Fatty Acid Profiling and In Vitro Antihyperglycemic Effect of Leucas cephalotes (Roth) Spreng via Carbohydrate Hydrolyzing Enzyme Inhibition

Anjali Verma, Anil Kumar, Dalip Kumar Upreti, Veena Pande, Mahesh Pal

Phytochemistry Division, CSIR-National Botanical Research Institute, Lucknow, Uttar Pradesh, 1Plant Diversity, Systematics and Herbarium Division, CSIR-National Botanical Research Institute, Lucknow, Uttar Pradesh, India, 2Department of Biotechnology, Kumaun University, Nainital, India

Submitted: 16-09-2016 Revised: 08-11-2016 Published: 07-04-2017

ABSTRACT

Background: Leucas cephalotes has been used by many tribes to treat variety of diseases and known to have many essential secondary metabolites. To the best of our knowledge, it is the first comparative analysis of total fatty acid (FA) composition and α-amylase inhibition activity of L. cephalotes. Objective: The present study is carried out to explore the antihyperglycemic activity and FA contents of all parts of L. cephalotes. Material and Method: Fruits, leaves, stems, and roots part of L. cephalotes have been extracted in hexane. Simultaneously, all plant parts have been extracted in hexane with Soxhlet extraction. Ethanolic extracts have been evaluated for antihyperglycemic activity and hexane extract have been analyzed for FA identification. Result: The present study indicated that ethanolic extract of fruit and leaves have shown significant α-amylase inhibitory activity with IC$_{50}$ value of 92.86 ± 0.89 and 98.09 ± 0.69 mg/mL, respectively. FA composition of all the parts of L. cephalotes was analyzed by GC/MS. Nineteen FAs have been identified in all parts of L. cephalotes in which palmitic acid, oleic acid, linolenic acid, and linoleic acid were major FAs. Conclusion: The study indicates that L. cephalotes has significant potential to inhibit α-amylase enzyme and it is a rich source of essential FAs.

Key words: α-amylase, diabetes, fatty acid, GC-MS

SUMMARY

L. cephalotes has significant antidiabetic activity and will be beneficial for diabetic patients to reduce the starch breakdown and helps in reduction of postprandial hyperglycemia. It can be used in the formulation of diabetic drugs.

INTRODUCTION

Diabetes mellitus (DM) is a global epidemic and most common endocrine disorder represents one of the most serious clinical as well as public health problem worldwide. Most common DM is type 2, which is rapidly rising as a global healthcare problem and is expected to reach pandemic levels by 2030.[1] There are many factors responsible for an active role in proper functioning of pancreatic β-cells and one of the most important role played by free fatty acids (FFAs) and inhibition of carbohydrate hydrolyzing enzymes (α-amylase, β-glucosidase). FFAs have an active role in lipid-signaling pathway in glucose-stimulated insulin secretion in healthy β-cells, while carbohydrate-hydrolyzing enzymes inhibition stops the starch breakdown. FFA stimulates insulin secretion from islets of Langerhans by coupling response with pancreatic β-cells receptors.[2] Chronicity of hyperglycemia is often associated with long term damage and initiator of diabetic macrovascular complications such as retinopathy, neuropathy and nephropathy;[3] Hyperglycemia characterized by rapid increase in postprandial blood glucose level due to the hydrolysis of starch by pancreatic α-amylase.[4] The most common therapeutic approach for decreasing the postprandial blood glucose level by the inhibition of carbohydrate hydrolyzing enzymes, α-amylase, and α-glucosidases in the digestive tract.[5] Therefore, targeting these enzymes will be key strategy in the control of diabetes. Many traditional medicinal plants are used by tribes for treatment of diabetes. Leucas cephalotes (Roth) Spreng. (synonym: Phlomis cephalotes) belongs to the family Labiatae or Lamiaceae; also known...
as “Dronapushpi” in Sanskrit and “Gumma” in local language, a rainy season weed and used as an edible extensively by Uttar Pradesh (tribal people), Bihar, and many other rural peoples in India. The plants as whole or different parts were used by many tribes to treat variety of diseases.[4] In Ayurveda, it has been recommended for inflammation, psoriasis, scabies, chronic skin eruptions, edema, diaphoresis, chronic malaria, asthma, eye diseases, jaundice, paralysis, and obstinate urinary troubles.[5] Leaves juice is used to treat psoriasis, skin eruptions, scabies, and urinary complaints. L. cephalotes whole herb contains new labdane, norlabdane, and abietane type diterpenes and protostane type triterpenes, together with common triterpene, five sterols, and eight flavones.[6] The seed oil of Dronapusphi containing 25% Labellenic acid (octadeca-5,6-dienoic acid), lauric acid, tridecanoic acid, adipic acid, and glutaric acid has been reported.[7]

