Asian Journal of Chemistry; Vol. 33, No. 4 (2021), 741-746

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2021.23067

Bioactive Phytochemical Compounds of *Physalis minima* L. and its Anticholinesterase and Antioxidant Activities

V. PRABHU[1], K. ARUNKUMAR[2], B. MONIKA[1], V. LATHA[3] and G. SIBI[4,*]

[1]P.G. Department of Chemistry, Nallamuthu Gounder Mahalingam College, Pollachi-642001, India
[2]Faculty of Chemistry, Hindusthan Polytechnic College, Coimbatore-641032, India
[3]Department of Chemistry, Indian Academy Degree College (Autonomous), Bengaluru-560043, India
[4]Department of Biotechnology, Indian Academy Degree College (Autonomous), Bengaluru-560043, India

*Corresponding author: E-mail: gsibii@gmail.com

Received: 16 November 2020; *Accepted*: 7 January 2021; *Published online*: 20 March 2021; AJC-20275

Physalis minima Linn. was investigated for its antioxidant and acetylcholine esterase inhibition activities. The methanolic extract of the whole plant was evaluated for radical scavenging and *in vitro* hydrolysis of acetylthiocholine iodide. Inhibitory concentration (IC_{50}) of the extract exhibited IC_{50} values of 78.6, 46.2, 76.7 and 296 µg/mL under 2,2-diphenyl-1-picrylhydrazyl (DPPH), ABTS (2,2′-azino-*bis*(3-ethylbenzothiazoline-6-sulfonic acid)), hydroxyl radical scavenging and acetylcholine esterase inhibition assays. GC-MS analysis revealed the presence of 11 compounds of which most of the compounds were reported with biological activities. The study suggests further investigations of *P. minima* for isolation, purification and characterization of valuable bioactive compounds related to their radical scavenging activity and for the treatment of neurodegenerative disease.

Keywords: *Physalis minima*, Antioxidant activity, Acetylcholine esterase.

INTRODUCTION

Cholinesterase regulates the neurotransmitters in synaptic cleft thereby playing a crucial role in cholinergic transmission. Acetylcholinesterase hydrolysis the neurotransmitter into acetyl and choline groups to regulate the transmission of nerve impulses hence acetylcholinesterase has been targeted to for therapeutic interventions in several diseases. Acetylcholine esterase (AChE) inhibitors enhance cholinergic neurotransmission through inhibition of the AChE and decreasing the cleavage of acetylcholine. Based on this inhibitory mechanism, AChE inhibitors represent the first option pharmacotherapy for Alzheimer's disease, senile dementia, ataxia, myasthenia gravis and Parkinson's disease [1]. Tacrine, donepezil and rivastigmine are commonly used to treat cognitive dysfunction but their adverse effects like gastrointestinal disturbances [2] necessitate to find out alternates from natural resources. Bioactive compounds from plants are sources of acetylcholine esterase inhibitors [3] and their favourable effects in cognitive disorders makes them potential candidates for treatment of Alzheimer's disease.

Cell damages caused by free radicals poses a threat to human health [4] and antioxidants play an important role in scavenging free radicals. Synthetic antioxidants such as butylhydroxyanisole and butylhydroxytoluene are effective in reducing the oxidative stress but their toxicity at high dosages and long-term treatment led to identification of natural antioxidants from plants, in particular. Based on the potentials of bioactive compounds from plant origin by inhibiting acetyl choline esterase activity and reducing oxidative stress, this study was intended to search a plant with both AChE inhibition and antioxidant activity. To determine the antioxidant activity, both DPPH (2,2 diphenyl-1-picrylhydrazyl) and $ABTS^+$ (2,2′-azino *bis*-3 ethylbenzothiazoline-6-sulfonic acid) assays were selected due to their highly stable reactions [5].

Physalis minima Linn. is an annual herbaceous plant belongs to the family Solanaceae. It is distributed in Asian countries, Africa and Australia [6]. The plants leaves are ovate, acute and shallowly toothed or lobed. Flower calyx is yellow in colour, ovary is ovoid in shape and styles are glabrous. Fruit calyx is 5 angular and berries of the plant are subglobose, reticu-

lately veined and 8 mm in size. Seeds are 2 mm in diameter and orange yellow in colour [7]. The plant as a whole has been reported with diverse ethnomedical uses in the history due to its biological and pharmacological activities. Aerial part of the plant has been used to treat fever, cough, pharyngitis and furuncle diseases [8]. Methanol extract of the aerial parts also exhibited cytotoxic activities [9]. Fruits of the plant showed strong quinone reductase inducing activity [10].

