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GC-MS Analysis, HPTLC Fingerprint Profile and Antidiabetic Activity of Methanolic Extract of *Perilla frutescens* Leaves

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The present study was aimed to investigate the phytochemical constituents of the different extracts of *Perilla frutescens* leaves and identification of the compounds by subjecting it to HPTLC and GC-MS analysis along with antidiabetic property of leaves of *P. frutescens.* The results from HPTLC finger print scanned at wavelength 200 nm for methanolic extract of *P. frutescens* leaf revealed the presence of seventeen polyvalent phytoconstituents. The HPTLC finger print scanned at wavelength 200 nm for ethanolic extract of *P. frutescens* leaves showed fifteen polyvalent phytoconstituents and corresponding ascending order of R_f values ranged from 0.02 to 0.78 in which highest concentration of the phytoconstituents was found to be 13.45 % and its corresponding R_f value was found to be 0.64. α-Amylase inhibition of methanolic extract of *P. frutescens* leaves was also performed. The standard (acarbose 0.5-2.0 mg/mL) revealed greatest α-amylase inhibitory action from 43.82 ± 0.12 to 88.14 ± 0.32% with IC_{50} value worth of 0.575 mg/mL. At a similar focus methanol concentrates of *P. frutescens* leaves showed the inhibitory action from 28.21 ± 0.11% to 68.01 ± 0.11%, with an IC_{50} worth of 1.15 mg/mL. The methanolic extract of *P. frutescens* leaves exhibited inhibition of α-amylase enzyme proved the antidiabetic potentiality of *P. frutescens* leaves.

Keywords: *Perilla frutescens,* Antidiabetic activity, α-Amylase inhibitors, HPTLC, GC-MS.

INTRODUCTION

Plants are used for the medicinal purposes in all countries of the world and are the source of a variety of potent drugs in the form of various primary and especially secondary metabolites. Plants have been known for thousands of years as the main source of medicine with absolute results [1]. Modern chemical and synthetic medications are frequently investigated for side effects [2], but plants are more natural, environmentally friendly and free of adverse effects [3]. People still choose plants based medications to synthetic medicines for the treatment of infectious and non-infectious disorders, despite all of the advantages of current synthetic treatments [4]. Diabetes mellitus is a non-infectious endocrine situation described *via* way of means of a disruption in carbohydrate metabolism [5,6]. It has been associated with plenty of great disorders, which include microvascular (nephropathy and retinopathy) and macrovascular peripheral vascular ailment and coronary heart diseases [7]. According to the International Diabetes Federation (IDF), diabetes is an ailment that impacts 415 million humans worldwide, with that range predicted to upward thrust to 642 million *via* way of means of 2040 [8]. India is likewise identified because the world's diabetes capital, affecting generally rural and concrete populations [9].

Herbal medications are becoming more popular in healthcare due to their safety, efficacy and lack of adverse effects [10]. Higher plants are being studied by researchers as a source for novel lead structures and the development of standardized phytotherapeutic agents [11]. Modern chromatographic techniques such as HPTLC and GC-MS fingerprinting are effective for identifying and quantifying the phytochemical contents of plant material, as well as for standardizing herbal medication formulation [12-14]. *In vitro* screening procedures are thought to be beneficial in providing the findings for selecting crude plant extracts with therapeutic potential for future chemical and pharmacological research [15].

Perilla frutescens L. is a Lamiaceae plant in the interior and remote villages of India's Uttarakhand state, generally referred to as Bhangira where people use the seeds as a food and folklore medicine to treat a variety of ailments including cough, allergy, depression, anxiety, tumors, intoxication and some intestinal problems, as well as cancers, infectious diseases and cardiovascular diseases. The plant's stem is used as a pain reliever and anti-abortive agent in northern India, while the leaves are thought to be beneficial for asthma, colds and flu treatment [16]. In Nepal, the leaf juice of this plant is used to remove intestinal worms and cure cuts and wounds [17].

