Acetylcholinesterase inhibition, antioxidant and identification of some chemical constituents of Phyllanthus atropurpureus cultivated in Egypt

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Investigation of the lipid constituents of the aerial parts of Phyllanthus atropurpureus resulted in isolation and identification of the fatty acid mixture which consists of eight acids with linolenic acid as major and the unsaponifiable fraction that contain a series of hydrocarbons, sterols, in addition to one triterpene (α-amyrin). The acetone insoluble fraction was found to contain two fatty alcohols and three n-hydrocarbons in which the n-eicosane is the most abundant (44.16%). The flavonoidal constituents were isolated from ethyl acetate and butanol fractions which were identified as: luteolin-7-O-glucoside, kaempferol 3-O-(p-coumaroylglucoside), kaempferitrin, luteolin and kaempferol. Evaluation of different extracts as acetylcholinesterase inhibitors (AChI), established the chloroform fraction as a promising inhibitor of the enzyme. The antioxidant testing with DPPH radical revealed the potential of precipitate from MeOH extract as a radical scavenger.

Keywords: Phyllanthus atropurpureus. Euphorbiaceae. Flavonoids. Acetylcholineesterase inhibitors. Antioxidant.

INTRODUCTION

Phyllanthus (Euphorbiaceae) is a large genus and widely distributed in tropical and subtropical zones like tropical Africa, tropical America and Asia. This genus, consisting of more than 700 species. It has been employed as herbal drugs for a long time in China, India, Brazil and Southeast Asian countries. The most abundant species are used and have a beneficial role for the treatment of digestive, genitourinary, respiratory, skin diseases, antioxidant, antihepatotoxic, anticancer, antidiabetic, diuretic, anti-inflammatory to treat hepatitis B, hypertension, dropsy, sore throat, to cure jaundice, renal calculus and malaria (Mao et al., 2016). Also, P. niruri contain many phytochemical constituents like alkaloids, anthocyanins, flavonoids, phenolic acids, lignans and saponins. Combined saponin and alkaloid of the plant showed more potency and may offer an alternative therapeutic agent against bacterial infections (Ajibade, 2014; Kaur, Kaur, Sirhindi, 2017).

The chemical review on genus Phyllanthus, revealed the presence of sterols, and/ or terpenes, flavonoids (5,6,8,4´-tetrahydroxy isoflavone, robustaside A and 6´-(3″,4″- dihydroxycinnamoyl), arbutin, demethoxysudachitin, polyphenolic compounds and tannins, in addition to minor alkaloids (EL-Saywd, 2009; Sarg et al., 2012).

Alzheimer’s disease (AD) is the most common form of dementia and accounts for approximately 60% of all dementia cases. Till now there is no discovered medication that can slow or stop the neurodegeneration process of AD, where current treatment medicines can only control and improve the disease-associated symptoms (Finn, 2017). In AD, there is an excessive activity of acetylcholinesterase (AChE) enzyme that hydrolyze acetylcholine, a potential neurotransmitter in cholinergic neurons of the cerebral cortex part of the brain, leading to acetylcholine deficiency, choline accumulation and loss of memory (Quirion, 1993;
Greenblatt et al., 1999). Therefore, current medicines for AD symptoms are essentially acetylcholinesterase inhibitors such as donepezil and galantamine that act by preventing the excessive AChE-catalyzed hydrolysis of acetylcholine and thereby improving cognitive symptoms (Ali et al., 2013). In addition, oxidative stress resulting from free radicals has been implicated in the pathogenesis of neurodegenerative diseases including AD (Omar et al., 1999; Mariani et al., 2005). Therefore, many antioxidant compounds have been very useful in neuroprotection against AD (Finn, 2017). There are many raw plant extracts/herbal formulations with immense uses as natural remedies in the treatment of AD and other neurodegenerative diseases. Many traditional natural medical systems have various brain tonics and memory enhancers (Perry et al., 1999).

In the present study, some chemical constituents such as lipids and some phenolics were isolated and identified in addition to the acetylcholinesterase inhibitors and antioxidant activities of some extracts of *P. atropurpureus* were evaluated.

**MATERIAL AND METHODS**

**Plant Material**

The aerial parts of *P. atropurpureus* was collected in June 2015 from botanical garden, Faculty of Agriculture, Cairo University and kindly identified by Dr. Mohammed Elgebaly at Phytochemistry and Plant Systematic Dept., National Research Center (NRC). Voucher specimen was deposited at the herbarium of the NRC (Herbarium specimen number: 11501).

