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FULL ARTICLE

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Polyphenol-rich Indian ginger cultivars ameliorate GLUT4 activity in C2C12 cells, inhibit diabetes-related enzymes and LPS-induced inflammation: An in vitro study

Meenakshi Venkateswaran[1] | Sasidharan Jayabal[1] | Shanmugam Hemaiswarya[2] | Selvakumar Murugesan[1] | Subudhi Enkateswara[3] | Mukesh Doble[2] | Sureshkumar Periyasamy[1]

[1]Department of Biotechnology, Anna University, BIT-Campus, Tiruchirappalli, India

[2]Bioengineering and Drug Design Lab, Department of Biotechnology, Indian Institute of Technology Madras, Chennai, India

[3]Center for Biotechnology, Siksha O Anusandhan University, Bhubaneswar, India

Correspondence
Mukesh Doble, Bioengineering and Drug Design Lab, Department of Biotechnology, Indian Institute of Technology Madras, Chennai 600036, India.
Sureshkumar Periyasamy, Department of Biotechnology, Anna University, BIT-Campus, Tiruchirappalli 620024, India.
Email: drsureshbiotech2003@gmail.com

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Abstract

Diabetes is a chronic metabolic disorder that results in distorted insulin signaling and microvascular complications. Current antidiabetic drugs possess harmful long term side effects, necessitating the need for alternate or compliment therapy with lesser issues. Medicinal plants such as ginger have been reported to possess several beneficial activities including antidiabetic activity. The antidiabetic efficacy of microwave-assisted polyphenolic extracts of Indian ginger cultivars from Odisha (MPO) and Tamil Nadu (MPT) is reported here. MPT and MPO showed insulin stimulated glucose uptake of 1.74 ± 0.25 and 1.47 ± 0.15 fold at 6.25 μg/ml of concentrations in C2C12 cells respectively when compared to control. MPT possessed α–amylase, α–glucosidase inhibitory and anti-glycation properties. It also showed DPPH radical scavenging activity (7.69 ± 0.001%), inhibited LPS-induced nitric oxide production (1.06 ± 0.004 fold) than the latter and increased the GLUT4 protein expression by 1.4 fold. Major active compounds such as shogaol and gingerol derivatives, curcumene, zingiberone were identified through GC-ESI/MS analysis and D-pinitol (cyclitol) was identified through HPLC analysis in this variety. This is the first paper to report the presence of an antidiabetic compound, D-pinitol, in the ginger variety. Polyphenol rich, biologically potent ginger extracts can be a good food and nutraceutical supplement to address diabetes and related complications.

Practical applications

Ginger is a native spice of South Asian Countries including India. Ginger extracts possess several medicinal properties such as anti-inflammatory, antidiabetic and antioxidant activities. It is used to treat nausea, vomiting and commonly used as a food flavouring agent and dietary food supplement. Our study shows the antidiabetic, anti-glycation and antioxidant efficacy of polyphenol rich Indian ginger cultivars grown in different geographical regions. Variations in the biological activities between the MPT and MPO ginger variety was observed. Different environmental conditions and their corresponding metabolite accumulation can be correlated with

Meenakshi Venkateswaran and Sasidharan Jayabal are the authors have contributed equally.

the better activity shown by MPT variety. It showed an increased GLUT4 expression even at a lower dose of 6.25 μg/ml. Ginger cultivar, especially MPT variety can be used as an adjuvant therapy for treating diabetes. Therefore, our study indicates that polyphenols rich ginger cultivar has major application in functional food product development.

KEYWORDS

active compounds, antioxidant, diabetes, GC-MS, ginger, GLUT 4, HPLC

1 | INTRODUCTION

Diabetes is a chronic metabolic disorder interrelated with inappropriate insulin levels, ageing, obesity and lifestyle changes (Maruthur, 2013). Nearly 700 million people are expected to be diabetic by the year 2045 Saeedi et al. (2019). Around, 75% of glucose uptake occurs in skeletal muscle via GLUT 4 translocation, which is the rate limiting step for regulated glucose homeostasis (Yoshizaki et al., 2009).

There is a strong interlink between hyperglycemia and advanced glycation end product product (AGEP) formation which increases oxidative stress, inflammation and leads to β-cell dysfunction (Ruiz et al., 2020). Oxidative stress produced as result of radical oxygen species such as hydroxyl ions, super oxide anions causes insulin resistance, a marked feature of type 2 diabetes and elevates the microvascular ailments (Ighodaro, 2018). The disrupted insulin signaling mechanism leads to hyperglycemic condition which needs proper antidiabetic therapy to efficiently sensitize the insulin action or reduce the post prandial blood glucose level. Though the current synthetic drugs like metformin, glibenclamide are efficient in treating diabetes, they have parallel complaints of side effects such as incidence of lactic acidosis in 10% of patients prescribed with metformin (DeFronzo et al., 2016). Herbal therapy approach is believed to be effective with lesser side effects and accessible to nearly 80% of global population (Pan et al., 2014).