FFAs have an important role in insulin secretion, and in this study, our aim was to envisage the FA composition and carbohydrate hydrolyzing enzyme via α-amylase inhibitory properties in plant parts such as fruits, leaves, stems, and roots.

**MATERIAL AND METHOD**

**Plant material**

The plant material was collected in August 2014 from the Sitapur district of Uttar Pradesh (India). The plant was identified by Dr. Anand Prakash, Scientist at Taxonomy Division of CSIR-National Botanical Research Institute (CSIR-NBRI), Lucknow. Each part of the plant was sliced into small pieces and air dried in the shade, grounded into powder, and subjected to hexane and ethanolic extraction. All chemicals and reagents used in this experiment were purchased from Sigma-Aldrich. All chemicals were analytical grade.

**Soxhlet extraction**

The powdered fruits, leaves, stems, and roots, each 100 g, were extracted with 500 mL of hexane (40–60°C) in a Soxhlet apparatus for 10 h. The extract was cooled to room temperature and evaporated (IKA-RV 10 digital) under reduced pressure at 40°C.

**Solvent extraction**

The powdered fruits, leaves, stems, and roots, each 400 g, were extracted with ethanol by maceration process for 72 h.

### Table 1: FA composition (%) in hexane extracts of different parts of L. cephalotes

| Fatty acid     | Fruits          | Roots          | Leaves         | Stems          |
|----------------|-----------------|----------------|----------------|----------------|
| Caprylic acid  | 1.69 ± 0.055    | 1.83 ± 0.31    | 0.55 ± 0.07    | 1.71 ± 0.37    |
| Capric acid    | 0.60 ± 0.050    | 0.13 ± 0.026   | 0.67 ± 0.05    | 0.55 ± 0.05    |
| Lauric acid    | 1.27 ± 0.047    | 0.34 ± 0.05    | 1.49 ± 0.34    | 0.36 ± 0.06    |
| Azelaic acid   | 0.16 ± 0.025    | 0.22 ± 0.12    | 1.55 ± 0.36    | 0.42 ± 0.31    |
| Myristic acid  | 5.42 ± 0.051    | 5.84 ± 0.18    | 10.49 ± 0.38   | 9.70 ± 1.04    |
| Palmitic acid  | 16.94 ± 0.18    | 17.47 ± 0.47   | 15.76 ± 0.46   | 19.85 ± 0.27   |
| Palmitoleic acid| 4.14 ± 0.38    | 3.48 ± 0.39    | 1.52 ± 0.21    | 2.53 ± 0.43    |
| Margaric acid  | 5.25 ± 0.28     | 5.51 ± 0.37    | 4.77 ± 0.11    | 3.63 ± 0.31    |
| Oleic acid     | 16.63 ± 0.51    | 15.64 ± 0.46   | 13.74 ± 0.35   | 9.49 ± 0.41    |
| Linoleic acid  | 8.41 ± 0.48     | 7.96 ± 0.11    | 8.50 ± 0.06    | 7.52 ± 0.47    |
| Linolenic acid | 14.60 ± 0.38    | 13.81 ± 0.22   | 11.80 ± 0.21   | 10.53 ± 0.33   |
| Arachidic acid | 1.66 ± 0.20     | 1.22 ± 0.32    | 1.86 ± 0.26    | 2.48 ± 0.32    |
| Behenic acid   | 2.51 ± 0.40     | 2.46 ± 0.44    | 4.55 ± 0.36    | 3.54 ± 0.31    |
| Tricosanoic acid| 1.53 ± 0.34    | 1.64 ± 0.32    | 0.53 ± 0.06    | 0.90 ± 0.06    |
| Lignoceric acid| 2.60 ± 0.50     | 2.69 ± 0.33    | 3.79 ± 0.19    | 4.45 ± 0.27    |
| Pentacosyl acid| 1.52 ± 0.41     | 1.45 ± 0.23    | 2.71 ± 0.25    | 2.44 ± 0.29    |
| Cerotic acid   | 1.25 ± 0.29     | 1.43 ± 0.39    | 0.37 ± 0.07    | 0.85 ± 0.05    |
| Montanic acid  | 1.55 ± 0.47     | 1.49 ± 0.46    | 0.54 ± 0.06    | 0.78 ± 0.10    |
| Melissic acid  | 0.76 ± 0.13     | 0.39 ± 0.073   | 0.22 ± 0.05    | 0.39 ± 0.06    |
| Total          | 88.48 ± 0.29    | 83.82 ± 0.60   | 83.89 ± 0.78   | 79.85 ± 0.71   |

