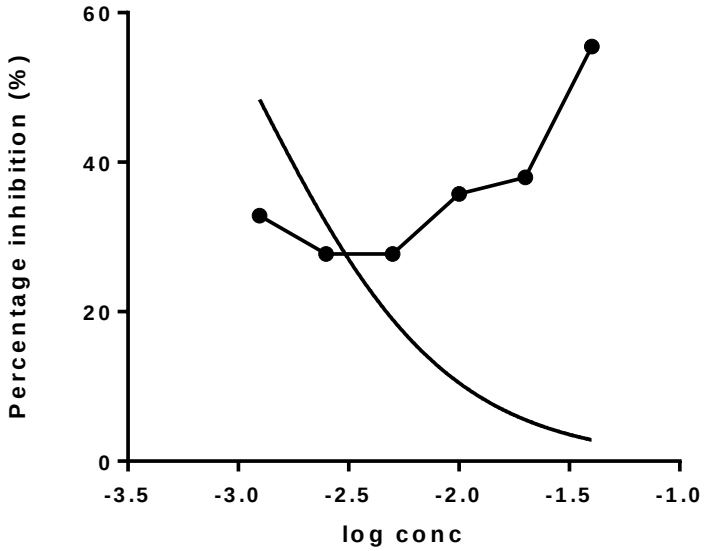

Fig. 5. Percentage inhibition over log concentration of Eserine (Standard)
IC_{50} determined to be 0.001171

Table 4. Determination of percentage cholinesterase Inhibition of methanolic leaf extract of *P.thonningii* and eserine (standard)

Concentration of P.*thonnigii*	Average	Inhibition (%)	Concentration of Eserine	Average	Inhibition (%)
20 mg/ml	0.42	16.05839	0.04 mg/ml	0.15	55.47445
10 mg/ml	0.49	5.839416	0.02 mg/ml	0.27	37.9562
5 mg/ml	0.48	7.29927	0.01 mg/ml	0.285	35.76642
2.5 mg/ml	0.45	11.67883	0.005 mg/ml	0.34	27.73723
1.25 mg/ml	0.455	11.67883	0.0025 mg/ml	0.34	27.73723
0.75 mg/ml	0.445	12.40876	0.00125 mg/ml	0.305	32.84672
Normal saline	0.685	-	-	-	-

4. 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 [16]. When compared to the limit for water content (8 - 14%) for herbal medications (African Pharmacopoeia, 1986), the plant sample had a moisture content of 7.73± 0.01% (Table 1), 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 *P. thonningii* 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 drug, 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 drug and can also be used to estimate which components are soluble in a given solvent [17]. 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 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 [12]. Chemo-microscopic test showed the presence of tannins, Starch, Oxalate crystals, Oil and Mucilage as seen in Table 2. 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 [18].

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 [11]. 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 (Fig. 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 (Fig. 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 Fig. 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 [19]. Phenolics, widely present in plants, serve multiple roles, including defence against pathogens, UV protection, and pollinator attraction [20]. 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 [21]. The ability of the methanolic 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 ability to neutralize free radicals [22]. Studies have demonstrated a positive correlation between the phenolic and flavonoid contents of plant extracts and their antioxidant capacity [23].

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 [24]. 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) [25].

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.The hyper-excitatory state at the site of action leads to heightened activation of the autonomic and central nervous systems. This increased activation, which varies with the degree of AChE inhibition, manifests as muscarinic, nicotinic, and central nervous system symptoms of intoxication [26]. There is an increasing inhibitory effect of *P. thonningii* methanolic leaf extract from 10 mg/ml to 0.75 mg/ml. However, eserine (standard) inhibition increases at increasing concentration. The potency of an inhibitor is often quantified by its IC_{50} value, which is the concentration required to inhibit 50% of the enzyme's activity. In this study, eserine has a much lower IC_{50} value (0.001171 mg/ml) [Fig. 5] compared to *P. thonningii* extract (0.1493 mg/ml) [Fig. 4], indicating that eserine is significantly more potent. However, natural extracts like *P. thonningii* may still offer therapeutic benefits, possibly with fewer side effects, depending on their specific bioactive components and overall safety profile [27,28].

5. CONCLUSION

The research on *P. thonningii* demonstrates significant pharmacognostic characteristics, substantial antioxidant potential, and notable anticholinesterase activity. These findings suggest that the plant holds promise as a neuroprotective agent, with potential applications in enhancing cognitive health and preventing neurodegenerative diseases such as Alzheimer’s. The detailed pharmacognostic analysis ensures accurate identification and quality control, while the antioxidant and anticholinesterase properties highlight the plant's therapeutic potential.

6. RECOMMENDATION

Based on the findings from the research, it is recommended that further in-depth studies be conducted to isolate and identify the specific bioactive compounds responsible for the neurodegenerative protective effects of *P. thonningii*. Additionally, clinical trials should be initiated to evaluate the safety and efficacy of *P. thonningii* extracts in humans. This will help to substantiate its potential as a neuroprotective agent and its application in managing neurodegenerative diseases such as Alzheimer's. Collaborations with pharmacologists and neurologists could accelerate the development of standardized herbal formulations, ensuring the consistent therapeutic quality of products derived from this plant.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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