Ginger (*Zingiber officinale Rosc.*), belonging to the Zingiberaceae family, is widely used in South East Asian countries especially in Indian medicinal system for its anti-inflammatory, anti-diabetic, antioxidant and anti-arthritic properties (Murugesan et al., 2020). As per the Indian spice board data, India is one of the largest producers and exporter (about 50,410 tonnes) of ginger worldwide (www.indianspices.com). Their pungent phenolic constituents including gingerol, shogaols have multiple reports for antidiabetic and antioxidant action (Mao et al., 2019).

1.1 | Nutritional and medicinal importance of ginger

Ginger is used as fresh or dried form in food as well as in traditional folkware medicines. It is used as a flavoring agent because of its pungent aroma and flavor from the active compounds like gingerol, its derivatives and essential oils. It is used to treat morning sickness, stomach aches, dyspepsia, cough, and bronchial infections (Ali et al., 2008).

Studies are available regarding the nutritive and medicinal value of Ginger. Jiang et al. (2008) reported that ginger extract (containing 53% of 6-gingerol) fed rats obtained maximum concentration of 6-gingerol within half an hour of oral feeding, rapidly absorbed and concentrated on gastro intestinal tracts. Clinical studies revealed that ginger extract had equal curative effect as diclofenac in osteoarthritis associated gastropathy and found to be safer than the steroids (Drozdov et al., 2012).

Fresh ginger contains polyphenolics such as 6, 8, 10-gingerol, which upon eventual storage and drying gets transformed to shagaol. They also possess other phenolics (zingerone, gingerenone-A), terpenes (α-curcumene, zingiberene, and β-sesquiphellandrene) and essential oils (Yeh et al., 2014).

Dietary polyphenols are potent antidiabetic agents whose therapeutic effect depends on the bioavailability. Though they are abundantly accumulated in the plant extracts, their absorption and distribution is only in the micromolar levels. It can be advantageous while utilizing polyphenols rich ginger extract for diabetic therapy as they have quicker bioavailability (Catalkaya et al., 2020).

Schwertner et al. (2006) reported the quantitative variations in 6, 8, 10-gingerol contents in the ginger varieties collected from different localities through HPLC metabolomics profiling and phylogenetic analysis. Definite differences in the gingerol contents were observed in the ginger-root dietary supplements obtained from pharmacies and health food stores Jiang et al. (2006). Recently, Gaur et al. (2016) found that agro-climatic variation and several environmental conditions influences the phytochemical accumulation and biological activities of the ginger cultivars.

In this present work, major ginger producing Indian cultivars from the states of Odisha and Tamil Nadu were chosen for the comparative antidiabetic study (Vijayan et al., 2020). Microwave-assisted polyphenolic extraction in ginger was carried out to obtain maximum yield and activity (Sun et al., 2020). Glucose uptake activity of the ginger cultivars was assessed in C2C12 mouse skeletal muscle cell lines. α-amylase and α-glucosidase inhibitory effect, advanced glycation end product formation (AGEP) activity, antioxidant activity (DPPH, LPS-induced nitric oxide (NO) scavenging assay) were investigated for both the varieties. The best variety was chosen for the GLUT 4 protein expression study and the polyphenolic compounds were determined through GC-ESI/MS and HPLC analysis.

2 | MATERIALS AND METHODS

2.1 | Materials

C2C12 skeletal muscle cell line was purchased from NCCS, Pune, India. Dulbecco modified Eagle's medium (DMEM), fetal bovine serum (FBS), 1% antibiotic-antimycotic liquid solution and trypsin were purchased from Gibco, Life Technologies Corporation, USA. Dimethyl sulfoxide (DMSO) and recombinant human insulin of molecular biology grade were brought from Himedia, India. 2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose (2-NBDG) was purchased from Molecular Probes labeling and detection technologies, USA. Radioimmuno precipitation assay buffer (RIPA buffer) was obtained from Thermo scientific, USA. Vinculin was purchased from Sigma Aldrich, USA and Rabbit anti-GLUT4 antibody from Novus Biologicals, USA. Solvents of analytical grade were used for microwave extraction and HPLC analysis (Himedia, India) and the plastic wares were purchased from Tarsons, India.