**Fatty acid methyl ester**

Fatty acid methyl ester (FAME) was prepared by the method described by Ichihara and Fukubayashi 2010.[8] The crude hexane extract (500 mg) of fruits, leaves, stems, and roots in concentrated sulfuric acid (2 mL) and methanol (20 mL) was heated under reflux on a water bath for 3 h. It was cooled to room temperature and extracted with petroleum ether (3 × 20 mL) and water in a separating funnel. The petroleum ether extract was dried over Na₂SO₄. The extract was dried under reduced pressure at 40°C. Prepared FAME was stored for further analysis. Qualitative and quantitative analysis of FAME of L. cephalotes were performed by using gas chromatography-mass spectrometry (GC-MS) on a Thermo Fisher TRACE GC ULTRA coupled with DSQ II Mass Spectrometer instrument using a TR 50MS column (30 m × 0.25 mm ID × 0.25 µm, film thickness). Identification of individual compounds was carried out by comparison of their mass spectra with those of the internal reference mass spectra library (NIST/Wiley) or with authentic compounds.

**α-Amylase inhibitory assay**

α-Amylase inhibitory activity assay was performed by using chromogenic method adopted from Sigma-Aldrich as described by Ali et al.[9] Crude 1.0 μg/mL α-amylase was dissolved in ice-cold distilled water. Starch (1% w/v) in 20 mM phosphate buffer (pH 6.9) containing 6.7 mM sodium chloride was used as a substrate solution. Experiments were performed in triplicate. Plant extract of 40 μL (1 mg/mL in DMSO), 160 μL of distilled water, and 500 μL of enzyme were added to the test tube, all tubes were incubated at 25°C for 10 min. After incubation, 500 μL of starch solution was added to the test tubes and again incubated at 25°C for 10 min. Now 1 mL coloring reagent was added to the test tubes and incubated at 80°C for 5 min; then the tubes were removed from the water bath and put in the ice flakes; and 9 mL of distilled water was added. The absorbance was recorded at 540 nm and calculated by the following formula:

\[
\text{Inhibition activity} \, (\%) = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{extract}} \times 100}{\text{Abs}_{\text{control}}}
\]

The IC₅₀ (inhibitor concentration at which 50% inhibition of the enzyme activity occurs) of all parts of the plant extracts was determined by performing the assay as described above with varying concentrations (40–160 μg/mL) of the ethanolic extracts. Acarbose was used as a
**RESULTS AND DISCUSSION**

Indian has a rich heritage of medicinal plants that have been used since the ancient times to treat many diseases, including diabetes. *L. cephalotes* is used by tribes for treatment of diabetes. In this present study, we focused on antihyperglycemic potential and essential FA study of *L. cephalotes*. All parts of *L. cephalotes* were extracted with ethanol and the percentage positive control in the concentration range 40–160 μg/mL. The IC$_{50}$ values were determined from plots of percent inhibition versus log inhibitor concentration and calculated by nonlinear regression analysis from the mean inhibitory values.

