Antioxidant and Anticholinesterase Activities of *Macrosphyra Longistyla* (DC) Hiern Relevant in the Management of Alzheimer’s Disease

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**Abstract:** *Macrosphyra longistyla* has been used in many traditional systems of medicine for its anti-hemorrhagic, antidiabetic, anti-ulcer, and anti-diarrhea properties. The acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitions of the crude methanol extracts and its various partitioned fractions were determined by a modified method of Ellman. An evaluation of the antioxidant activity was carried out using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging, ferric reducing power, and nitric oxide scavenging assays. The total flavonoids were estimated based on the aluminum chloride method, while the total tannins and phenolics were estimated based on the vanillin–HCl and Folin–Ciocalteu method, respectively. The ethyl acetate fraction had the highest DPPH radical scavenging activity, and the highest ferric reducing power with a concentration providing 50% inhibition (IC\(_{50}\)) of 0.079 mg/mL and 0.078 mg/mL, respectively, while the crude methanol extract had the highest nitric oxide scavenging activity with an IC\(_{50}\) of 0.008 mg/mL. The methanol extract had the highest phenolics and flavonoids contents, while the aqueous fraction had the highest tannin content. The crude methanol extract had the best AChE and BuChE inhibitory action, with an IC\(_{50}\) of 0.556 µg/mL and 5.541 µg/mL, respectively, suggesting that the plant had a better AChE inhibiting potential. A moderate correlation was observed between the phenolic content and DPPH radical scavenging, NO radical scavenging, and AChE inhibitory activities (\(r^2 = 0.439, 0.430, \text{and } 0.439, \) respectively), while a high correlation was seen between the flavonoid content and these activities (\(r^2 = 0.695, 0.724, \text{and } 0.730, \) respectively), and the ferric reducing antioxidant power correlated highly with the proautocyanidin content (\(r^2 = 0.801\)). Gas chromatography mass spectrometry (GCMS) revealed decanoic acid methyl ester (24.303%), 11,14-eicosadienoic acid methyl ester (16.788%), linolealaidic acid (10.444%), pentadecanoic acid (9.300%), and 2-methyl-hexadecanal (9.285%). Therefore, we suggest that *M. longistyla* contain bioactive chemicals, and could be a good alternative for the management of Alzheimer’s and other neurodegenerative diseases.

**Keywords:** *Macrosphyra longistyla*; cholinesterase; antioxidant; total phenolic; total flavonoid

1. Introduction

Alzheimer’s disease and other neurodegenerative conditions are usually characterized by the slow, but progressive, dysfunction and loss of neurons in the central nervous system [1]. About 55 million people are suffering from one form of neurodegenerative disease (ND) or another, with an expected rise in this figure with increasing age of the population [2,3]. Despite the volume of research on the pathogenesis of neurodegenerative conditions, appropriate treatment is yet to be found [4]. However, several factors, including aging [5] and some pathological conditions, such
as impaired mitochondrial function [6], aggregated proteins deposit [7], neuroinflammation [8], cholinergic deficit [9], and oxidative stress [10], have been associated with NDs. Thus, the management of NDs involves addressing one or more of the associated conditions. The currently available therapies for Alzheimer’s disease (AD) are cholinesterase inhibitors such as rivastigmine and donepezil, which only reduce disease progression and provide symptomatic relieve [11]. Thus, efforts are still being made to find alternative and better therapeutic options.

Macrosphyra longistyla is a shrub found in several tropical countries. It has long, arching stems that are about 4 m long [12]. M. longistyla has been used traditionally as an antihemorrhagic in Benin [13], as an antidiabetic in Nigeria and Côte d’Ivoire [14,15], as a contraceptive and for the restoration of fertility [16], for ulcers [17], and for diarrhea [18]. Fresh wildly-collected leaves are eaten as a vegetable by the Gourmantché, Aïzo, and Cotafo people in Benin [19]. It is also widely consumed in Togo [20]. The leaves have been suggested an indigenous food ingredient for complementary food formulations to combat infant malnutrition [21]. To the best of our knowledge, the chemical constituents and biological activity of this plant have not been reported in the literature. This study therefore investigates its anticholinesterase and anti-oxidant potentials, as well as phytochemical characterization.