2.2 | Preparation of extracts

Indian ginger cultivars were collected from Koraput district, Odisha and Nilgiris district, Tamil Nadu (Authentication number: HARS-S-56 and BSI/SRC/5/23/2018/TECH/2,242 respectively) and subjected to polyphenol extraction. Initially, ginger was washed, shadow dried and powdered. Nearly, 35 g of ginger powder was defatted with 125 ml of hexane for 3 hr, dried and the residual ginger powder was extracted in microwave at a power of 40 watts for 10 min with intermittent on and off using methanol-water mixture (80:20) and continuously extracted for 48 hr. Supernatant was collected and the residue was again extracted with aqueous methanolic extract for 24 hr. The collected supernatants were fractionated using ethyl acetate, concentrated in rotary vacuum evaporator and stored at −20°C for further studies (Venkateswaran et al., 2019; Yang et al., 2009).

2.3 | Determination of total polyphenol content

The total polyphenol content in the MPT and MPO extracts was estimated using modified protocol of Akinola et al. (2014). Ginger extracts (1 mg/ml) was added to 0.5 ml of Folin–Ciocalteu reagent, made up to 3 ml with distilled water and allowed to stand for 5 min. Later, 2 ml of 20% sodium carbonate and incubated for 60 min at room temperature. Gallic acid was used as the standard and the total polyphenol content was expressed as milligram of gallic acid equivalent per gram of the extract (mg GAE/g). Absorbance was measured at 760 nm.

2.4 | Cell culture

C2C12 mouse skeletal muscle cell lines were taken for the study. Myoblasts were cultured in DMEM having 10% FBS, 1% antibiotic solution under 5% CO_2 condition at 37°C. For myotube differentiation, media was replaced with 2% FBS every alternative day. Formation of myotubes were observed at day 5 of differentiation and used for further experiments.

2.5 | MTT assay

C2C12 cells were incubated with different concentrations of MPO and MPT varieties prepared in DMSO (1–100 μg/ml) for 24 hr to determine the cell cytotoxicity using the 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay Hemaiswarya and Doble (2013).

2.6 | NBDG assay

Based on the cell viability assay, three different concentrations (12.5, 6.25, and 3.125 μg/ml) were tested for the glucose uptake efficiency. The basal and insulin stimulated glucose uptake activities were assessed using NBDG, fluorescence based assay. Myotubes were serum starved for 4 hr and then cultured in low glucose medium supplemented with ginger extracts for 16 hr. The cells treated with 80 μM NBDG prepared in 1% BSA for 1 hr and stimulated with 100 nM insulin for 30 min and the cells were washed with ice cold PBS followed by cell lysis buffer and the lysates were added to 96 black well microtiter plate to measure the fluorescence at an excitation wavelength of 467 nm and emission wavelength of 542 nm using fluorescence microtiter plate reader (PerkinElmer, USA) Kim et al. (2017).

2.7 | In vitro anti diabetic (α –amylase and α-glucosidase enzyme inhibitory) assays

The inhibitory effect of ginger extracts to regulate the postprandial sugar elevation was studied here. The percentage of inhibition was calculated using the formula. % of inhibition = ((Absorbance $_{control}$ − Absorbance $_{sample}$)/Absorbance $_{control}$) × 100). Acarbose was used as the standard. MPO and MPT (6.25 μg/ml) were incubated with 250 μl of α-amylase enzyme solution (0.5 mg/ml) for 10 min at 25°C. Then, 250 μl of 1% starch solution was added and incubated for 10 min at 25°C. The reaction was terminated with the addition of 500 μl of 3, 5- dinitrosalicylic acid (DNSA) reagent (10 g sodium potassium tartrate, 0.05 g sodium sulfate, 0.2 g phenol, 5 N NaOH, and 1 g DNSA) and kept in a boiling water bath for 5 min, diluted with distilled water and the absorbance was measured at 540 nm..

Regarding α- glucosidase inhibitory activity, ginger extracts (80 μl of 6.25 μg/ml of concentration), 100 μl of 4 mM 4-nitrophenyl beta-D-glucopyranoside (PNPG) substrate and 20 μl of 1U/ml of yeast alpha-glucosidase enzyme was added, incubated at 37°C for 20 min and the ability of ginger extracts to prevent the enzyme binding with PNPG was tested by measuring the absorbance at 405 nm Apostolidis et al. (2007).