**Extraction of lipid and phenolic constituents**

About 1.5 kg of the air dried powdered plant material of *P. atropurpureus* were extracted with hexane in a Soxhlet for two days. The hexane extract was passed over Fuller’s earth to remove the colored pigments and evaporated in vacuo to give a yellowish residue (6.5 g), which was dissolved in hot acetone to afford two fractions, acetone insoluble fraction which was filtered and analyzed by Gas chromatography-Mass spectrometry (GC/MS) and acetone soluble fraction which subjected to saponification process to afford the unsaponifiable materials and fatty acid methyl esters fraction which were identified using Gas liquid chromatography (GLC) (Gören et al., 2003). The defatted plant material (1400 g) was divided into two portions (700 g each), the first portion was extracted with methanol: chloroform mixture (50:50) for two days, filtered and the precipitated material during the concentration was separated and washed with chloroform to afford a dark brown precipitate and chloroform/methanol extract. The second portion was macerated in aqueous methanol (70%) for five days to produce a brownish extract, concentrated up to 700 mL which increased to 1000 mL with hot distilled water, the precipitated material was filtered off and the filtrate was partitioned with successive portions of chloroform, ethyl acetate and butanol. The butanol fraction was applied into a Polyamide column (5x75) eluted with 100% water, water/methanol gradients and decreasing the polarity by increasing the amount of methanol up to methanol 100%. The different fractions which contain the main compounds were further purified using small columns of Polyamide and Sephadex LH-20 to afford three compounds (PA-I, PA-II and PA- III). The ethyl acetate fraction (about 1.5 g) was dissolved in methanol and subjected to preparative paper chromatography, developed with AcOH (20%). Two main bands were localized, cut off and eluted with hot methanol to afford two main compounds which were further purified by passing through Sephadex LH-20 column eluted with methanol (95%) to afford compounds PA-IV and PA-V.

**Gas liquid chromatography (GLC) analysis of unsaponifiable matters**

The GLC analyses were carried out using the GC instrument, Varian model 3700. The GC was equipped with capillary column (AG-Bp-70). Polysilphenylene-siloxane, 60 m length, 320 µmL internal diameter, 0.25 µmL film thickness. Analyses were carried out using the following temperature program: 70 °C, rising at 4 °C/min to 270 °C and the injector and detector were held at 280 °C and 290 °C, respectively. Flow rates of N₂ and H₂ 30 mL/min and Air 300 mL/min.

**Gas liquid chromatography (GLC) analysis of fatty acid methyl esters (FAMEs)**

The GLC analyses were carried out using the GC instrument, Varian model 3700. The GC was equipped with capillary column (AG-Bp-70). Polysilphenylene-siloxane, 60 m length, 320 µmL internal diameter, 0.25 µmL film thickness. Analyses were carried out using the following temperature program: 70 °C, rising at 4 °C/in to 190 °C and the injector and detector were held at 240 °C and 280 °C, respectively. Flow rates of N₂ and H₂ 30 mL/min, Air 300 mL/min.

**AChE Inhibitory activity**

The AChE inhibitory activity of each extract was
tested using 96-well micro-plate assay based on previously published methods (Ingkaninan et al., 2000; Rhee et al., 2001) with minor modifications. Each extract (25 μL of 10× of final concentrations in DMSO) was dispensed in duplicates onto 96 well microplate and mixed with 200 μL of Ellman’s mixture [final concentrations of 10 mM Tris–HCl, pH 8.0, 0.1% bovine serum albumin (BSA, fraction V), 1.5 mM acetylthiocholine iodide (ATCI, Carboxsynth, UK) and 150 μM 5,5’-dithio-bis-(2-nitrobenzoic acid), DTNB, Sigma-Aldrich, Germany]. The control wells contained the extract vehicle (DMSO) instead of the extract. The reaction was started with the addition of AChE enzyme solution (0.02 U/mL, type VS –from electric eel, Sigma-Aldrich, Germany). Non-enzymatic autohydrolysis of the substrate was corrected by replacing the enzyme with 25 μl of enzyme buffer (50 mM Tris–HCl, pH 8, 0.1% BSA) in duplicate wells. The enzymatic activity was monitored kinetically at 405 nm in a microplate reader (Zenyth 200, Biochrom® Anthos, Cambridge, UK), every 20s intervals for 20 cycles. The enzyme rate was calculated from the slope of the curve of absorbance change vs. time. Neostigmine methyl sulphate (0-31.25 μg/mL) was used as reference AChE inhibitor. As screening strategy, final concentration of 50 μg/mL from each extract was examined and the average % inhibition was calculated relative to the enzyme rate of the vehicle control wells according to the equation:

\[
\% \text{ACHE Inhibition} = \frac{\left(\text{mean slopes of the vehicle control}\right) - \left(\text{mean slopes of the sample}\right)}{\left(\text{mean slopes of the vehicle control}\right)}
\]

Microwell assay of DPPH radical scavenging

Extracts were prepared in DMSO as 10x stocks from each test concentration and briefly sonicated when necessary in an ultrasonic water bath. In a preliminary screen, extracts that produced radical scavenging activities ≥ 50% at 100 μg/mL were taken for further concentration-response testing to determine the EC_{50} (concentration of the extract producing 50% scavenging of the DPPH). Quercetin dihydrate was used as reference antioxidant. The method used in the present study was based on previously published methods (Braca et al., 2001; Nara et al., 2006). The extract stock solutions (20 μL/well) were pipetted in triplicate onto 96-well plates (flat-bottomed, Grene Bio-one, Germany). The assay was started with the addition of DPPH reagent (0.004% wt/v in methanol, 180 μl /well). Appropriate blanks were prepared using the solvent only in addition to the same amount of DPPH reagent to get rid of any inherent solvent activity. Negative controls were run in parallel to correct for sample color by OD subtraction. The plate was incubated in dark for 30 min at room temperature and then absorbances were read on a Zenyth 200 microplate reader (Biochrom®, Anthos, Cambridge, UK).

The percentage of antioxidant activity (% AA) was calculated using the following equation:

\[
\% \text{DPPH Scavenging} = 100 - \frac{\text{100} \times \left(\text{color corrected OD of test sample}\right)}{\text{color corrected OD of vehicle control}}
\]

EC_{50} values (concentrations that scavenge 50% of the DPPH in the vehicle control) were derived using non-linear regression analysis of the concentration-% DPPH scavenging plot on GraphPad Prism® V6.0 (San Diego, USA). Data were presented as means ± S.E of three experiments.

RESULTS AND DISCUSSION

Identification of lipoidal matter

The lipid constituents of the aerial parts of *P. atropurpureus* were extracted with hexane (Table I-III) and fractionated to acetone insoluble fraction, unsaponifiable matter and fatty acid methyl esters. The acetone insoluble fraction was analyzed using Gas chromatography-Mass spectrometry (GC/MS) (Table I) and it was found to contain fatty alcohols and hydrocarbons with n-eicosane is the main hydrocarbon while octadecanol is the main fatty alcohol. 

The Gas liquid chromatography (GLC) analysis of the unsaponifiable fraction (Table III) revealed the presence of n-hydrocarbons mixture (66.76%) in which n-heptacosane (C_{27}) is the main (28.1%) and saturated with palmitic acid as major (17.90%). These findings were in agreement with that reported by Rao, Sino (1992), where they found that linolenic acid was the major component in *P. calycinus*. 

The data in Table II proved that the fatty acid fraction was found to consist of eight acids, three of them were unsaturated (73.38%) in which linolenic acid the main one (48.19%) and five acids are saturated (26.62%) with palmitic acid as major (17.90%). These findings were in agreement with that reported by Rao, Sino (1992), where they found that linolenic acid was the major component in *P. calycinus*.

The Gas liquid chromatography (GLC) analysis of the unsaponifiable fraction (Table III) revealed the presence of n-hydrocarbons mixture (66.76%) in which n-heptacosane (C_{27}) is the main (28.1%), a sterol fraction (26.10%) contain three components [campasterol, stigmasterol (14.22%) and β-sitosterol] and α-amyrin (4.45%) as a triterpene. These data were in agreement with that reported by Subraya, Satyanarayana, Yamin (2013), where they isolated β-sitosterol, stigmasterol and α-amyrin acetate from aerial part of *P. lawii*. 