Steroid compounds such as withanolides and physalin X were isolated from *P. minima* which were found to be effective in scavenging free radicals [11,12] and abortificient activity [13,14]. Antifertility effect of the leaves was reported by Sudhakaran *et al.* [15]. Plant extract prepared from ethanol [16-18], chloroform [19], methanol [10] were exhibited anti-inflammatory activities at various levels. Cytotoxic activity of *P. minima* against human lung adenocarcinoma NCI-H460 cells [20], breast carcinoma T-47D cells [21,22], hepatic carcinoma SMMC-7721 cells [23], human colorectal-carcinoma HCT-116 cells [24], HepG2, SK-LU-1 and MCF7 [9] were reported elsewhere. Apart from the mentioned pharmacological activities, the plant has been reported for its antimicrobial [25,26], leishmanicidal [27,28] and antidiabetic [29] activities.

Based on the diverse pharmacological activities, an effort was made to identify the active compounds from *Physalis minima* by extracting the whole plant in methanol and further analysis by gas chromatography-mass spectroscopy (GC-MS). The biological activity of *P. minima* was determined in terms of antioxidant and acetylcholine esterase inhibitory activities *in vitro*.

EXPERIMENTAL

Whole plant of *Physalis minima* was collected from the different areas of Pollachi (10.669823 ºN, 76.980639 ºE), India. The plant was identified and authenticated in the Department of Botany, Bangalore University, Bengaluru, India.

Reagents and standards of analytical grade such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino*bis*-(3-ethylbenzthiazolin-6-sulfonic acid) [$ABTS^+$], 5,5-dithio*bis*-2-nitrobenzoic acid (DTNB) and acetylcholinesterase were purchased from Sigma-Aldrich (India).

Sample preparation: Whole plant of *Physalis minima* was dried at room temperature for 7 days, finely powdered and used for extraction. The powder (100 g) was mixed with a 500 mL methanol using a shaker for 15 h. Then the mixture was centrifuged at 2850 ×g in a centrifuge and the supernatant was decanted. The pellet was mixed again with a 250 mL methanol and the entire process was repeated once again. The supernatants collected from the two phases were mixed in a round-bottom flask and concentrated under reduced pressure in a rotary evaporator. The dried extract was kept at -20 ºC for further studies.

DPPH assay: *Physalis minima* extract was tested for its antioxidant properties using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay [30]. DPPH solution was prepared in methanol (40 µg/mL). The scavenging activity was determined in 96 well microplate by mixing 100 µL each of DPPH solution and plant extract. The mixture was incubated in dark at room temperature for 30 min and the absorbance (n = 3) was read at 514 nm using an ELISA reader (Genetix, GMB-580). Ascorbic acid was used as a reference. DPPH scavenging effect was measured using the following formula to calculate the percentage of inhibition.

$$\text{Inhibition } (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

ABTS assay: 2,2′-Azino-*bis*(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation assay was done by preparing ABTS reagent (7 mM) in potassium persulfate (140 mM). The scavenging activity was determined in 96 well microplate by mixing 100 µL each of ABTS reagent and plant extract. The mixture was incubated in dark at room temperature for 6 min and the absorbance (n = 3) was read at 734 nm using an ELISA reader.

$$\text{Radical scavenging } (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Hydroxyl radical scavenging activity: Hydroxyl radical scavenging activity of the extractives was determined by the method of Halliwell & Gutteridge [31]. The reaction mixture contained 0.8 mL of phosphate buffer solution (50 mM, pH 7.4), 0.2 mL each of plant extract, EDTA (1.04 mM), $FeCl_3$ (1 mM) and 2-deoxy-D-ribose (28 mM). The mixtures were kept in a water bath at 37 ºC and the reaction was started by adding 0.2 mL each of ascorbic acid (2 mM) and H_2O_2 (10 mM). After incubation at 37 ºC for 1 h, 1.5 mL of cold thiobarbituric acid (1%) was added to the reaction mixture followed by 1.5 mL of HCl (25%). The mixture was heated at 100 ºC for 15 min and then cooled down with water. The absorbance of solution was measured at 532 nm with a spectrophotometer. The hydroxyl radical scavenging capacity was evaluated with the inhibition of percentage of 2-deoxy-D-ribose oxidation on hydroxyl radicals. Ascorbic acid was used as the positive control. The percentage of hydroxyl radical scavenging activity was calculated according to the following formula:

$$\text{Scavenging activity } (\%) = A_0 - \frac{A_1 - A_2}{A_0} \times 100$$

where A_0 is the absorbance of the control without a sample; A_1 is the absorbance after adding the sample and 2-deoxy-D-ribose; and A_2 is the absorbance of the sample without 2-deoxy-D-ribose.