**FA analysis**

Hexane extract of *L. cephalotes* plant parts were derivatized into FAME in which fruits (0.22%) and leaves (0.21%) showed the highest yield of FAs percentage. FAs analysis of hexane extract of all parts of *L. cephalotes* was analyzed by GC-MS. The analysis enabled the identification of 19 FAs in fruits, leaves, stems, and roots. Comparative study of FAs in *L. cephalotes* fruits, leaves, stems, and roots are represented in Table 1. Major FFA present in all parts of *L. cephalotes* are represented in Figure 1B, in which the concentration of palmitic acid was higher in all parts of the plant. The present study revealed that *L. cephalotes* is a rich source of unsaturated and essential FA. Percentage yields of saturated FA, mono-unsaturated FA, and polyunsaturated FA presented in Figure 1A.

To the best of our knowledge, it is the first study of comparative analysis of FA of *L. cephalotes* plant parts. Insulin secreted from pancreatic β-cells response to elevated plasma glucose level, which is modified by various factors and one of the major factor is FFAs. FFAs is an important source of nutrients and also acts as signaling molecules in various cellular processes including insulin secretion.[12]

**Table 2**: Half maximal inhibitory concentration (IC$_{50}$) of *L. cephalotes* plant parts ethanolic extract in comparison with standard drug on porcine pancreatic α-amylase inhibitory activities. The results are represented as mean ± SD

| Plant extract | IC$_{50}$ value (μg/mL) |
|---------------|------------------------|
| Fruits        | 92.86 ± 0.89           |
| Leaves        | 98.09 ± 0.69           |
| Stems         | 218 ± 0.93             |
| Roots         | 109.00 ± 0.97          |
| Acarbose      | 88.28 ± 0.94           |

**Figure 1**: (A) The major classes of FAs. (B) Percentages of major FAs present in different parts of *L. cephalotes*

Major essential FAs in *L. cephalotes* are oleic acid, linoleic acid, linolenic acid, palmitic acid, and stearic acid, which are all beneficial for lowering body cholesterol.[13] Linoleic acid is one of the major constituent of all plant parts; it also helps to prevent diabetes and its late complications.[14]

Most of the saturated FAs like palmitic and stearic acid are used for dietary supplements; they increase the nutritional value of the product and add to the overall health benefits. Although FA composition of *L. cephalotes* seed has already been reported,[15] in the present study, whole plant has been found to have beneficial saturated and unsaturated FAs; thus, the study proves that *L. cephalotes* whole plant may be used as a novel source of beneficial FAs. Many studies have concluded when FFA level decreased in diabetic and obese patients; the level of insulin secretion is also decreased up to 30–50%.[16]

**α-Amylase inhibition assay**

The dose-dependent α-amylase inhibitory activity of ethanolic extracts of *L. cephalotes* all parts was tested in triplicates. Among these ethanolic extracts of all parts of the plants, maximum inhibition percentage was shown by fruits (79%) then leaves (78.59%) in compression to standard acarbose (80.34%) at 160 μg/mL, while roots (68.26%) showed moderate and stems (36.38%) showed the minimum inhibition percentage. Acarbose at concentrations (40–160 μg/mL) showed α-amylase inhibitory activity from 29.37 ± 0.69 to 80.34 ± 0.67 μg/mL, with an IC$_{50}$ value of 88.28 ± 0.94 μg/mL [Table 2]. Ethanolic extract of fruit showed highest inhibitory activity, which varied from 25.94 ± 0.68 to 78.83 ± 0.67 μg/mL, with an IC$_{50}$ value of 92.86 ± 0.89 μg/mL, whereas inhibitory activity in leaves varied from 21.80 ± 0.96 to 78.91 ± 0.37 μg/mL inhibition with IC$_{50}$ (98.09 ± 0.69 μg/mL) [Figure 2]. The significant (*P* < 0.001) decrease was found in starch breakdown at dose range from 120 to 160 μg/mL. Table 2 shows IC$_{50}$ (μg/mL) values of ethanolic extracts of different parts of *L. cephalotes*.

The approach to reduce postprandial glucose level by inhibiting α-amylase is an effective strategy for diabetes management.[17] This study revealed that the ethanolic extract of the fruits and leaves of *L. cephalotes* have potent α-amylase inhibitory activity. The present study, therefore, seems to be the first endeavor to reveal the potential α-amylase inhibitory activity in the ethanolic extract of fruits and leaves of *L. cephalotes*.