2.8 | Advanced glycation end product (AGEP) inhibition assay

AGEP is produced as a result of a non-enzymatic action between the endogenous glucose and protein, which upon binding to its receptor produces radical scavengers that aggravates diabetic complications like diabetic retinopathy and neuropathy. The antiglycation effect of ginger extracts is evaluated as per the protocol of (Vinson & Howard, 1996). The reaction mixture contains 10 mg/ml of BSA, which acts as a model protein in causing glycation of sugars. To that, sodium benzoate (0.02%), glucose (0.2M) and fructose (0.2M), ginger extracts (6.25 μg/ml) were added and incubated at 37°C for 7 days. Ascorbic acid was used as the standard. The inhibitory fold calculation was done by measuring the absorbance at an excitation/emission wavelength of 350/450 nm.

2.9 | In vitro antioxidant assays

2.9.1 | DPPH scavenging assay

The antioxidant activity of the ginger cultivars (MPT and MPO-6.25 μg/ml) was tested using DPPH assay. To 0.1 ml of 0.4 μM DPPH (2,2-diphenyl-1-picrylhydrazyl) methanolic solution, 6.25 μg/ml of MPO and MPT extracts were added, incubated in dark for 30 min and the absorbance was read at 517 nm (Blois, 1958).

2.9.2 | LPS-induced nitric oxide scavenging assay in RAW cells

Nitric oxide is the major mediator of inflammation, which is investigated here. RAW 264.7 cells were treated with ginger extracts (6.25 μg/ml) for 1 hr and stimulated with LPS (1 μg/ml) for 1 hr. After incubation, equal volume of Griess reagent (1% sulfanilamide, 0.1% napthyl ethylene diamine hydrochloride, and 2.5% ortho phosphoric acid) was added and the absorbance was read at 540 nm to test the presence of nitric oxide evolved during the reaction. Ascorbic acid was used as the standard. Untreated cells and cells treated with LPS alone were used as the control (Han et al., 2013).

Western blot analysis

Western blot analysis was performed for the samples, MPT with insulin, untreated control, and insulin alone. Briefly, C2C12 myotubes were treated with better acting extract (MPT-6.25 μg/ml) for 16 hr and stimulated with insulin (100 nm) for 30 min. Cells lysates were extracted with RIPA buffer, sonicated, and quantified using Bradford assay and subjected to western blot analysis to study the GLUT 4 protein expression (Nankar & Doble, 2015). Samples including MPT with insulin, untreated control, and insulin alone were run through SDS–PAGE, separated and transferred to polyvinylidene fluoride membrane. Blocking was done for 1h with 5% skimmed milk prepared in 1X PBST. Primary antibody (GLUT 4– 1:2000 dilution in 3% skim milk) was added, incubated overnight at 4°C, washed thrice in 1X TBST (Tris-Buffered Saline with Tween-20) for 10 min. Furthermore, it was incubated with HRP (Horse Radish Peroxidase) conjugated secondary antibody for 1h (1:5,000 dilution in 3% skim milk) and rinsed thrice in 1X TBST. Vinculin of 1:10,000 was used for normalization. Enhanced chemiluminescence kit was used for protein bands detection and quantitation was done using image lab software (BioRad, U.S.A).

Gas chromatography- electron spray ionization mass spectroscopy (GC-ESI MS) analysis

GC- MS analysis was performed for the MPT extract with initial silylation using N, O- bis (trimethylsilyl) trifluoroacetamide (BSTFA) in a Clarus 500 Gas Chromatograph (Perkin Elmer, USA).The GC capillary column of ZB-5 ms (30 m × 0.25 mm, film), USA was used and the column temperature was maintained at 250°C with an injection flow volume of 1 μl Proestos et al. (2008). Helium was used as the carrier gas at a flow rate of 1 ml/min. Mass analyzer was used in the scanning range *m/z* 100–1000 at a scan time of 250 ms with electron impact mode (70 eV) injector. The bioactive compounds were identified in the MPT ginger variety based on their elution order in the column, fragment ions at m/z and literature reports. The retention time, molecular formula, m/z value and the area (%) of the bioactive compounds in MPT variety were tabulated.

High performance liquid chromatography (HPLC) analysis

For the identification and quantitation of D-pinitol in MPT extract, Phenomenex Rezex ROA with organic acid H^+ ion-exclusion column was used. Different concentrations of D-pinitol (100, 250, 500, 750, and 1,000 μg/ml in water) were prepared and standard calibration curve was obtained to concentration D-pinitol concentration in MPT extract (1 mg/ml). 5mM of H_2SO_4 was used as an isocratic mobile phase at a flow rate of 0.6 ml/min and maintained at 50°C (Ravindran et al., 2020).