2. Materials and Methods

2.1. Plant Material

The leaves of Macrosphyra longistyla were collected from Agbogi village in Osun State in December 2017. The plant was identified and authenticated by Mr. Odewo of the Forest Herbarium Ibadan (FHI), with voucher number FHI 112042. The voucher specimen were deposited at the herbarium of the Department of Pharmacognosy, University of Ibadan.

2.2. Plant Extraction and Partitioning

The leaves were air-dried and pulverized. About 2.25 kg of the powdered leaf was macerated using 100% methanol. The extract was filtrated using a Buchner funnel, and concentrated in vacuo so as to obtain a crude methanol extract. Then, 80 g of the crude methanol extract was partitioned into n-hexane, ethyl acetate, and water, to obtain the respective fractions, which were concentrated in vacuo and used for the subsequent experiments. The percentage yield of both extract and fractions were determined.

2.3. Phytochemical Screening

The preliminary phytochemical screening of the crude methanol extract was carried out using standard procedures. These include tests for alkaloids using Dragendorff and Wagner reagents, the Borntrager’s test for anthraquinones, and a ferric chloride test for phenolic compounds [22,23].

2.4. Determination of the Total Phenol Content (TPC)

The total phenol content in the methanolic extract and various fractions of M. longistyla were determined based on a previously described procedure [24]. Then, 2.5 mL of 10% Folin–Ciocalteau’s reagent was mixed with 2 mL of 2% sodium carbonate solution (Na₂CO₃), followed by the addition of 0.5 mL of methanolic extract and fractions of M. longistyla (1 mg/mL). The mixture was incubated at 45 °C for 15 min, and absorbance was taken at 765 nm. The quantification was done with respect to the standard of gallic acid at different concentrations (1, 0.5, 0.25, 0.125, 0.063, and 0.031 mg/mL). The content of the total phenolic compounds was calculated based on a standard curve prepared using gallic acid and expressed as milligrams of gallic acid equivalent (GAE) per gram of sample.

2.5. Determination of Total Flavonoid Contents (TFC)

The total flavonoid content was determined using the aluminum chloride colorimetric method [25]. In this method, 1 mL of crude extract or fractions of M. longistyla were mixed with 3 mL of methanol,
followed by 0.2 mL of 10% aluminum chloride (AlCl$_3$), 0.2 mL of potassium acetate (1 M), and 5.6 mL of distilled water, and left at room temperature for 30 min. Absorbance was taken at 420 nm. Quantification was done with respect to the standard of gallic acid at different concentrations (1, 0.5, 0.25, 0.125, 0.063, and 0.031 mg/mL). The total phenolic content was calculated based on a standard curve prepared using gallic acid, and expressed as milligrams of gallic acid equivalent (GAE) per gram of sample.

2.6. Determination of Pro-Anthocyanidin Content (PAC)

The vanillin–HCl method was used for the quantitative determination of condensed tannins (proanthocyanidins) [26]. In this method, 3 mL of 4% vanillin in methanol, and 1.5 mL of hydrochloric acid (HCl) was added to 0.5 mL of extract/fractions (1 mg/mL). The mixture was vortexed thoroughly and allowed to stand for 15 min at room temperature. Absorbance was read at 500 nm. A calibration curve was prepared using a standard gallic acid solution. All of the results were expressed as mg gallic acid equivalents (GAE) per gram of sample.