A thorough literature review of the plant used in this study revealed that few published publications on the probable phytochemical components of *Perilla frutescens* leaves around the world [18-20]. As a result, the current study attempted to evaluate the probable phytochemical contents by first creating different extracts of *Perilla frutescens* leaves and then separating and identifying the compounds using HPTLC and GC-MS analysis, as well as the antidiabetic property of *Perilla frutescens* leaves.

EXPERIMENTAL

Perilla frutescens L. was collected from the local village Sahiya of Chakrata, Dehradun, India, during the months of October and November 2021 for this study. The plant was validated and a voucher specimen was sent to The Himalaya Drug Company's Division of Pharmacognosy in Dehradun, India. The leaves were separated and sun-dried after being rinsed thoroughly with distilled water. After the removal of foreign matters, dust and grime, the leaves were dried. A grinder was used to ground the leaves into powder. Zurera *et al*. [21] approach was used with minor modifications.

Preparation of extracts: In a closed flask, powdered plant material of *Perilla frutescens* leaves (5 g) was extracted individually with 100 mL of methanol, ethanol, chloroform and *n*-hexane. For continuous shacking, the flasks were left on the shaker for 6 h and then the entire flask was left for 24 h. All extracts were filtered and concentrated before being used.

High performance thin-layer chromatography (HPTLC) study was reformed by following Reich & Schibli's guidelines [22].

Sample prepartion: Previously prepared extracted were diluted in organic solvents and 10 µL of the solution was loaded as a 10 mm band length in the plate format 100 mm Merck, TLC plate's silica gel 60 F_{254} using a LINOMAT 5 applicator connected to a CAMAG HPTLC machine controlled by winCAT software. The chromatogram was generated in a 20 × 10 cm Twin Trough Chamber (TTC) saturated with mobile phase. Methanol: chloroform = 90:10, saturation time 20 min. After development, the plate was dried at room temperature for 5 min.

UV-visible analysis: The plate in photo-documentation, images were captured at white light, UV 254 nm and UV 366 nm.

Scanning: The plate was mounted on the CAMAG TLC Scanner 3 and scanning was performed at a wavelength of 200 mm. Each compound's retention factor (R_f) value was separated on a plate and the percent peak area of each band was recorded.

Derivatization: The generated plate was placed in an iodine chamber saturated with vapours of resublimed iodine for 5 min before being photographed under white light.

GC-MS analysis

Sample preparation: Using the cold maceration process, a 5 g powder of leaves was extracted into 50 mL of AR grade methanol. After that, the mixture was filtered and the solvent was evaporated on a water bath at 80 ± 2 ºC until dry. The semisolid liquid/extract was kept at 4 ºC in refrigerator until used.

GC-MS analysis: A methanolic extract of *P. frutescens* L. leaves was injected into a Shimadzu GCMSQP2010 Ultra system with a selective mass detector with an ion source of 230 and 270 ºC surface temperature. The following was the analysis instrument's operation: Preheated the oven to 140 ºC for 6 min, then increase to 280 ºC for 31 min at 10 ºC/min. The transporter gas was helium at a velocity of 41.6 cm/s and the sample injection was 1.0 µL. The elucidation of mass spectra was carried out by comparing the obtained spectral fragmentation with the database provided by WILEY8 and the NIST Library and the evaluation of compounds/components was based on the retention time (RT) for GC.

α-Amylase inhibitor assays: The starch-iodine test was carried out as described by Xiao *et al.* [23], with a few changes, to screen methanolic leaves extract of perilla for α-amylase inhibitors. The chromogenic DNSA technique was also used to perform the inhibitory assay by following Miiller [24] and Hara & Honda [25] methods with few modifications.