Statistical analysis

All the experiments were performed in triplicate and results expressed as mean ± S.D. One way ANOVA was employed for analysis of the data.

3 | RESULTS AND DISCUSSION

3.1 | Total polyphenol contents in ginger cultivars

Polyphenols are potent antioxidants that have therapeutic activity in delaying diabetes pathogenesis, which is primarily evaluated in the two Indian ginger cultivars. The total polyphenol content was found to be higher in MPT variety (286.0 ± 0.28 mg GAE/g) than the MPO variety (195.28 ± 1.89 mg GAE/g) as shown in Table 1. In general, quantitative and qualitative variations in active compounds amongst ginger varieties were reported, which depends on the physiological conditions and environmental factors such as soil nature,

temperature and precipitation levels Tao et al. (2009). Ghasemzadeh et al. (2010) reported variation in the accumulation of phenol, flavonoid content in Malaysian ginger varieties cultured under controlled environmental condition as similar to our study.

3.2 | Effect of ginger extracts on glucose uptake assay

Cell culture study suggests that both the ginger varieties did not show cytotoxicity against C2C12 cells up to 100 μg/ml of concentration (Data not shown). From sub inhibitory concentrations tested (12.5, 6.25, and 3.125 μg/ml), 6.25 μg/ml showed better glucose uptake activity which was chosen for further studies. Both the MPO and MPT ginger variety increased the insulin stimulated glucose uptake in the C2C12 myotubes as shown in Figure 1. MPT ginger extract significantly increased the glucose uptake up to 1.74 ± 0.25 fold which is significantly higher than the MPO variety (1.47 ± 0.15 fold) denoting that the ginger varieties can be used as an adjuvant therapy with insulin in diabetes treatment. Both the varieties exhibited better activity than the standard drug, metformin tested at 800 μm concentration (1.09 ± 0.12). A similar kind of dose dependent glucose uptake activity was shown by the freeze-dried ethyl acetate ginger extract. Nearly, 2 fold of glucose uptake was observed at 20 μg/ml in the L6 myotubes (Li et al., 2014).

TABLE 1 Total Polyphenol Content in MPT and MPO ginger extracts. (MPO- Microwave assisted Polyphenolic extract of Odisha variety and MPT- Microwave assisted Polyphenolic extract of Tamil Nadu variety)

Total Polyphenol Content (1 mg/ml)	
MPT	286 ± 0.0007 mg GAE/g
MPO	194 ± 0.004 mg GAE/g

3.3 | In vitro anti diabetic assays

In the α-amylase inhibitory assay, MPT and acarbose inhibited α-amylase (by 22.8 ± 0.02% and 31.2 ± 0.01%, respectively). MPO and MPT showed 55.4 ± 0.001% and 56.1 ± 0.0002% of α-glucosidase activity, respectively, but not higher than acarbose (62.2 ± 0.0007%) ($p < .005$) (Figure 2a,b). This study clearly shows that both the varieties could act as potent glucosidase inhibitors. During the prevention of complex sugars breakdown by synthetic inhibitors, the unconverted sugars get fermented in colon and cause flatulence and gastrointestinal disturbances in patients (Derosa & Maffioli, 2012). It is expected that ginger extracts could efficiently inhibit these enzymes and reduce the post prandial sugar surge with lower side effects.

3.4 | AGEP inhibitory reaction

MPO and MPT varieties showed 3.39 ± 0.12 and 3.50 ± 0.33 fold inhibition with respect to the control, respectively which were not statistically different ($p = .61$), but they were better than the activity of the standard ascorbic acid (2.11 ± 0.40 fold) ($p < .01$) (Figure 3). Since the AGEP can actively increase the free radical production, it is expected that a drug acting as an antidiabetic and antioxidant agent may curtail the diabetic complications. Starowicz and Zieliński (2019) reported the lesser inhibitory action by aqueous ethanolic extract of ginger with bovine serum on AGEP formation. It is expected that polyphenols have better affinity with proteins and prevent the glycosylation and AGEP formation than any other phytocompounds (Sadowska-Bartosz et al., 2014).