2.7. DPPH (2,2-Diphenyl-1-Picrylhydrazyl Hydrate) Radical Scavenging Assay

The radical scavenging ability of the fractions was determined using the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl hydrate), as previously described [27]. In this assay, 1 mL of 0.1 mM DPPH was mixed with 1 mL of crude extract and fractions of _M. longistyla_ at different concentrations (1, 0.5, 0.25, 0.125, 0.063, and 0.031 mg/mL), as well as the positive controls (ascorbic acid and 2,6-di-tert-butyl-4-methylphenol (DDM)) at different concentrations (1, 0.5, 0.25, and 0.125 mg/mL). The reaction was vortexed and left in the dark at room temperature for 30 min, after which the absorbance was taken at 517 nm. The percentage inhibition was calculated as follows:

\[
I% = \left(\frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}}\right) \times 100
\]

where \(A_{\text{blank}}\) is the absorbance of the control reaction (containing all reagents except the test compound), and \(A_{\text{sample}}\) is the absorbance of the test compound. The sample concentration providing 50% inhibition (IC$_{50}$) was also calculated.

2.8. Nitric Oxide (NO) Scavenging Assay

The nitric oxide scavenging assay was carried out as previously described [28]. First, 2 mL of sodium nitroprusside was mixed with 0.5 mL of phosphate buffer pH 7.4 and 0.5 mL of different concentrations of extract (0.0031–1.0 mg/mL). The mixture was incubated at 25 °C for 150 min, and an initial absorbance (\(A_0\)) was taken at 540 nm. Thereafter, 0.5 mL of the incubated mixture was mixed with 1 mL of a sulfanilic acid reagent and 1 mL of naphthylethylenediamine dichloride (0.1% w/v), and incubated at room temperature for 30 min, before another absorbance (\(A_1\)) was taken at 540 nm. The same reaction mixture without the extract but with the equivalent amount of methanol served as the negative control. Ascorbic acid and DDM at various concentrations were used as the standard. All of the experiments were in triplicates. The percentage nitrite radical scavenging activity of the extracts and standard were calculated using the following formula:

\[
\% \text{ inhibition of NO} = \frac{A_0 - A_1}{A_0} \times 100
\]

where \(A_0\) is the absorbance before the reaction, and \(A_1\) is the absorbance after the reaction.

2.9. Ferric Reducing Antioxidant Assay

The reducing power was determined according to the method of Oyaizu [29]. Substances with a reducing ability react with potassium ferricyanide (Fe$^{3+}$) to form potassium ferrocyanide (Fe$^{2+}$), which then reacts with ferric chloride to form a ferric ferrous complex that has an absorption maximum at
700 nm. Briefly, 0.2 mL of various concentrations of plant extract and fractions was mixed with 0.2 mL of phosphate buffer and 0.2 mL of potassium ferricyanide. The mixture was vortexed and incubated at 50 °C for 20 min. After cooling, 0.2 mL of 10% trichloroacetic acid (TCA) was then added to the mixture and centrifuged at 4500 rpm for 10 min. Then, 100 µL of the upper solution was mixed with 20 µL of the ferric chloride solution and 100 µL of distilled water. The absorbance was taken at 700 nm. The control was prepared in a similar manner, but without the test sample. Ascorbic acid and DDM at various concentrations were used as the standard. The experiments were done in triplicates.

2.10. Cholinesterase Inhibitory Assay

Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitions were determined spectrophotometrically using acetylcholine iodide and butyrylcholine iodide as substrates, respectively, by a modified method of Ellman [30]. The serial dilutions of the fractions were subjected to this test using eserin and donepezil as the positive control. Then, 5 mg of both the extract and fractions were dissolved in 1 mL of methanol. Serial dilutions of each sample were done in order to obtain the final concentrations of 1, 0.5, 0.25, 0.125, and 0.0625 mg/mL, while the positive controls (eserin and donepezil) were also diluted serially to obtain the final concentrations of 0.1, 0.05, 0.025, 0.0125, 0.00625, and 0.003125 mg/mL. Thereafter, 20 µL of each concentration was pipetted into the microplates, followed by 240 µL of the phosphate buffer (pH 8) and 20 µL of the enzyme, which was then vortexed. The plates were then incubated at 37 °C for 30 min. After incubation, 20 µL of 25 mM of the substrate (acetylthiocholine iodide (ATChI) or butyrylthiocholine chloride (BTChCl)) was added to the reaction mixture, followed by the addition of 20 µL of 10 mM 5,5′-Dithiobis-2-nitrobenzoic acid (DTNB). The hydrolysis of acetylcholine iodide or butyrylthiocholine chloride was determined spectrophotometrically at 412 nm. The assay was carried out in triplicates, with methanol as the negative control. The percentage inhibition was computed using the following formula:

$$\frac{\Delta a - \Delta b}{\Delta a} \times 100$$

where $\Delta a$ is the change in absorbance of the negative control, and $\Delta b$ is the change in absorbance of the sample.

2.11. Gas Chromatography Mass Spectrometry (GCMS) Analysis

One microliter (1 µL) of the sample diluted in hexane was analyzed on a Bruker 450 gas chromatography-300 mass spectrometer (GCMS) system operating in EI mode at 70 eV, equipped with a HP-5 MS fused silica capillary system with a 5% phenylmethysiloxane stationary phase. The capillary column parameter was 30 m by 0.25 mm, while the film thickness was 0.25 µm. The initial temperature of the column was set at 70 °C, and heated to 240 °C at a rate of 5 °C/min, with the final temperature kept at 450 °C. The run time was 66.67 min, and helium was used as the carrier gas at a flow rate of 1 min/min. The split ratio was 100:1. The scan time was 78 min, with a scanning range of 35 to 450 amu.

2.12. Statistical Analysis

All of the data were analyzed using GraphPad Prism 6.0, and were expressed as mean ± standard error of the mean (SEM). The correlation and regression analysis of the activities (Y) versus the total phytochemical content (X) were carried out using the online Quest Graph™ Linear, Logarithmic, Semi-Log Regression Calculator [31].

3. Results and Discussion

*M. longistyla* has been reportedly used for managing different ailments in traditional medicine [13–18]. In an ethnomedical survey carried out by us, the plant was mentioned as a memory enhancer.
Thus, the present study was carried out to investigate its phytochemical content, as well as evaluate the antioxidant and cholinesterase inhibitory activities of the extracts and partitioned fractions. The preliminary phytochemical screening of the methanol extract revealed the presence of tannins, flavonoids, phenolics, terpenoids, and saponins. Anthraquinones and alkaloids were, however, found absent in the plant (Table 1).

| Tests                        | Observations            | Inferences               |
|------------------------------|-------------------------|--------------------------|
| 1. Alkaloids                 |                         |                          |
| a. Dragendorff              | Deep yellow color       | Alkaloid absent          |
| b. Wagner test              | Orange color            | Alkaloid absent          |
| 2. Anthraquinones           |                         |                          |
| a. Borntrager’s test        | Milky color             | Anthraquinone absent     |
| 3. Flavonoids               | Yellow coloration       | Flavonoids present       |
| 4. Phenols                  | Dark coloration         | Phenols present          |
| 5. Tannins                  | Blue black coloration   | Tannin present           |
| 6. Saponin                  | Frothing which disappear after sometime | Saponin present         |
| 7. Terpenoid                | Dark green coloration   | Terpenoids present       |

The percentage yield of the extract and fractions (expressed as weight of extract/fraction relative to the weight of the initial plant material) ranged from 4.70% to 40.00%, with the highest being the aqueous fraction (Table 2). This suggests that the polar solvent was able to extract more constituents, probably because of the solubility of the polar compounds present in the plant material.