Acetylcholinesterase inhibition activity: Acetylcholinesterase (AChE) inhibition activity of *P. minima* crude extract was determined based on Ellman's method [32]. *P. minima* extract was (20 µL) was added with 10 µL of acetylcholinesterase (1 U/mL), 160 µL of 5,5-dithio*bis*-2-nitrobenzoic acid (0.33 mM DTNB in 0.1 M phosphate buffer, pH 8.0) in microtiter plate followed by incubation at room temperature for 10 min. Then, 10 µL of acetylthiocholine iodide (20 mM) was added and the rate of hydrolysis of acetylthiocholine iodide was then determined spectrophotometrically by measuring the change in the absorbance per minute due to the formation of 5-thio-2-nitrobenzoate anion (yellow colour) at 412 nm over

a period of 20 min. Any increase in absorbance due to the spontaneous hydrolysis of the substrate was corrected by subtracting the rate of reaction before adding the enzyme from the rate after adding the enzyme. Rivastigmine was used as a reference and the percentage of inhibition of AChE was calculated by the formula:

$$\text{Inhibition } (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Determination of IC_{50} values: The concentration of *P. minima* extract required to inhibit 50% of radical-scavenging effect and acetylcholinesterase (IC_{50}) has been determined by testing a series of concentrations. IC_{50} values for DPPH, ABTS and AChE were obtained through extrapolation from non-linear regression of the % relative activity of enzymes *versus* the sample concentrations.

Statistical analysis: The results are expressed as mean ± SD values (n = 3). Statistical significance was compared for each treated group with the control and determined by Student's test. Each experiment was repeated at least three times to yield comparable results. Values with $p < 0.01$ and $p < 0.001$ were considered significant.

GC-MS analysis: GC-MS analysis of the phytoconstituents of *P. minima* methanol extract was carried out using thermo GC-trace ultraversion: 5.0 coupled with thermo MS DSQ II instrument. Compounds were separated on DB-35, MS capillary standard non-polar column (30 × 0.25 mm), film thickness 0.25 µm. Helium was used as the carrier gas and the temperature programming was set with initial oven temperature at 70 ºC and held for 2 min and the temperature of the oven was raised to 260 ºC for 10 min and raised 6 ºC per min and final temperature was 300 ºC for 10 min. The sample of 100 µL was dissolved in 1 mL of acetone injected with split less mode. Mass spectra were recovered over 50-500 amu range with electron impact ionization energy 70 eV, while injector and MS transfer line temperature were set at 230 and 280 ºC, respectively. Interpretation of mass spectrum of GC-MS was conducted using the database of National Institute Standard and Technology (NIST) library by comparing with the spectrum of unknown with known compound.

RESULTS AND DISCUSSION

Consumption of herbs are beneficial due to the presence of antioxidant compounds present in them [33]. Interest in natural antioxidants due to the increasing doubt towards synthetic antioxidants, which may be carcinogenic and unstable [34]. Free radical scavenging assays of plant extract determines the antioxidant capability of molecules that prevent the detrimental effects caused by free radicals in human body. Hydrogen ion from antioxidants transform the unstable violet DPPH and ABTS free radicals into stable yellow radicals. In both assays, absorption of proton free radicals decreases upon exposure to radical scavengers. This study evaluated the ability of *P. minima* extract concentration required to inhibit 50% of the radical scavenging effect by testing a series of concentrations. It was determined that *P. minima* exhibited better scavenging activity against ABTS radicals than DPH radicals.

The results of the determination of DPPH radical scavenging revealed that the methanol extract of *P. minima* scavenged the free radical. As can be seen, scavenging activity increased with the higher concentrations of the extract with highest radical scavenging activity by 85%. The results proved that DPPH radical scavenging activity by *P. minima* was dose dependent (Fig. 1). IC_{50} value was observed 78.5 µg/mL for the methanol extract of *P. minima* and 8.8 µg/mL for ascorbic acid. Antioxidant activity of *P. minima* determined by ABTS assay was compared with that of standard ascorbic acid at varying concentrations. Similar to DPPH assay, scavenging activity was increased when the plant extract concentration was increased (Fig. 2). A maximum ABTS scavenging of 58.7% was observed at 320 µg/mL concentration with an IC_{50} value of 46.2 µg/mL. Reduction of free radicals indicated the presence of biologically active substances with antioxidant potential in the extract. The results also provide a comparison of antioxidant activity of *P. minima* through DPPH and ABTS assay. Similarly, antioxidant activity of leaves extract of *P. minima* was identified by Fatima *et al*. [35].