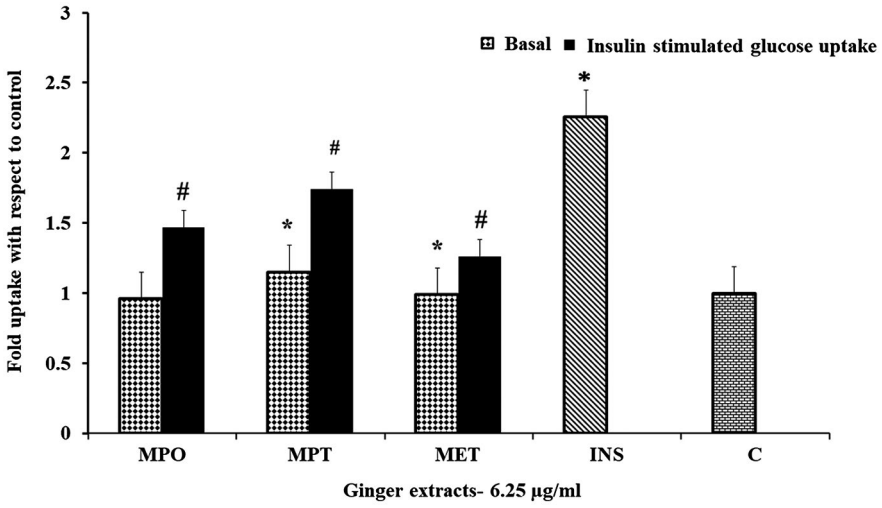

FIGURE 1 Effect of ginger extracts at 6.25 μg/ml on basal and insulin stimulated glucose uptake in C2C12 cells. (*)$p < .05$ when compared to untreated control, (#)$p < .05$ when compared to untreated control. Microwave-assisted Polyphenolic extract of Odisha variety (MPO) and Microwave-assisted Polyphenolic extract of Tamil Nadu variety (MPT), Metformin (MET) (800 μM), Insulin (INS) (100 μM), C-Untreated control

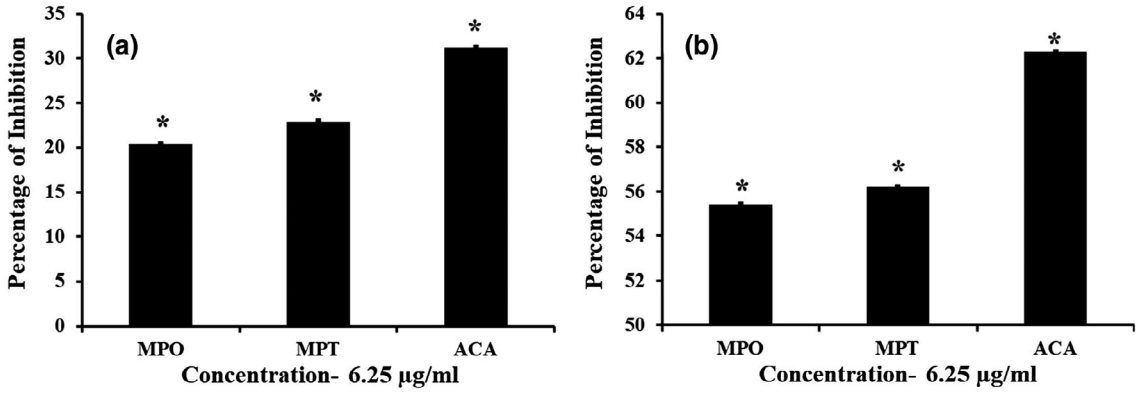

FIGURE 2 Effect of ginger extracts at 6.25 µg/ml (a) α-amylase inhibitory activity; (b) α-glucosidase inhibitory activity. (*)$p < .05$ when compared to untreated control. Microwave-assisted Polyphenolic extract of Odisha variety (MPO) and Microwave-assisted Polyphenolic extract of Tamil Nadu variety (MPT), Acarbose (ACA)