| Assays                     | ME           | HF           | EF           | AF           |
|----------------------------|--------------|--------------|--------------|--------------|
| % Yield                    | 6.18         | 4.70         | 7.11         | 40.00        |
| Total phenolics (mg GAE/g) | 18.30 ± 0.04 | 7.56 ± 0.12  | 16.06 ± 0.13 | 9.02 ± 0.02  |
| Total flavonoids (mg GAE/g)| 16.07 ± 0.14 | 5.02 ± 0.01  | 10.49 ± 0.014 | 11.62 ± 0.01 |
| Total tannins (mg GAE/g)   | 24.44 ± 0.32 | 2.99 ± 0.06  | 9.12 ± 0.17  | 26.11 ± 0.02 |

Data are expressed as mean ± standard error of the mean (SEM; n = 3). GAE—gallic acid equivalent; ME—methanol extract; HF—hexane fraction; EF—ethyl acetate fraction; AF—aqueous fraction.

Furthermore, the content of the phenols (TPC), flavonoids (TFC), and the tannins (PAC) was estimated quantitatively. The TPC, as determined by the Folin–Ciocalteu method, ranged from 7.56 ± 0.12 to 18.30 ± 0.04 mg GAE/g of extract (Table 2). Both the crude extract and the various fractions had an appreciable total phenolic content, with the methanol extract and the ethyl acetate fractions having the highest TPC, while the n-hexane fraction had the least TPC. The total flavonoid and proauthocyanidin contents, also reported as mg GAE/g of extract, showed that the TFC ranged from 5.02 ± 0.01 to 16.07 ± 0.14 mg GAE/g of extract, while the PAC ranged from 2.99 ± 0.06 to 26.11 ± 0.02. In both cases, the hexane fraction had the least amount (Table 2).

Phenolic compounds are present in plant tissues and serve as antioxidants [32], because of the presence of hydroxyl groups, which are responsible for their scavenging ability. Thus, they are capable of reacting with active oxygen radicals such as hydroxyl radicals [33]. Flavonoids are polyphenolic compounds, and are responsible for some of the health benefits of vegetable and fruits [34]. They are known to play an active role in the quenching of free radicals, because of their redox properties [35].
Tannins, however, are a high molecular weight polyphenolic that have also been implicated as antioxidants [36].

The antioxidant activity of the extract and fractions was evaluated by the DPPH and NO radical scavenging activity, as well as the ferric reducing power, while the AChE inhibitory activity was evaluated by Ellman’s colorimetric assay.

DPPH is usually reduced by a hydrogen donating compound, leading to its change in color, from deep violet to light yellow, which can be monitored spectrophotometrically [37]. The DPPH radical scavenging activity results are as shown in Figure 1, while the IC$\text{_{50}}$—the concentration of antioxidant (extract/fractions) required for 50% scavenging of DPPH radicals—values are given in Table 3. From the results, the ethyl acetate fraction had the highest activity, with an IC$\text{_{50}}$ value of 0.078.

![DPPH radical scavenging activity](image)

**Figure 1.** 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity of extract and fractions of *M. longistyla*. Data are expressed as mean ± standard deviation (SD; \( n = 3 \)). ME—methanol extract; HF—hexane fraction; EF—ethyl acetate fraction; AF—aqueous fraction; DDM—2,6-di-tert-butyl-4-methylphenol.

**Table 3.** The concentration providing 50% inhibition (IC$\text{_{50}}$) values of the different antioxidant assays.

| Assays             | ME  | HF  | EF  | AF  | Ascorbic Acid | DDM  |
|--------------------|-----|-----|-----|-----|---------------|------|
| DPPH scavenging    | 0.090 | 0.363 | 0.079 | 0.089 | 0.006         | 0.050 |
| NO scavenging      | 0.008 | 5.678 | 0.056 | 0.010 | 0.072         | 0.063 |
| Ferric reducing    | 0.051 | 0.087 | 0.078 | 0.009 | 0.053         | 0.003 |

ME—methanol extract; HF—hexane fraction; EF—ethyl acetate fraction; AF—aqueous fraction; DDM—2,6-di-tert-butyl-4-methylphenol; DPPH—1,1-diphenyl-2-picryl-hydrazyl; NO—nitric oxide.