The IC_{50} values were calculated as per the concentration of extract containing the α-amylase was applied to calculate the Inhibition percentage.

$$\text{Inhibition } (\%) = \frac{A_{540\ (control)} - A_{540\ (extract)}}{A_{540\ (control)}} \times 100$$

RESULTS AND DISCUSSION

The HPTLC chromatograms of the four distinct *Perilla frutescens* leaf extracts (methanol, chloroform, ethanol and *n*-hexane) are displayed in Fig. 1. The greatest results were observed at 200 nm after scanning and the absorbance values at 254 nm, 366 nm and the visible light range (400-600 nm after spraying with iodine). All the four HPTLC chromatograms revealed that all sample constituents are well separated, with no tailing or diffuseness. The presence of 17 polyvalent phytoconstituents was detected in a methanol extract of perilla leaves using an HPTLC fingerprint scanned at 200 nm (Table-1). The R_f values were in the range of 0.02 to 0.91 (Table-1) and the chromatogram in Fig. 1a demonstrate that out of 17 components, the components with R_f values of 0.81 and 0.72 were determined to be the most prominent, with percentage areas of 21.86% and 14.18%, respectively. The HPTLC fingerprint for ethanolic extract of *P. frutescens* leaves scanned at 200 nm revealed 15 phytoconstituents with corresponding ascending order R_f values ranging from 0.02 to 0.78, with the maximum phytoconstituent concentration of 13.45 % and its associated R_f value of 0.64 (Table-3, Fig. 1c). For chloroform and *n*-hexane