Identification of flavonoidal components

The flavonoidal constituents were isolated from the butanol and ethyl acetate fractions using different columns chromatography as follow:

**Compound PA-I**

*Luteolin-7-O-glucoside:* It was obtained as yellowish powder, appeared as a brown spot turned yellow on spraying with AlCl$_3$ under UV light and it’s behavior on paper chromatography proved it’s glycosidic nature. The UV spectra in methanol and shifts reagents showed that it is a flavone type with occupation at C-7, with an ortho-dihydroxy system in ring B. UV spectrum of the aglycone of NaOAc/H$_3$BO$_3$ relative to methanol spectrum confirmed the presence of a free OH group at C-3´ and C-4´. The presence of free OH group at C-5 is confirmed by the bathochroic shift of band-1 with AlCl$_3$ relative to methanol. The absence of free OH group at C-7 was confirmed through NaOAc spectrum (Mabry, Markham, Thomas, 1970). The EI-MS displayed M$^+_{+}$ at m/z = 448 which fit to the formula C$_{21}$H$_{20}$O$_{11}$. Another important fragments at m/z = 420 and 286 are due to M$^+_{+}$ CO and hexose moiety respectively. The $^1$H NMR (400 MHz ,

**TABLE I** - GC/MS analysis of acetone insoluble fraction of *Phyllanthus atropurpureus*

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>R$_t$ (min.)</th>
<th>%</th>
<th>Mol. formula</th>
<th>Mol. Wt.</th>
<th>Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20.92</td>
<td>9.54</td>
<td>C$<em>{16}$H$</em>{14}$O</td>
<td>242</td>
<td>hexadecanol</td>
</tr>
<tr>
<td>2</td>
<td>25.57</td>
<td>15.87</td>
<td>C$<em>{18}$H$</em>{10}$O</td>
<td>270</td>
<td>octadecanol</td>
</tr>
<tr>
<td>3</td>
<td>30.50</td>
<td>2.50</td>
<td>C$<em>{20}$H$</em>{32}$</td>
<td>282</td>
<td>9-methylnonadecane</td>
</tr>
<tr>
<td>4</td>
<td>32.60</td>
<td>44.16</td>
<td>C$<em>{20}$H$</em>{32}$</td>
<td>282</td>
<td>eicosane</td>
</tr>
<tr>
<td>5</td>
<td>37.67</td>
<td>9.67</td>
<td>C$<em>{20}$H$</em>{32}$</td>
<td>386</td>
<td>cholesterol</td>
</tr>
<tr>
<td>6</td>
<td>40.57</td>
<td>15.50</td>
<td>C$<em>{20}$H$</em>{32}$</td>
<td>352</td>
<td>pentacosane</td>
</tr>
<tr>
<td>7</td>
<td>-----</td>
<td>2.76</td>
<td>-----</td>
<td>-----</td>
<td>unknowns</td>
</tr>
</tbody>
</table>

**TABLE II** - GLC analysis of FAME fraction of *Phyllanthus atropurpureus*

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>R$_t$ (min.)</th>
<th>Rel. %</th>
<th>Mol. formula</th>
<th>Mol. Wt.</th>
<th>Compounds</th>
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<tbody>
<tr>
<td>1</td>
<td>9.47</td>
<td>1.20</td>
<td>C$<em>{16}$H$</em>{22}$O$_{2}$</td>
<td>200</td>
<td>Lauric acid , n-C$_{12}$:0</td>
</tr>
<tr>
<td>2</td>
<td>10.47</td>
<td>1.93</td>
<td>C$<em>{18}$H$</em>{26}$O$_{2}$</td>
<td>214</td>
<td>Traidecanoic acid , n-C$_{13}$:0</td>
</tr>
<tr>
<td>3</td>
<td>11.95</td>
<td>3.61</td>
<td>C$<em>{18}$H$</em>{23}$O$_{2}$</td>
<td>228</td>
<td>Myristic acid , n-C$_{14}$:0</td>
</tr>
<tr>
<td>4</td>
<td>14.77</td>
<td>17.90</td>
<td>C$<em>{18}$H$</em>{24}$O$_{2}$</td>
<td>256</td>
<td>Palmitic acid , n-C$_{16}$:0</td>
</tr>
<tr>
<td>5</td>
<td>17.85</td>
<td>1.98</td>
<td>C$<em>{18}$H$</em>{26}$O$_{2}$</td>
<td>284</td>
<td>Stearic acid , n-C$_{18}$:0</td>
</tr>
<tr>
<td>6</td>
<td>18.35</td>
<td>7.89</td>
<td>C$<em>{18}$H$</em>{23}$O$_{2}$</td>
<td>282</td>
<td>Oleic acid , n-C$_{18}$:1</td>
</tr>
<tr>
<td>7</td>
<td>19.36</td>
<td>17.30</td>
<td>C$<em>{18}$H$</em>{24}$O$_{2}$</td>
<td>280</td>
<td>Linoleic acid , n-C$_{18}$:2</td>
</tr>
<tr>
<td>8</td>
<td>20.62</td>
<td>48.19</td>
<td>C$<em>{18}$H$</em>{23}$O$_{2}$</td>
<td>278</td>
<td>Linolenic acid , n-C$_{18}$:3</td>
</tr>
</tbody>
</table>