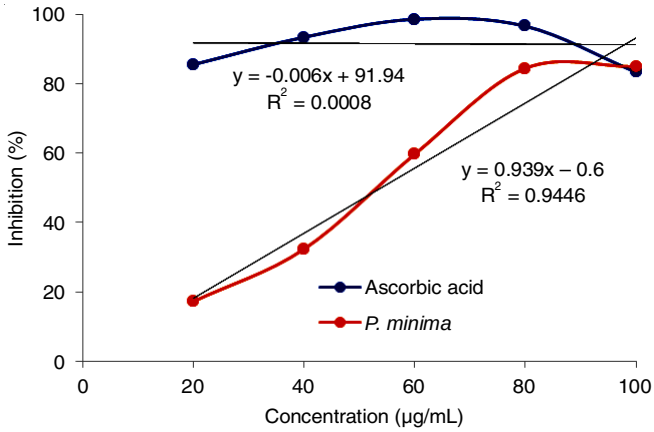


Fig. 1. DPPH radical scavenging activity of *P. minima*

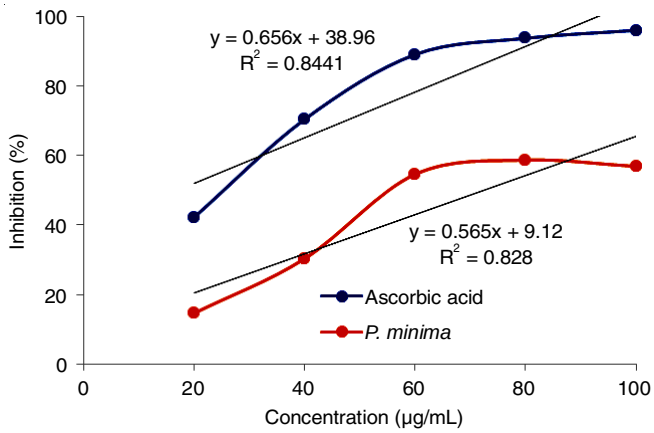


Fig. 2. $ABTS^+$ radical scavenging activity of *P. minima*

In the hydroxyl radical scavenging assay, free radical was generated by Fe^{3+}-ascorbate-EDTA-H_2O_2 system (Fenton reaction). The assay is based on the quantification of 2-deoxy-D-ribose degradation product, which forms a pink chromogen upon heating with thiobarbituric acid at low pH. Hydroxyl

radical scavenging activity of the methanolic extract of *P. minima* was dose dependent with the scavenging activity ranged between 9.4 and 79% (Fig. 3). The IC_{50} of 76.7 µg/mL was greater than the standard used (11.3 µg/mL). Free radicals cause mutagenesis due to the direct interactions of hydroxyl radicals with DNA, resulting in DNA breakdown [36]. Addition of methanolic extract to the reaction mixture removed hydroxyl radicals in this study and could be inhibiting the interaction of hydroxyl radicals with DNA.

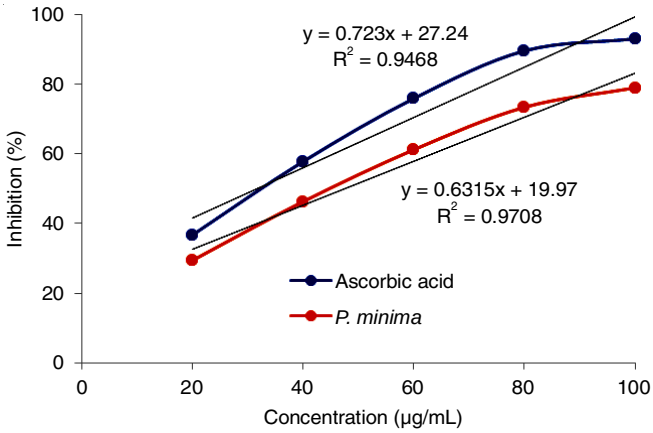


Fig. 3. Hydroxyl radical scavenging activity of *P. minima*

Enzymes are the primary targets for the development of new drugs because of the simplicity of enzyme-based assays. The inhibitor interacts with the enzyme or enzyme-substrate complex with a decrease in the rate of reaction [3]. Acetylcholinesterase hydrolyzes the acetylcholine released on central nervous system synapses regulating its concentration and effect. In acetylcholine esterase inhibition activity, hydrolysis of acetylthiocholine iodide leads to production of acetic acid and thiocholine, that reacts with DTNB producing 5-thio-2-nitrobenzoic acid anion, which is determined spectrophotometrically. AChE inhibitors are used clinically on the treatment of Alzheimer's disease, because they increase the availability of acetylcholine present in cholinergic synapses, enhancing the cholinergic functions [37,38]. In this study, *P. minima* at the concentrations of 20, 40, 60, 80 and 100 µg/mL was used for the acetylcholine esterase inhibition studies. The extract exhibited inhibitory percentage between 24.2 and 76.3%, which was also dose dependent (Fig. 4). Though the IC_{50} of *P. minima* extract (296 µg/mL) was greater than the standard, rivastigmine (26 µg/mL), the AChE activity of the plant is reported for the first time through this study. Hence, *P. minima* could be a source of alternative acetylcholinesterase inhibitory drugs or serve as starting points for synthesizing AChE inhibitors.