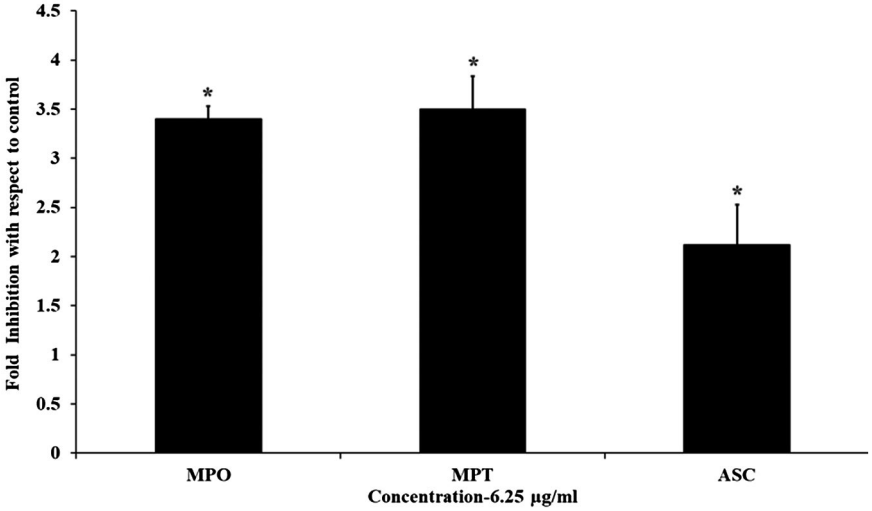

FIGURE 3 Advanced Glycation End Product inhibitory (AGEP) activity of ginger extracts. (*)$p < .05$ when compared to untreated control. Microwave-assisted Polyphenolic extract of Odisha variety (MPO) and Microwave-assisted Polyphenolic extract of Tamil Nadu variety (MPO), Ascorbic Acid (ASC)

3.5 | In vitro antioxidant assays

3.5.1 | DPPH scavenging assay

The DPPH inhibitory activity at 6.25 µg/ml of concentration was found to be 3.86 ± 0.001% for MPO and 7.69 ± 0.001% for MPT extract that was similar to the activity of ascorbic acid of 7.71 ± 0.03% ($p < .005$) as denoted in Figure 4a. Oboh et al. (2010) reported that Nigerian variety of red ginger showed increased antioxidant property than the white ginger due to the increased flavonoids content in the former variety at 12.5 mg/ml of concentration.

3.5.2 | Nitric oxide scavenging assay

In the NO scavenging assay, LPS acted as a potential inducer of inflammation and free radical production. MPO variety could significantly reduce the nitrite production up to 1.06 ± 0.004 fold with respect to control ($p < .005$) and ascorbic acid showed 1.14 ± 0.02 fold of inhibition (Figure 4b). Though Tamil Nadu variety showed 1.14 ± 0.001 fold inhibition, it was not statistically significant. Improved activity can be possibly due to microwave extraction at optimized conditions to yield abundant polyphenolics that act as ROS scavenger (Rahath Kubra et al, 2013).

3.6 | Selection of potent extract

It could be observed that Tamil Nadu variety showed consistently better activity than the Odisha variety. MPT showed increased polyphenol content, better glucose uptake, antioxidant, and AGEP inhibitory activities than MPO. In general, ginger varieties possesses active constituents such 6, 8, 10- gingerol, shogals, but quantitatively varies in different cultivars depending on the physiological conditions and environmental factors such as soil nature, humidity, temperature, and precipitation levels (Tao et al., 2009). To support

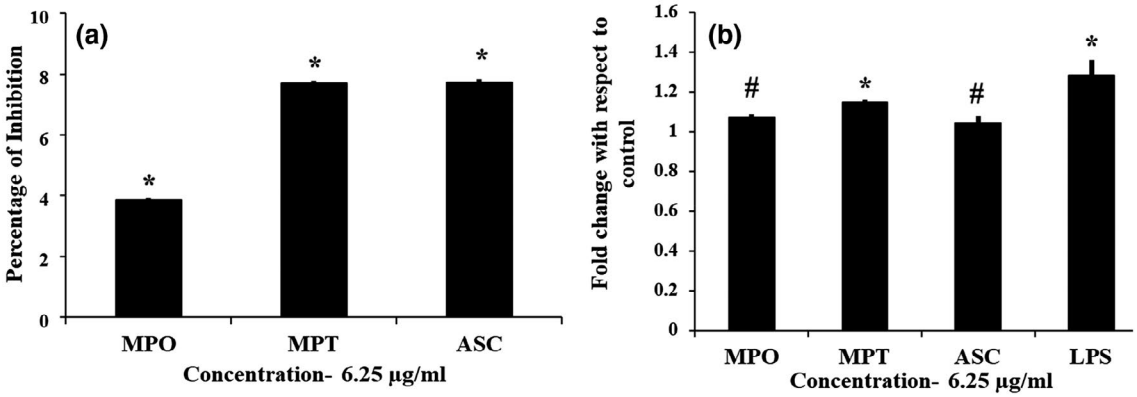

FIGURE 4 Effect of ginger extracts at 6.25 µg/ml of concentration. (a) DPPH scavenging assay. (*)$p < .05$ when compared to untreated control; (b) Inhibition of LPS-induced nitric oxide production (*)$p < .05$ when compared to untreated control, (#)$p < .05$ when compared to LPS-treated control. Microwave-assisted Polyphenolic extract of Odisha variety (MPO) and Microwave-assisted Polyphenolic extract of Tamil Nadu variety (MPT), Ascorbic acid (ASC)