**TABLE III** - GLC analysis of the unsaponifiable fraction of *Phyllanthus atropurpureus*

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>R$_t$ (min)</th>
<th>%</th>
<th>Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.4</td>
<td>21.58</td>
<td>n-tetradecane (C$_{14}$)</td>
</tr>
<tr>
<td>2</td>
<td>12.5</td>
<td>0.9</td>
<td>n-pentadecane (C$_{15}$)</td>
</tr>
<tr>
<td>3</td>
<td>15.2</td>
<td>0.9</td>
<td>n-heptadecane (C$_{17}$)</td>
</tr>
<tr>
<td>4</td>
<td>18.7</td>
<td>0.9</td>
<td>n-cosane (C$_{20}$)</td>
</tr>
<tr>
<td>5</td>
<td>19.8</td>
<td>1.22</td>
<td>n-hencosane (C$_{22}$)</td>
</tr>
<tr>
<td>6</td>
<td>20.8</td>
<td>1.67</td>
<td>n-dodicosacone (C$_{22}$)</td>
</tr>
<tr>
<td>7</td>
<td>21.8</td>
<td>2.22</td>
<td>n-triacosane (C$_{22}$)</td>
</tr>
<tr>
<td>8</td>
<td>22.8</td>
<td>3.11</td>
<td>n-tetracosan (C$_{24}$)</td>
</tr>
<tr>
<td>9</td>
<td>23.4</td>
<td>2.34</td>
<td>n-pentacosan (C$_{25}$)</td>
</tr>
<tr>
<td>10</td>
<td>24.6</td>
<td>1.33</td>
<td>n-hexacosane (C$_{26}$)</td>
</tr>
<tr>
<td>11</td>
<td>25.8</td>
<td>28.81</td>
<td>n-heptacosan (C$_{27}$)</td>
</tr>
<tr>
<td>12</td>
<td>28.6</td>
<td>1.78</td>
<td>n-nonacosan (C$_{29}$)</td>
</tr>
<tr>
<td>13</td>
<td>31.9</td>
<td>7.45</td>
<td>Campasterol</td>
</tr>
<tr>
<td>14</td>
<td>32.5</td>
<td>14.22</td>
<td>Stigmasterol</td>
</tr>
<tr>
<td>15</td>
<td>33.4</td>
<td>4.45</td>
<td>α-sitosterol</td>
</tr>
<tr>
<td>16</td>
<td>36.4</td>
<td>4.45</td>
<td>α-amyrin</td>
</tr>
</tbody>
</table>
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DMSO) gave signals at: 7.56 (1H, dd, J=7.6, 1.842, H-6’’), 6.93 (d, J=2.5, H-2’’), 6.92 (1H, d, J=7.5, H-5’’), 6.24 (s, H-3), 6.16 (d, J=2.0 Hz, H-6), 6.28 (d, J=2.0 Hz, H-8), 5.45 (1H, d, J=7.5, H-1’’), 3.35-2.75 (as complex m, rest of glucose protons). The acid hydrolysis proved the presence of glucose as a sugar and luteolin as an aglycone. The UV spectra of the aglycone proved the presence of the glucose moiety at C-7. So, the structure of compound PA-I could be elucidated as *luteolin 7-O-glucoside* (Giannasi, 1988; Harborne, Baxter, 1999; Saxena, Aprajita, 2008).