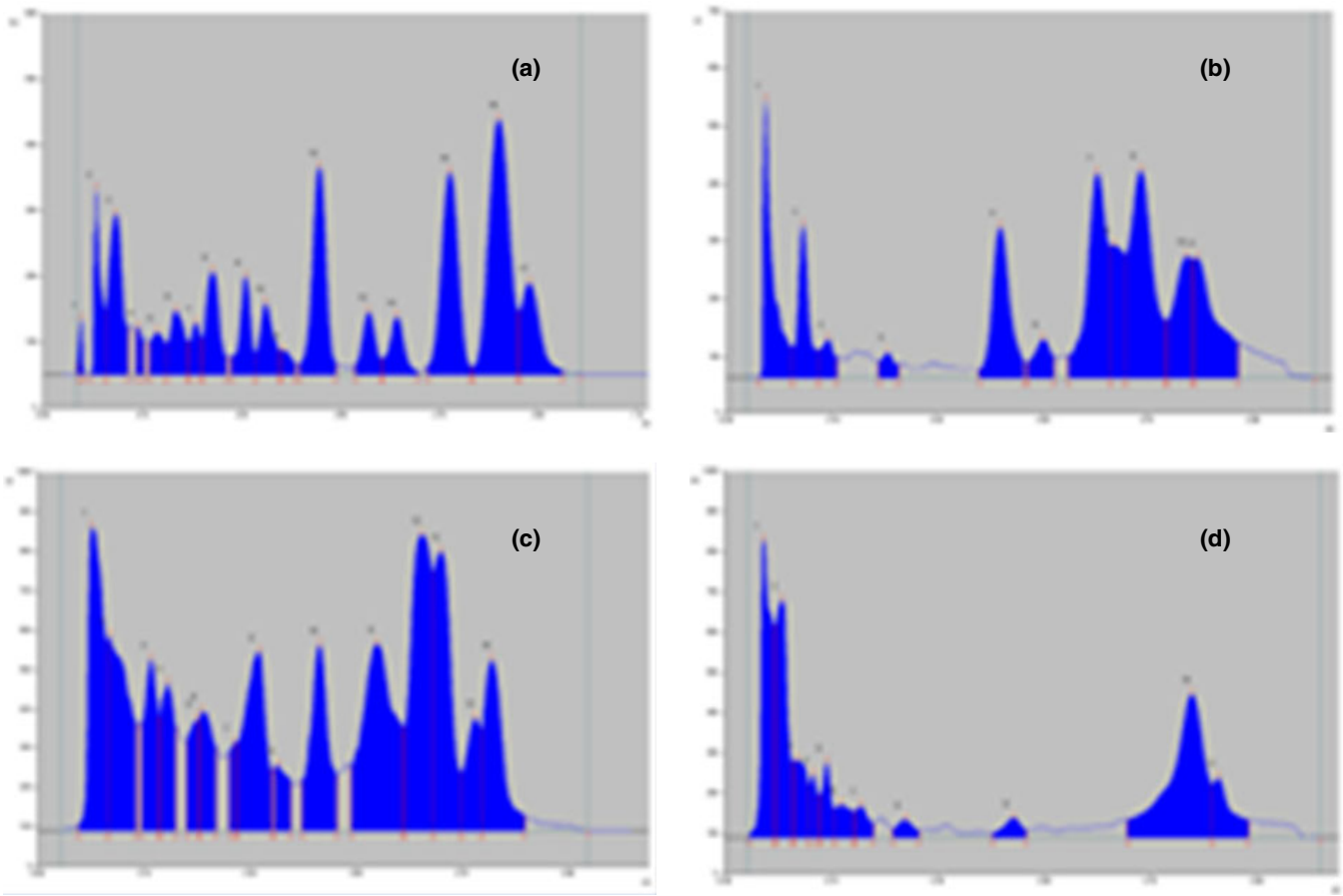

Fig. 1. HPTLC chromatograms of (a) methanol, (b) chloroform, (c) ethanol, (d) *n*-hexane of *Perilla frutescens* extracts

TABLE-1
PEAK TABLE WITH R_f VALUES, HEIGHTS AND AREA OF SCAN DATA OF METHANOLIC LEAVES EXTRACT OF *Perilla frutescens* (Bhangira) AT 200 nm

Peak	Start position (R_f)	Start height (AU)	Max position (R_f)	Max height (AU)	Max (%)	End position (R_f)	End height (AU)	Area (AU)	Area (%)
1	0.02	19.4	0.02	80.0	3.11	0.03	0.2	909.4	0.72
2	0.04	0.90	0.05	281.9	10.44	0.07	37.9	5909.0	1.68
3	0.07	98.7	0.09	243.6	9.02	0.12	71.3	11221.9	8.88
4	0.13	69.9	0.14	71.5	2.65	0.15	49.7	1500.5	1.19
5	0.16	48.9	0.18	63.1	2.34	0.19	47.0	2866.3	2.27
6	0.20	47.2	0.21	96.5	3.57	0.24	48.9	4922.2	3.89
7	0.24	48.9	0.26	77.4	2.87	0.27	53.9	2405.9	1.90
8	0.27	54.1	0.29	156.0	5.78	0.32	26.8	7209.0	5.70
9	0.33	26.6	0.36	148.8	5.51	0.37	31.6	5418.3	4.29
10	0.37	32.1	0.40	107.9	4.00	0.43	35.1	5039.7	3.99
11	0.43	35.1	0.43	38.8	1.36	0.46	15.9	1310.6	1.04
12	0.46	15.3	0.50	315.9	11.69	0.54	17.0	14625.2	11.57
13	0.58	13.9	0.60	95.3	3.53	0.63	23.4	4263.2	3.37
14	0.63	23.4	0.66	87.6	3.24	0.70	3.7	4378.5	3.46
15	0.72	11.0	0.77	307.4	11.38	0.81	10.5	17926.6	14.18
16	0.81	10.6	0.87	387.7	14.35	0.91	95.9	27630.7	21.86
17	0.91	96.2	0.93	139.4	5.16	1.00	3.6	8849.7	7.00

extract, the HPTLC fingerprint scanned at 200 nm revealed eleven polyvalent phytoconstituents (Table-2, Fig. 1b; and Table-4, Fig. 1d). The R_f values for chloroform ranged from 0.01 to 0.83, with a maximum concentration of 20.14 % at R_f 0.71 (Table-2, Fig. 1b) and -0.01 to 0.86 for *n*-hexane extract, with a maximum concentration of 30.93 % at R_f 0.70 (Table-4, Fig. 1d).