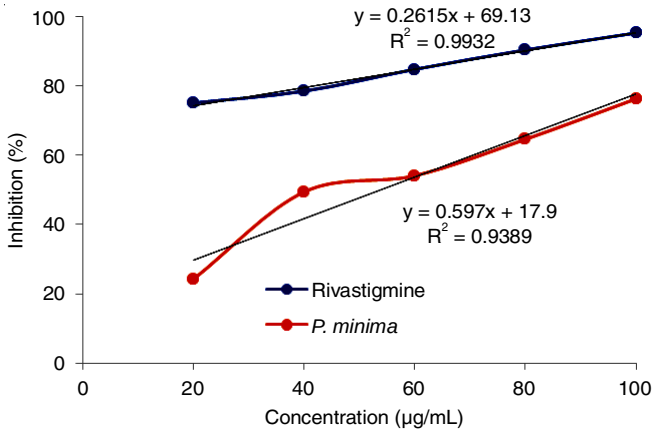
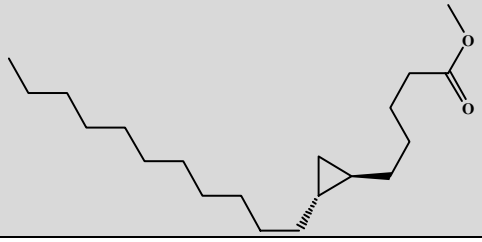


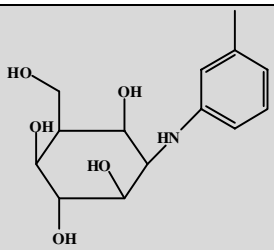
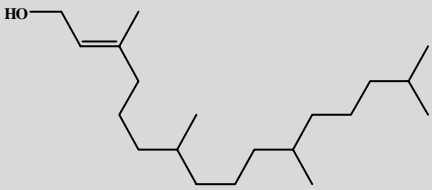
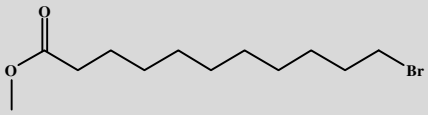
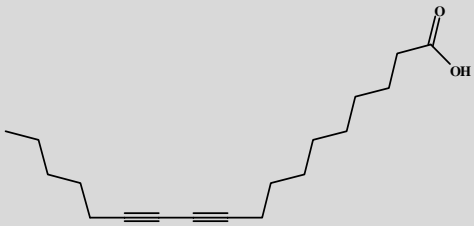
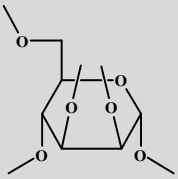
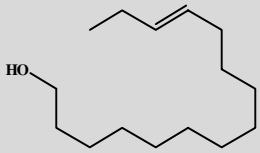
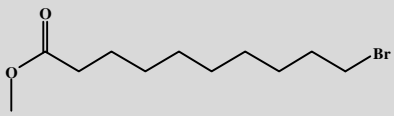
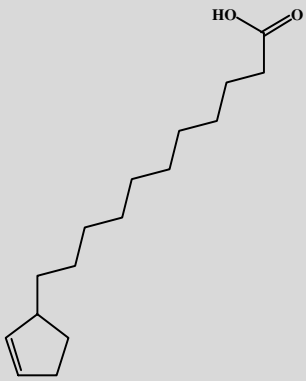
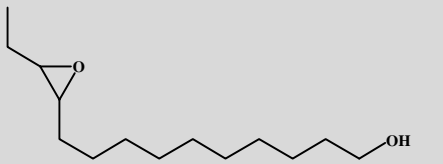
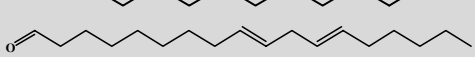
Fig. 4. Acetylcholine esterase inhibition activity of *P. minima*