**CONCLUSION**

In our study, we found total 19 FAs, in which fruits showed highest area percentage 88.97% of FFAs as well as highest inhibition percentage of α-amylase. The result indicates that *L. cephalotes* has potential to inhibit α-amylase and this therapeutic potential could be beneficial in the
management of postprandial hyperglycemia in the treatment of type 2 DM. Further, this study directs future research in separating the bioactive compound responsible for this activity. The present investigation revealed that the FAs from the *L. cephalotes* can be used in various pharmaceutical products, as it contains different bioactive FAs.

**Acknowledgements**

The authors are thankful to the Director, CSIR-National Botanical Research Institute, Lucknow, India, for facilities and encouragements.

**Financial support and sponsorship**

The financial support received from Council and Scientific and Industrial Research, New Delhi, under the project “Bio-prospection PR (BSC-0106)” is duly acknowledged.

**Conflicts of interest**

There are no conflicts of interest.

**REFERENCES**

1. Salwa S, Ibrahim A, Mafauzy M, Syed ASS, Ayman SA, Mohamed AH. Current clinical status and complications among type 2 diabetic patients in Universiti Sains Malaysia hospital. Int J Diab Mell 2010;2:184-8.  
2. Gravena C, Mathias PC, Ashcroft S J H. Acute effects of fatty acids on insulin secretion from rat and human islets of Langerhans. J Endocrinol 2010;172:73-80.  
3. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 2001;414:813-20.  
4. Chipili T, Ibrahim MA, Singh M, Islam MS. *In vitro* α-amylase and α-glucosidase inhibitory effects and cytotoxic activity of Albizia antunesiana extracts. Pharmacogn Mag 2015;11:231-6.  
5. Charles LP, Rao KVB. Postprandial antihyperglycemic and antioxidant activities of Acalypha indica Linn stem extract: an in-vivo study. Pharmacogn Mag 2016;14:475-81.  
6. Ansari MY, Wadud A, Jabeen U, Irshad S. Pharmacognostic evaluation of Leucas cephalotes spreng leaves. Phcog J 2012;3:15-21.  
7. Kumar S, Mishra PK, Sori A, Sharma B. An Ayurvedic approach to diabetes mellitus-a review article. Int Ayur Med J 2016;4:419-21.  
8. Katara A, Pradhan CK, Tyagi AK, Singh P. Phytochemical investigation and antimicrobial activity of Leucas cephalotes Roth Spreng whole herb. Der Pharm Lett 2010;2:284-96.  
9. Bhoria R, Kansa S, Chaudhary M. Antifertility activity of chloroform and alcoholic flower extracts of Leucas cephalotes (Roth.) Spreng in albino rats. Int J Drug Dev Res 2013;5:168-72.  
10. Ishihara K, Fukubayashi Y. Preparation of fatty acid methyl ester for gas-liquid chromatography. J Lipid Res 2010;51:635-40.  
11. Ali H, Houghton PJ, Sournyanath A. α-Amylase inhibitory activity of some Malaysian plants used to treat diabetes, with particular reference to Phyllanthus amarus. J Ethnopharmacol 2004;107:449-55.  
12. Henquin JC. Triggering and amplifying pathways of regulation of insulin secretion by glucose. Diabetes 2000;49:1751-60.  
13. Albishri HM, Almaghrabi OA, Moussa TAA. Characterization and chemical composition of fatty acids content of watermelon and muskmelon cultivars in Saudi Arabia using gas chromatography/mass spectroscopy. Pharmacogn Mag 2013;9:58-66.  
14. Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev 2009;2:270-8.  
15. Daley CA, Abbott A, Doyle PS, Nader GA, Larson S. A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. Nutr J 2010;9:10.  
16. Campillo JE, Valdivia MM, Rodriguez E, Osoiro C. Effect of oleic and octanoic acids on glucose-induced insulin release in vitro. Diab Metab 1979;5:183-7.  
17. Unnikrishnan PS, Suthanthiran K, Jayasri MA. Alpha-amylase inhibition and antioxidant activity of marine green algae and its possible role in diabetes management. Pharmacogn Mag 2015;11:511-6.