GC-MS studies: Fig. 2 shows the gas chromatogram and mass spectra of a methanolic extract of *Perilla frutescens* leaves. The extract was subjected to GC-MS analysis, which

TABLE-2
PEAK TABLE WITH R_f VALUES, HEIGHTS AND AREA OF SCAN DATA OF CHLOROFORM LEAVES EXTRACT OF *Perilla frutescens* (Bhangira) AT 200 nm

Peak	Start position (R_f)	Start height (AU)	Max position (R_f)	Max height (AU)	Max (%)	End position (R_f)	End height (AU)	Area (AU)	Area (%)
1	0.01	0.9	0.02	483.6	18.85	0.07	57.7	14131.0	10.61
2	0.07	55.1	0.09	264.3	10.30	0.12	49.2	8806.2	6.61
3	0.12	49.4	0.14	68.2	2.66	0.16	37.6	2938.3	2.21
4	0.23	30.6	0.25	46.1	1.80	0.27	25.2	2235.3	1.68
5	0.43	15.8	0.47	262.5	10.23	0.51	28.8	14866.4	11.16
6	0.52	29.0	0.55	69.2	2.70	0.57	37.2	3753.4	2.82
7	0.59	40.0	0.65	357.7	13.94	0.68	28	2307.23	17.32
8	0.68	228.1	0.69	231	9.01	0.71	15.7	8786.2	6.60
9	0.71	215.6	0.74	362.8	14.14	0.78	2.2	26824.1	20.14
10	0.78	101.7	0.82	211.5	8.24	0.83	6.2	12511.1	9.39
11	0.83	206.3	0.84	208.7	8.14	0.92	53.6	15283.9	11.47

TABLE-3
PEAK TABLE WITH R_f VALUES, HEIGHTS AND AREA OF SCAN DATA OF ETHANOL LEAVES EXTRACT OF *Perilla frutescens* (Bhangira) AT 200 nm

Peak	Start position (R_f)	Start height (AU)	Max position (R_f)	Max height (AU)	Max (%)	End position (R_f)	End height (AU)	Area (AU)	Area (%)
1	0.02	13.3	0.05	771.0	11.60	0.08	77.7	36248.0	9.79
2	0.08	478.3	0.08	495.9	7.46	0.13	71.7	32998.7	8.92
3	0.14	276.7	0.16	435.9	6.56	0.18	92.5	17832.9	4.82
4	0.18	293.0	0.19	375.8	5.65	0.21	34.4	15262.1	4.12
5	0.23	228.7	0.25	285.4	4.29	0.25	33.3	9247.3	2.50
6	0.25	283.2	0.26	306.2	4.61	0.28	5.8	13043.5	3.52
7	0.31	193.0	0.32	224.9	3.38	0.32	17.6	4439.1	1.20
8	0.32	219.9	0.36	455.5	6.86	0.39	54.4	31721.0	8.57
9	0.39	158.7	0.40	164.4	2.47	0.42	21.7	6776.9	1.83
10	0.45	126.2	0.48	472.3	7.11	0.51	38.9	26683.1	7.21
11	0.54	165.4	0.59	474.8	7.15	0.64	64.2	48802.5	13.19
12	0.64	264.9	0.67	751.3	11.31	0.69	58.6	49778.3	13.45
13	0.69	658.8	0.71	708.5	10.66	0.75	46.3	38242.2	10.33
14	0.75	146.4	0.77	288.2	4.34	0.78	59.9	13188.4	3.56
15	0.78	260.7	0.80	434.7	6.54	0.87	36.9	25819.7	6.98

TABLE-4
PEAK TABLE WITH R_f VALUES, HEIGHTS, AND AREA OF SCAN DATA OF HEXANE LEAVES EXTRACT OF *Perilla frutescens* (Bhangira) AT 200 nm