In order to ascertain the active compounds present in *P. minima*, methanol extract of the whole plant was quantified. Gas chromatography-mass spectroscopy (GC-MS) analysis of methanol extract of whole plant indicated the presence of 11 compounds (spectra not shown). The compounds identified were 10,12-octadecadienoic acid; decanoic acid, 10-bromo-, methyl ester; 11-tetradecen-1-ol, (E); *cis*-Z-11,12-epoxytetradecan-1-ol; 9,12-octadecadienal; undecanoic acid, 11-bromo-, methyl ester; α-D-mannopyranoside, methyl 2,3,4,6-tetra-*O*-methyl; 1-(*p*-toluidino)-1-deoxy-β-D-idopyranose; cyclopropanepentanoic acid, 2-undecyl-, methyl ester, *trans*; cyclopentaneundecanoic acid, methyl ester and 11-(2-cyclopenten-1-yl) undecanoic acid, (+) (Table-1). Cyclopropanepentanoic acid, 2-undecyl-, methyl ester, *trans*; 1-(*p*-toluidino)-1-deoxy-β-D-idopyranose and cyclopentaneundecanoic acid, methyl ester accounted for 91.1% of the total bioactive compounds were identified in this study. The minor compounds present were 11-tetradecen-1-ol, (E); decanoic acid, 10-bromo-, methyl ester; 11-(2-cyclopenten-1-yl)undecanoic acid, (+); *cis*-Z-11,12-epoxytetradecan-1-ol and 9,12-octadecadienal (Table-1).

Most of the compounds identified in the methanol extract of *P. minima* were reported to possess biological activities (Table-1). Fatty acid containing cyclopropane rings could display biological activity. herein, 2-hexyl-cyclopropane-decanoic acid increased human cyclooxygenase activity [39]. Cyclopentane-

TABLE-1
COMPOSITION OF BIOACTIVE COMPOUNDS OF THE METHANOL EXTRACT OBTAINED FROM *Physalis minima*

Compound name	Structure	Retention time	Compound (%)	m.f.	Biological activity
Cyclopropanepentanoic acid, 2-undecyl-, methyl ester, *trans*		37.649	31.425	$C_{20}H_{38}O_2$	Cyclooxygenase activity [39]

1-(*p*-Toluidino)-1-deoxy-β-D-idopyranose		37.156	31.290	$C_{13}H_{19}NO_5$	No activity reported
Cyclopentaneundecanoic acid, methyl ester		40.676	28.427	$C_{17}H_{32}O_2$	Antioxidant, anticancer [40,41]
Undecanoic acid, 11-bromo-, methyl ester		33.820	4.564	$C_{12}H_{23}BrO_2$	Antioxidant [42]
10,12-Octadecadienoic acid		16.731	1.884	$C_{18}H_{28}O_2$	No activity reported
α-D-Mannopyranoside, methyl 2,3,4,6-tetra-O-methyl		35.286	1.109	$C_{11}H_{22}O_6$	Antimicrobial [43,44]
11-Tetradecen-1-ol, (E)		28.969	0.654	$C_{14}H_{28}O$	Anti-inflammatory, Antipyretic [45]
Decanoic acid, 10-bromo-, methyl ester		27.581	0.357	$C_{11}H_{21}BrO_2$	No activity reported
11-(2-Cyclopenten-1-yl) undecanoic acid, (+)		42.648	0.210	$C_{16}H_{28}O_2$	No activity reported
cis-Z-11,12-Epoxytetradecan-1-ol		29.880	0.072	$C_{14}H_{28}O_2$	No activity reported
9,12-Octadecadienal		31.283	0.008	$C_{18}H_{32}O$	Anti-inflammatory, antioxidant [46,47]

undecanoic acid was found as major component in leaves of *Lepidagathis keralensis* with reported antioxidant activity [40]. Antioxidant properties of methyl 10-undecenoate based lipoconjugates were determined by Narra *et al.* [42]. Mannopyranoside may be targeted for future studies for its usage as a broad spectrum antibiotic [43].

