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ORIGINAL ARTICLE

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

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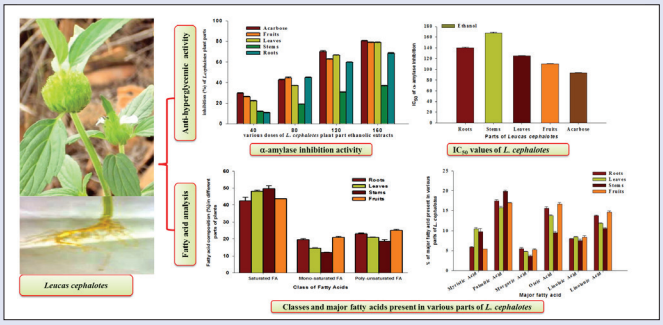
ABSTRACT

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 ethanol. 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 µg/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.
- *L. cephalotes* is rich source of essential FAs and may be used as a nutraceutical.
- Ethanol extract of fruits and leaves of *L. cephalotes* are showed the maximum α-amylase inhibition when compared with standard drug acarbose.



Abbreviations used: DM: Diabetes Mellitus, FA: Fatty Acid, FFAs: Free Fatty Acids, FAME: Fatty Acid Methyl Ester, IC50: Inhibitor Concentration, GC-MS: Gas Chromatography- Mass Spectrophotometer

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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 has 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

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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.[6] 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.[7] 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.[8] The seed oil of Dronapusphi containing 25% Labellenic acid (octadeca-5,6-dienoicc acid), lauric acid, tridecanoic acid, adipic acid, and glutaric acid has been reported.[9]

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.

Formation and quantification of FA methyl ester

Fatty acid methyl ester (FAME) was prepared by the method describe by Ichihara and Fukubayashi 2010.[10] 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 Na_2SO_4. 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.*[11] 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:

$$Inhibition\ activity\ (\%) = \frac{Abs_{(control)} - Abs_{(extract)} \times 100}{Abs_{(control)}}$$

The IC_{50} (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

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
Pentacosylic 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 ± 0.078	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

Table 2: Half maximal inhibitory concentration (IC_{50}) of L. cephalotes plant parts ethanolic extract in comparison with standard drug on acarbose 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

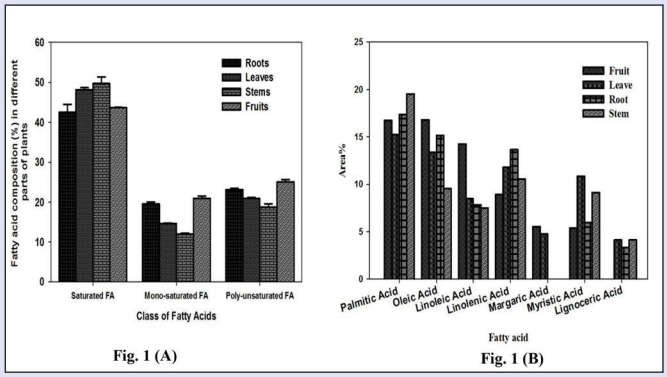

Figure 1: (A) The major classes of FAs. (B) Percentages of major FAs present in different parts of *L. cephalotes*

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.

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 extractive yield was 8.57% w/w (fruit), 6.93% w/w (leaves), 4.23% w/w (stem), and 3.35% w/w (roots), respectively.

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 listed in Table 1. Major FFAs 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 [Figure 1B]. 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 represented 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]

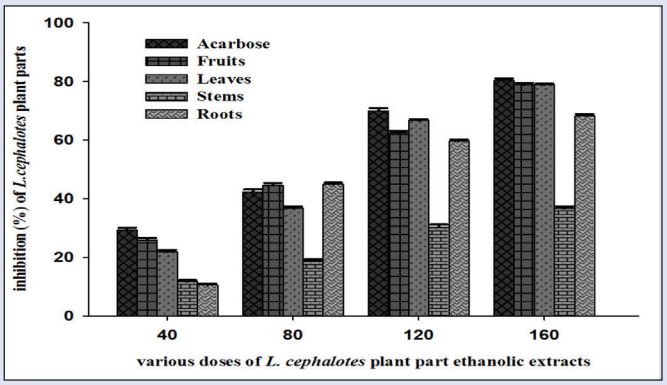

Figure 2: α-Amylase inhibitory activities of ethanolic extracts of *L. cephalotes* various plant parts by using acarbose as a standard

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 part 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.

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Conflicts of interest

There are no conflicts of interest.

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