Nitric oxide is important in the regulation of several physiological processes, and several diseases have been associated with a high concentration of NO [38]. The nitric oxide scavenging activity can be determined by estimating for nitrate and nitrite, using the Greiss Illosvoy reaction [39]. At a physiological pH (7.2), sodium nitroprusside decomposes in an aqueous solution to produce NO, which reacts with oxygen to form stable products—nitrate and nitrite. Scavengers of NO compete with oxygen, leading to a reduced production of nitrite ions [40].
In the nitric oxide scavenging assay, all of the extract and fractions exhibited a good scavenging effect, with the methanol extract having the best scavenging effect (IC$_{50}$ = 0.008), followed by an aqueous fraction (IC$_{50}$ = 0.010) and then the ethyl acetate fraction (IC$_{50}$ = 0.056; Figure 2 and Table 3).

![Figure 2. Nitric oxide (NO) radical scavenging activity of the extract and fractions of M. longistyla. Data are expressed as mean ± SD (n = 3). ME—methanol extract; HF—hexane fraction; EF—ethyl acetate fraction; AF—aqueous fraction; DDM—2,6-di-tert-butyl-4-methylphenol.](image)

Ferric reducing power is well linked with antioxidant activity [41], and compounds with a reducing effect are usually electron donors that can reduce oxidized intermediates of lipid peroxidation processes, thus acting as primary or secondary antioxidants [33]. In the ferric reducing antioxidant assay, the methanol extract, aqueous fraction, and the ethyl acetate fraction had good reducing activity (Figure 3), with IC$_{50}$ values of 0.051, 0.009, and 0.078 respectively.

![Figure 3. Ferric reducing activity of the extract and fractions of M. longistyla. Data are expressed as mean ± SD (n = 3). ME—methanol extract; HF—hexane fraction; EF—ethyl acetate fraction; AF—aqueous fraction; DDM—2,6-di-tert-butyl-4-methylphenol.](image)

On the whole, a better antioxidant activity was observed in the polar fractions, and this could be because of the abundant presence of major secondary metabolites, such as tannins and flavonoids, in these fractions, as supported by the higher TPC and TFC in these fractions. Phenolics are free-radical terminators [33], thus having protective effects against many infectious and neuro degenerative diseases such AD [42].
The inhibition of cholinesterase enzymes is considered promising in the management of neurological and neurodegenerative disorders such as AD, senile dementia, ataxia, and myasthenia gravis, where a deficit in cholinergic neurotransmission is often observed [43,44]. Compounds with a dual inhibitory effect on AChE and BuChE are also considered better, as BuChE also plays a minor role in the regulation of AChE [45,46].

In this study, the methanol extract inhibited the acetylcholinesterase enzyme the most, followed by the ethyl acetate and aqueous fractions, with respective percentage inhibitions of 81.629 ± 0.02, 76.985 ± 0.04, and 71.778 ± 0.01 (Figure 4). The hexane fraction had the least inhibitory action, suggesting that the active constituents are likely to be polar. The study also suggests a better inhibition of AChE as compared to BuChE, as both the crude extract and the various fractions had a lower percentage inhibition and higher IC$_{50}$ values in the later enzyme (Figure 5 and Table 4).

**Figure 4.** Acetylcholinesterase inhibitory activity of the extract and fractions of *M. longistyla*. Data are expressed as mean ± SD (n = 3). ME—methanol extract; HF—hexane fraction; EF—ethyl acetate fraction; AF—aqueous fraction.

**Figure 5.** Butyrylcholinesterase inhibitory activity of the extract and fractions of *M. longistyla*. Data are expressed as mean ± SD (n = 3). ME—methanol extract; HF—hexane fraction; EF—ethyl acetate fraction; AF—aqueous fraction.
Table 4. IC₅₀ values for the cholinesterase inhibitory assay. AChE—acetylcholinesterase; BuChE—
butyrylcholinesterase.

| Assays | IC₅₀  |
|--------|-------|
|        | ME    | HF   | EF   | AF   | Eserin | Donepezil |
| AChE   | 0.556 | 25.871 | 0.914 | 0.846 | 0.002 | 0.001 |
| BuChE  | 5.541 | 11.957 | 23.338 | ND   | 0.002 | 0.001 |

ND: Not determined.