Peak	Start position (R_f)	Start height (AU)	Max position (R_f)	Max height (AU)	Max (%)	End position (R_f)	End height (AU)	Area (AU)	Area (%)
1	-0.01	3.4	0.02	737.4	28.30	0.04	26.7	24797.7	20.38
2	0.04	527.0	0.06	587.9	22.56	0.07	36.7	21658.6	17.80
3	0.07	186.9	0.08	191.7	7.36	0.11	30.1	8242.4	6.77
4	0.11	130.8	0.11	155.3	5.96	0.12	5.6	3457.0	2.84
5	0.12	108.8	0.14	184.2	7.07	0.15	70.9	5408.2	4.44
6	0.15	71.2	0.17	79.9	3.07	0.19	35.7	3896.7	3.20
7	0.19	65.4	0.2	77.2	2.96	0.23	34.9	3156.0	2.59
8	0.26	21.5	0.29	43.6	1.67	0.31	16.8	2246.6	1.85
9	0.45	8.7	0.49	46.8	1.80	0.51	17.6	2745.6	2.26
10	0.7	43.8	0.83	354.8	13.62	0.86	31.9	37639.8	30.93
11	0.86	132.1	0.87	146.6	5.63	0.93	43.0	8451.8	6.94

confirmed the presence of 16 main components (Table-5). Many more minor peaks in the extract of *Perilla frutescens* leaves reflect different phytochemicals in trace amounts. The chemicals discovered in GC-MS analysis have a wide range of biological characteristics. *n*-Hexadecanoic acid (7.03%) present in the this extract has anti-inflammatory, antioxidant, hypocholesterolemic nematicide, pesticide, antiandrogenic taste, hemolytic, 5-α reductase inhibitor and powerful mosquito larvicide among the discovered phytochemicals [26-28]. 1,2-Benzenedicarboxylic acid, *bis*(2-methylp) (11.05 %) inhibits α-glucosidase

TABLE-5
MAJOR COMPOUNDS IDENTIFIED IN THE METHANOL EXTRACT FROM *Perilla frutescens* (Bhangira) LEAVES

	Peak#	Retention time	Area (%)	Name	
1.0	2	8.253	1.68	*cis*-3-Ethyl-endo-tricyclo[5.2.1.0 (2.6)]decane	Not reported
2.0	6	9.511	2.39	1-Adamantylm-tolyloxyacetate	Not reported
3.0	7	9.934	14.04	1- (Furan-2-yl)-4-methylpentan-1-one	Not reported
4.0	10	10.471	1.98	4- (2-Methylcyclohex-1-enyl)-but-2-enal	Not reported
5.0	11	10.707	16.98	4- (2-methyl-1-cyclohexen-1-yl)-2-butenal	Not reported
6.0	15	11.493	1.03	2-Furancarboxamide,N-propyl-	Not reported
7.0	16	12.661	2.11	(2,6,6-Trimethylcyclohex-1-enylmethanesulfonyl) benzene	Not reported
8.0	18	13.086	4.69	*trans*-.α-Bergamotene	Not reported
9.0	21	14.373	1.39	3,6-Diethyl-3,6-dimethyltricyclo[3.1.0.0~2,4~]h	Not reported
10.0	28	17.407	11.05	1,2-Benzenedicarboxylicacid,*bis* (2-methylp	Antimicrobial and α-glucosidase inhibition and the *in vivo* hypoglycemic efficacy [23]
11.0	32	18.440	7.03	*n*-Hexadecanoic acid	Anti-inflammatory, antioxidant [24], hypocholesterolemic nematicide, pesticide, antiandrogenic flavor, hemolytic, 5α-reductase inhibitor [25], potent mosquito larvicide [26]
12.0	37	19.761	3.70	2-Hexadecen-1-ol,3,7,11,15-tetramethyl-,[r-[r	Not reported
13.0	38	20.156	10.47	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	Antieczemic, antiacne hypocholesterolemic, nematicide, antiarthritic, hepatoprotective, antiandrogenic, hypocholesterolemic, nematicide, 5α-reductase inhibitor antihistaminic, anticoronary, insectifuge, antieczemic [23]
14.0	42	21.955	1.25	Galactopyranosid,1-thiooctyl-	Not reported
15.0	51	28.285	3.21	Squalene	Oxygen-scavenging agent [27], antitumor [28]
16.0	56	36.869	1.46	γ-Sitosterol	Antihyperlipidemic activity, antidiabetic [23]