Conclusion

In this study, *Physalis minima* Linn. was investigated to identify the presence of bioactive compounds. A total of 11 compounds were identified from the methanolic extract with reported biological activities. The extract exhibited significant acetylcholine esterase inhibition and antioxidant activities *in vitro*. Isolation and translation of individual bioactive compounds from *P. minima* would help to find new drugs with potential biological activity.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. M.I. Choudhary, *Pure Appl. Chem.*, **73**, 555 (2001); https://doi.org/10.1351/pac200173030555
2. V. Schulz, *Phytomedicine*, **10**, 74 (2003); https://doi.org/10.1078/1433-187X-00302
3. M. Ashraf, K. Ahmad, I. Ahmad, S. Ahmad, S. Arshad, S.M.A. Shah and F.H. Nasim, *J. Med. Plants Res.*, **5**, 2086 (2011).
4. A. Favier, *Ann. Pharm. Fr.*, **64**, 390 (2006); https://doi.org/10.1016/S0003-4509(06)75334-2
5. Z. Chen, R. Bertin and G. Froldi, *Food Chem.*, **138**, 414 (2013); https://doi.org/10.1016/j.foodchem.2012.11.001
6. B.K. Gopalkrishna, Flora of Udupi, Indian Naturalist (Regd.) Publications: Udupi, India, edn 1, p. 418 (2003).
7. K.R. Kirtikar and B.D. Basu, Indian Medicinal Plants, Lalit Mohan Basu Publication: Allahabad, India, edn 2, p. 1766 (2003).
8. V.V. Chi, The Dictionary of Medicinal Plant in Vietnam, Medical Publishing House: Hanoi, Vietnam, p. 929 (2012).
9. V.C. Le Canh, V. Le Ba,P.T.H. Yen, L.L. Thi, P.T.T. Hoai, T.T.H. Dat, D.T. Thao, L.G. Bach, Y.H. Kim and H.L.T. Anh, *Nat. Prod. Res.*, (2019); https://doi.org/10.1080/14786419.2019.1650360
10. S.S. Wei, C.Y. Gao, R.J. Li, L.Y. Kong and J. Luo, *Chin. J. Nat. Med.*, **17**, 469 (2019).
11. Y.Z. Guan, S.M. Shan, W. Zhang, J.G. Luo and L.Y. Kong, *Steroids*, **82**, 38 (2014); https://doi.org/10.1016/j.steroids.2014.01.004
12. A.K. Gupta, K.P. Shivalinge Gowda, R.N. Umashankar, R. Nandeesh and S. Sreedhar, *Res. J. Pharmacol. Pharmacodyn.*, **5**, 332 (2010).
13. S.N. Yoganarasimhan, Medicinal Plants of India, Karnataka: Interline Publishing Pvt. Ltd., vol. 1, p. 363 (1996).
14. K. Mohana, R. Uma and K.K. Purusothaman, *Indian J. Exp. Biol.*, **17**, 690 (1979).
15. S. Sudhakaran, B. Ramanathan and A. Ganapathi, *Pharm. Biol.*, **37**, 269 (1999); https://doi.org/10.1076/phbi.37.4.269.5809
16. J. Wu, X. Li, J. Zhao, R. Wang, Z. Xia, X. Li, Y. Liu, Q. Xu, I.A. Khan and S. Yang, *Phytochemistry*, **155**, 164 (2018); https://doi.org/10.1016/j.phytochem.2018.08.009
17. R.J. Li, C.Y. Gao, C. Guo, M.M. Zhou, J. Luo and L.Y. Kong, *Inflammation*, **40**, 401 (2017); https://doi.org/10.1007/s10753-016-0485-1
18. R. Lin, Y.Z. Guan, R.J. Li, X.M. Xu, J.G. Luo and L.Y. Kong, *Chem. Biodivers.*, **13**, 884 (2016); https://doi.org/10.1002/cbdv.201500282
19. M.A. Khan, H. Khan, S. Khan, T. Mahmood, P.M. Khan and A. Jabar, *J. Enzyme Inhib. Med. Chem.*, **24**, 632 (2009); https://doi.org/10.1080/14756360802321120
20. O.K. Leong, T.S.T. Muhammad and S.F. Sulaiman, *Evid. Based Complement. Alternat. Med.*, **2011**, 185064 (2011); https://doi.org/10.1093/ecam/nep057
21. K.L. Ooi, T.S. Tengku Muhammad and S.F. Sulaiman, *J. Ethnopharmacol.*, **150**, 382 (2013); https://doi.org/10.1016/j.jep.2013.09.014
22. K.L. Ooi, T.S.T. Muhammad, C.H. Lim and S.F. Sulaiman, *Integr. Cancer Ther.*, **9**, 73 (2010); https://doi.org/10.1177/1534735409356443
23. J. Wu, T. Zhang, J. Si, Q. Xu, Y. Gu, S. Yang and Z. Zou, *Fitoterapia*, **140**, 104413 (2020); https://doi.org/10.1016/j.fitote.2019.104413