The cholinesterase inhibitory activity of several medicinal plants has been reported in the
literature [47–51]. Also, antioxidants such as vitamin E and vitamin C have been reportedly associated
with a decrease in AD incidence and prevalence, [52] and AD patients on high doses of antioxidants
were reported to have a slower rate of cognitive deterioration [53]. Thus, the good antioxidant and
anticholinesterase activities of polar fractions in this study suggest that these fractions are good
sources of phenolic compounds, with potential cholinesterase inhibitory and antioxidant properties
that may find usefulness in the management of AD. This is the first report of such activities in
Macrosphyra longistyla.

We also correlated the phytochemical content with the observed activities of the plant. Several
pharmacological effects of the plant extract such, as being anti-inflammatory, antioxidant, and
 antimicrobial, have also been associated with the presence of phenolic compounds [54,55], and r²
values have been used to show the relationship between the phytochemical constituents and activities
of medicinal plants [56]. There was a moderate correlation between the total phenolic content and the
DPPH and NO radical scavenging, as well as the AChE inhibitory activities (r² = 0.439, 0.430, and
0.439, respectively). However, a better correlation was observed between the flavonoid content and
these activities (r² = 0.695, 0.724, and 0.730, respectively), while the ferric reducing antioxidant power
correlated with the proautocyanidin content (r² = 0.801; Table 5).

Table 5. Correlation of the total phenolic, total flavonoid, and proautocyanidin contents with antioxidant
and anticholinesterase activities.

| Assays       | r² Values |
|--------------|-----------|
|              | Total Phenolics | Total Flavonoids | Proautocyanidin |
| DPPH scavenging | 0.439     | 0.695          | 0.515          |
| NO scavenging   | 0.430     | 0.724          | 0.558          |
| Ferric reducing | 0.012     | 0.276          | 0.801          |
| AChE inhibition | 0.439     | 0.730          | 0.557          |
| BuChE inhibition| 0.00154   | 0.131          | 0.325          |

Finally, the identification of possible compounds in the non-polar (hexane) fraction using GC-MS
revealed the presence of twenty-three compounds (Table 6). The most abundant was decanoic acid
methyl ester (24.303%), followed by 11,14-eicosadienoic acid methyl ester (16.788%), linoelaidic acid
(10.444%), pentadecanoic acid (9.300%), and 2-methyl-hexadecanal (9.285%).
Table 6. Compounds identified through gas chromatography mass spectrometry (GCMS).