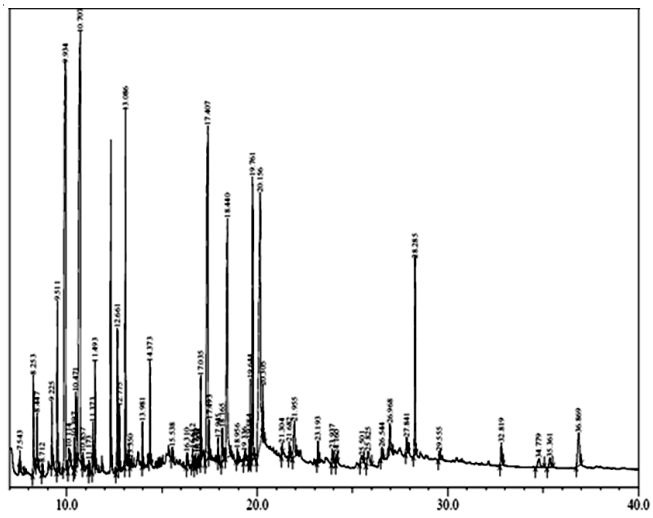

Fig. 2. GC-MS analysis of methanolic extract of leaves of *Perilla frutescens* (Bhangira)

and has hypoglycemic effect *in vivo* [29]. γ-Sitosterol (1.46%) is an unsaturated plant sterol with antihyperlipidemic and antidiabetic properties [29]. Squalene (3.21%) is a triterpene which exhibit anticancer and oxygen-scavenging properties [30,31]. 9,12,15-Octadecatrienoic acid, (*Z*,*Z*,*Z*)-10.47% known as linolenic acid have the property of antieczemic, antiacne hypocholesterolemic, antiarthritic, hepatoprotective, antiandrogenic, hypocholesterolemic, nematicide, 5-α reductase inhibitor antihistaminic, anticoronary and insectifuge [32].

Antidiabetic activity: The α-Amylase inhibition of methanolic extract of *Perilla frutescens* leaves was also performed. The standard (acarbose, 0.5-2.0 mg/mL) revealed the greatest α-amylase inhibitory action from 43.82 ± 0.12 to 88.14 ± 0.32% (Table-6) with IC_{50} value worth of 0.575 mg/mL. At a similar focus methanol concentrates of *P. frutescens* leaves showed the inhibitory action from 28.21 ± 0.11% to 68.01 ± 0.11% with an IC_{50} value of 1.15 mg/mL (Table-7).

TABLE-6
α-AMYLASE INHIBITION OF STANDARD DRUG

Concentration (mg/mL)	Inhibition (%)
0.00	0.0
0.50	43.82 ± 0.12
1.00	78.12 ± 0.22
1.50	82.55 ± 0.18
2.00	88.14 ± 0.32

TABLE-7
α-AMYLASE INHIBITION OF METHANOLIC EXTRACT OF *Perilla frutescens* (L.) LEAVES

Concentration (mg/mL)	Inhibition (%)
0.00	0.0
0.50	28.21 ± 0.11
1.00	43.47 ± 0.18
1.50	65.12 ± 0.09
2.00	68.01 ± 0.11

Conclusion

A methonlic extract of *Perilla frutescens* leaves revealed a plethora of active chemical compounds, which contribute to the plant's primary and secondary metabolic processes, resulting in a wide range of medicinal properties. The present study suggested that extract possess the secondary metabolites, which have different activities like antimicrobial, antioxidant, anti-

cancer, antidiabetic activities, and thus supports their folkloric use of *P. frutescens* leaves. The *in vitro* antidiabetic activity of methanolic extract of *P. frutescens* leaves was assessed using α-amylase inhibitory assay. Based on the inhi-bitory assay, it was estimated that the methanolic extract leaves inhibited the α-amylase enzyme. The results of the α-amylase inhibitory assay demonstrated the antidiabetic potential of *P. frutescens* leaves. Thus, the HPTLC and GC-MS analysis is the first step toward understanding the nature of active principles in medicinal plants and will be useful for further detailed research. However, isolating individual secondary metabolites and investigating their biological activity would provide impetus for further research into this plant's antidiabetic potential.

CONFLICT OF INTEREST

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

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