**Compound PA-II**

*Kaempferol 3-O-(p-coumaroylglucoside)*: The compound was isolated from the butanol fraction as a yellowish powder and it is a glycosidic in nature through its chromatographic behavior in different solvent system (Rf 0.77 in BAW and 0.34 in 15% AcOH). It is a flavonol type of kaempferol structure occupied at C-3 where it’s UV spectrum in MeOH gave band-I at 359, also the spectrum of the NaOMe reagent proved the presence of the glucose moiety appeared at 5.2 as (1H, dd, J=8.7 Hz, H-6’’), finally complex multiplet signals at 3.69-4.31 for two sugar moieties at 5.54 (1H, d, J=6.2 Hz, H-6’’), 0.83 (3H, d, J=6.32 Hz, H-6’’), 5.69 (1H, d, J=6.8 Hz, H-5’’), 6.15 (1H, d, J=1.75 Hz, H-8) and 5.42 (1H, d, J=1.3 Hz, H-1’’), while the two methyl protons at 1.05 (3H, d, J=6.32 Hz, H-6’’), 0.83 (3H, d, J=6.32 Hz, H-6’’), the anomeric protons of two sugar moieties at 5.54 (1H, d, J=1.3 Hz, H-1’’), 5.69 (1H, d, J=1.75 Hz, H-8), 6.15 (1H, d, J=1.75 Hz, H-8). The p-coumaric acid at 7.5 (2H, d, J=8.8 Hz, H-2’’), 6.75 (1H, d, J=10.7 Hz, H-7’’) and 5.69 (1H, d, J=11.2 Hz, H-8’’), while the anomic proton of the glucose moiety appeared at 5.2 as (1H, d, J=6.7 Hz). The acid hydrolysis revealed the presence of kaempferol as an aglycone with glucose and p-coumaric acid with aid of paper chromatography and authentic samples. All these data were agree with that reported and supported the identification of compound PA-II as kaempferol 3-O-(p-coumaroylglucoside) (Felipe et al., 2014).

**Compound PA-III**

*Kaempferitrin*: The compound was isolated as a dark yellow powder and it is a glycosidic in nature where it’s Rf is 0.68 in AcOH (15%) and a flavonol type structure where it displayed band-I at 350 nm with free OH group at C-4’’ where band-I was shifted to 415 nm with increasing in intensity. The absence of an ortho dihydroxy system was confirmed through both AlCl3/ACl3/HCl spectrum where there is no hypsochromic shift in band-I relative to AlCl3, and no bathochromic shift in band -I in NaOAc/H2BO3.

**Acetylcholinesterase inhibition**

The screening of the effect of *P. atropurpureus* extracts and fractions against AChE enzyme activity is presented in (Figure 1). The results revealed that the chloroform fraction produced the highest inhibition of the enzyme activity among other tested samples, recording 82.5% inhibition at 50 µg/mL. Weak inhibition was produced by the rest of samples as shown in Figure 1. To determine the IC50 of the chloroform extract, dose-response experiment was conducted and revealed a concentration-dependent inhibition of AChE enzyme. Non-linear regression analysis of the concentration-% inhibition plot...
revealed the IC\textsubscript{50} value to be 13.7 µg/mL (Figure 2). The IC\textsubscript{50} for the reference AChE inhibitor was calculated as 3.3 µg/mL (Figure 3). The obtained activity of the chloroform extract could be attributed to its alkaloidal contents which are known to cause AChE inhibition (Elgorashi, Stafford, Van Staden, 2004; Halldorsdottir, Jaroszewski, Olafsdottir, 2010; Kulhankova et al., 2013; Dong et al., 2015; Benamar et al., 2016). Kaur, Kaur, Sirhindi (2017) stated that \textit{P. niruri} contain alkaloids of neuro-pharmacological activity.

**Antioxidant activity**

The prescreen of the effect of \textit{P. atropurpureus} extracts and fractions on the scavenging of DPPH radical revealed the activity of three fractions, namely the precipitate from MeOH extract, BuOH fraction and water fraction. Concentration-response experiments were conducted to reveal the EC\textsubscript{50} values (Figure 4 and Table IV). The obtained EC\textsubscript{50} revealed the most potent
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**CONCLUSION**

This study resulted in isolation and identification of lipid constituents (fatty alcohols, fatty acids and unsaponifiable matters), and the flavonoidal compounds (aglycone and glycosides). Evaluation of different extracts and fractions as acetylcholinesterase inhibitors proved that the chloroform fraction is the most active one, while the precipitate from MeOH extract exhibited the highest antioxidant activity.

**CONFLICT OF INTEREST**

All authors declare no conflicts of interest.

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