| S/N | Name of Identified Compounds | Retention Time (min) | % Abundance | Molecular Formula | Class of Compound | Reported Biological Effect | References |
|-----|-----------------------------|----------------------|-------------|-------------------|-------------------|---------------------------|------------|
| 1   | 2,6,8-trimethyl-decane       | 27.084               | 0.084       | C₁₃H₂₈          | Alkane            | Antifungal                | [57]       |
| 2   | 2-methyl-hexadecanal         | 30.833               | 9.285       | C₁₇H₃₄O        | Aldehyde          | Antifungal                | [56]       |
| 3   | Z,Z,L-1,4,6,9-nonadecatetraene| 34.147               | 0.349       | C₁₉H₃₂          | Alkene            | Antioxidant               | [59]       |
| 4   | 2-dodecanone                 | 34.831               | 0.357       | C₁₂H₂₄O        | Aliphatic ketones | Nematocidal               | [60]       |
| 5   | 2-pentadecanone              | 35.591               | 1.630       | C₁₅H₃₀O        | Ketone            | Cytotoxic and repellant   | [61,62]    |
| 6   | 17-octadecanal               | 39.211               | 0.119       | C₁₈H₃₆O        | Long-chain aldehyde| NR                        | NR         |
| 7   | Hexadecanoic acid            | 39.564               | 0.152       | C₁₆H₃₂O₂        | Saturated fatty acid| Anticancer and antihelminic| [63,64]    |
| 8   | 2-methyl-dodecanoic acid     | 43.071               | 0.283       | C₁₁H₂₃O₂        | Fatty acid        | Antimicrobial             | [65]       |
| 9   | Neophytadiene                | 43.482               | 0.109       | C₁₉H₃₈           | Sesquiterpene     | Anti-inflammatory         | [66]       |
| 10  | 2-nonadecane                 | 43.611               | 1.942       | C₁₉H₃₈O        | Alkanone          | Antimicrobial             | [67]       |
| 11  | Decanoic acid methyl ester   | 46.414               | 24.303      | C₁₁H₂₂O₂        | Fatty acid ester  | Antimicrobial             | [68]       |
| 12  | Phytol                      | 47.053               | 0.202       | C₂₀H₄₂O        | Diterpene alcohol | Antinociceptive, antioxidant, and anticholinesterase| [69,70]    |
| 13  | Eicosanoic acid ethyl ester  | 48.585               | 5.265       | C₂₂H₄₄O        | Fatty acid        | Anticancer                | [71]       |
| 14  | Pentadecanoic acid           | 48.592               | 9.300       | C₁₅H₃₀O        | Saturated fatty acid| Anthelmintic          | [64]       |
| 15  | Tetradecanoic acid-12-methyl-methyl ester | 49.580 | 0.048 | C₁₆H₃₄O₂ | Fatty acid | Anticancer and antifungal | [72,73] |
| 16  | 11,14-eicosadienoic acid methyl ester | 51.562 | 16.788 | C₂₁H₃₂O₂ | Fatty acid | Antioxidant and anti-amylase | [74] |
| 17  | 8,11,14-eicosatrienoic acid | 51.712               | 2.299       | C₂₀H₃₄O₂        | Omega fatty acid  | Atopic dermatitis and malignant hypertension| [75,76] |
| 18  | Z-methyl-hexadec-11-enoate   | 51.963               | 4.204       | C₁₇H₃₄O₂        | Fatty acid methyl ester | Antimicrobial | [77] |
| 19  | Dodecanoic acid-10-methyl-methyl ester | 52.643 | 1.957 | C₁₄H₂₈O₂ | Fatty acid methyl ester | Anticoagulant | [78] |
| 20  | Linoelaidic acid             | 53.559               | 10.444      | C₁₈H₃₄O₂        | Omega-6 trans fatty acid | Anticholinesterase, anti-mycobacterium, anticancer, and antitoxicant | [79–82] |
| 21  | Z,E-3,13-octadecadien-1-ol   | 53.763               | 2.707       | C₁₈H₃₄O        | Fatty alcohol     | Antimicrobial             | [83]       |
| 22  | (Z)-methyl-Heptadec-9-enoate | 53.953               | 0.179       | C₁₆H₃₂O₂        | Fatty acid        | Antibiotic                | [84]       |
| 23  | Hexadecanoic acid-2-methyl-methyl ester | 54.615 | 1.139 | C₁₈H₃₄O₂ | Fatty acid methyl esters | Antimicrobial and antitoxicant | [85] |

NR: Not reported. The various identified compounds have been reported to have different biological effects, such as being antimicrobial, antioxidant, anticoagulant, anticholinesterase, anticancer, and anthelmintic. All of these ultimately contribute to the overall activity of the plant.

4. Conclusions

This study revealed the antioxidant and anticholinesterase activities of the compounds present in *M. longistyla*, and suggest the potential use of extracts from this plant for the management of neurodegenerative conditions. The polar fractions had the highest antioxidants and anticholinesterase constituents, which can be further exploited. Also, the GCMS analysis identified the compounds likely to contribute to the observed activities.
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