24. L. Ma, X.W. Gan, Q.P. He, H.Y. Bai, M. Arfan, F.C. Lou and L.H. Hu, *Helv. Chim. Acta*, **90**, 1406 (2007); https://doi.org/10.1002/hlca.200790143
25. N. Shrivastava, K. Shah, T. Patel and K. Jiwan, *Indian J. Pharm. Sci.*, **73**, 111 (2011); https://doi.org/10.4103/0250-474X.89770
26. N. Shariff, M.S. Sudarshana, S. Umesha and P. Hariprasad, *Afr. J. Biotechnol.*, **5**, 946 (2006).
27. M.I. Choudhary, S. Yousaf, S. Ahmed, S. Samreen, K. Yasmeen and Atta-ur-Rahman, *Chem. Biodivers.*, **2**, 1164 (2005); https://doi.org/10.1002/cbdv.200590086
28. M.I. Choudhary, S. Yousuf, Samreen, S. Ahmed and Atta-Ur-Rahman, *Nat. Prod. Res.*, **21**, 877 (2007); https://doi.org/10.1080/14786410701315147
29. D. Sathish Kumar, S.N. Raju, A. Harani, B. David, K.N. Rao and B. Otalia, *Pharmacogn. J.*, **4**, 273 (2009).
30. N. Pellegrini, R. Ke, M. Ying and C. Rice-Evans, *Methods Enzymol.*, **299**, 379 (1999); https://doi.org/10.1016/S0076-6879(99)99037-7
31. B. Halliwell and J.M.C. Gutteridge, Free Radicals in Biology and Medicine, Clarendon Press, vol. 3, p. 617 (1989).
32. S. Padilla, T.L. Lassiter and D. Hunter, *Methods Mol. Med.*, **22**, 237 (1999); https://doi.org/10.1385/0-89603-612-X:237
33. V. Lobo, A. Patil, A. Phatak and N. Chandra, *Pharmacogn. Rev.*, **4**, 118 (2010); https://doi.org/10.4103/0973-7847.70902
34. S. Chandra, S. Khan, B. Avula, H. Lata, M.H. Yang, M.A. El Sohly and I.A. Khan, *Evid. Based Complement. Alternat. Med.*, **2014**, 1 (2014); https://doi.org/10.1155/2014/253875
35. I. Fatima, T. Hussain, M. Rafay, M. Akram, S. Bano and S. Shabbir, *Pak. J. Pharm. Sci.*, **30**, 1625 (2017).
36. C. Scully, *Dent. Update*, **20**, 95 (1993).
37. N.O. Martins, I.M. de Brito, S.S.O. Araujo, G. Negri, E.A. Carlini and F.R. Mendes, *BMC Complement. Altern. Med.*, **18**, 172 (2018); https://doi.org/10.1186/s12906-018-2222-9
38. E. Giacobini, *Neurochem. Int.*, **32**, 413 (1998); https://doi.org/10.1016/S0197-0186(97)00124-1
39. L. Dong, A.J. Vecchio, N.P. Sharma, B.J. Jurban, M.G. Malkowski and W.L. Smith, *J. Biol. Chem.*, **286**, 19035 (2011); https://doi.org/10.1074/jbc.M111.231969
40. L. Palakkal, N.H. Zeinul Hukuman and J. Mullappally, *J. Appl. Pharm. Sci.*, **7**, 182 (2017); https://doi.org/10.7324/JAPS.2017.70626
41. J.K. Akintunde and A.K. Babaita, *Middle East Fertil. Soc. J.*, **22**, 211 (2017); https://doi.org/10.1016/j.mefs.2017.02.005
42. N. Narra, S.S. Kaki, R.B.N. Prasad, S. Misra, K. Dhevendar, V. Kontham and P.V. Korlipara, *Beilstein J. Org. Chem.*, **13**, 26 (2017); https://doi.org/10.3762/bjoc.13.4
43. S.M. Kawsar, M.O. Faruk, M.S. Rahman, Y. Fujii and Y. Ozeki, *Sci. Pharm.*, **82**, 1 (2014); https://doi.org/10.3797/scipharm.1308-03
44. P.R. Ashton, E.F. Hounsell, N. Jayaraman, T.M. Nilsen, N. Spencer, J.F. Stoddart and M. Young, *J. Org. Chem.*, **63**, 3429 (1998); https://doi.org/10.1021/jo9804184
45. H.H. Imad, J.H. Hussein, A.K. Muhanned and S.H. Nidaa, *J. Pharmacog. Phytother.*, **7**, 107 (2015); https://doi.org/10.5897/JPP2015.0349
46. B.R. Kim, H.M. Kim, C.H. Jin, S.Y. Kang, J.B. Kim, Y.G. Jeon, K.Y. Park, I.S. Lee and A.R. Han, *Plants*, **9**, 717 (2020); https://doi.org/10.3390/plants9060717
47. N. Gurnani, M. Gupta, D. Mehta and B.K. Mehta, *J. Taibah Univ. Sci.*, **10**, 462 (2016); https://doi.org/10.1016/j.jtusci.2015.06.011
48. F. Mazhar, R. Khanum, M. Ajaib and M. Jahangir, *Pak. J. Pharm. Sci.*, **28**, 2053 (2015).