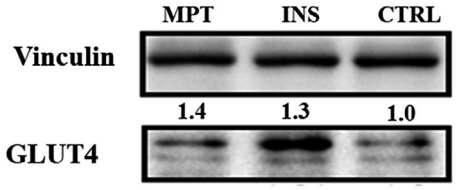

FIGURE 5 GLUT4 protein expression normalized with vinculin. Microwave-assisted Polyphenolic extract of Tamil Nadu variety (MPT), Insulin control (INS), CTRL: Untreated control

our findings, Gaur et al. (2016) reported differential expression of bioactive compounds in Odisha ginger cultivars grown in different climatic conditions through transcriptome analysis. These environmental factors may influence the phytoconstituents presence and the activities in the ginger varieties collected from different parts of India. Based on the above observations, MPT variety was chosen for further studies.

3.7 | Western blot analysis

Western blot was performed for the MPT variety in C2C12 cells at 6.25 µg dose. As denoted in Figure 5, MPT showed an increased GLUT 4 expression of 1.4 fold. The insulin control showed 1.3 fold of increase when normalized with vinculin. Li et al. (2012) reported that 8-gingerol (ginger's active fraction) showed dose dependent increase in the GLUT 4 expression at 20 µM concentration up to 2 fold. It is noteworthy mentioning that the whole ginger extract shows better GLUT 4 expression even at the lower dose and fewer reports are available regarding the GLUT 4 activity of ginger extracts.

3.8 | GC-ESI MS analysis

Based on the promising results, bioactive compounds were identified in the MPT ginger variety. Eight metabolites such as 8, 10- shogaol, 6, 10- gingerol were identified and characterized based on their elution order in the column, fragment ions at m/z and literature reports (Asamenew et al., 2019). The retention time, molecular formula, m/z value and the area (%) of these bioactive compounds was presented in Table 2, supplementary Figure 1. Based on the molecular ions at m/z value and abundance, the major compound was identified as 8-Shogaol with 28.06% of area at the RT-6.92 min displaying a molecular mass ions m/z 328 $[M + Na+H]^+$ with the addition of sodium and hydrogen ions, 6-Gingerol at 12.21 min RT displayed the m/z 919 $[3M- H_2O + 3Na]^+$, molecular mass ion m/z 356 $[M-H_2O + Na+H]^+$ with the removal of water molecule and addition of sodium ion and protonated precursor yielded 10-gingerol, 6-gingerdione (6.74%) showed m/z 601 $[2M + Na]^+$, 6-Zingiberine (5.14%) showed displayed m/z 326 $[M-CH3 + 2Na]^+$, 10-shogaol (3.71%) with m/z 354 $[M + Na]^+$, Ar- Curcumene (3.55%) showed m/z 428 $[M + Na]^+$ and methyl-6-isogingerol was found in low amount of 2.01% with molecular mass ions at $[2M-H_2O-CH_3-CH_3]^+$. Though 6-gingerol was identified as the major phenolic compound through GC-MS and HPLC analysis in Odisha cultivar, only a negligible amount of 10-gingerol and 10-shogaol (<0.2%) was observed Kizhakkayil and Sasikumar (2012).

6-shogaol (pungent non-volatile compound) was reported to enhance glucose uptake activity via AMPK pathway in 3T3-L1 adipocytes at 100 µM concentration Wei et al. (2017) which is identified to be the important active compound of MPT variety. Apart from the pungent phenolics, sesquiterpenes hydrocarbons such as zingiberene, ar-curcumene was found in both the MPO and MPT variety that is reported for antioxidant, anti-inflammatory and anti hyperglycemic activities. This study shows that MPT variety contains abundant polyphenolics than the MPO variety which is responsible for its better activity.

Among the several active constituents of ginger, gingerol has many supporting reports for the antidiabetic activity. Chakraborty et al. (2012) reported that 6-Gingerol improved the expression of insulin signaling genes both at the mRNA and protein levels in arsenic

TABLE 2 GC-ESI/MS analysis of MPT (Microwave assisted Polyphenolic extract of Tamil Nadu variety) ginger

S.No	Retention time	Area %	*m/z*	Assignment	Identified compound
1	5.90	2.01	570	$[2M\text{-}H_2O\text{-}CH_3\text{-}CH_3]^+$	Methyl-6-isogingerol
2	6.92	28.06	328	$[M + Na+H]^+$	8-Shogaol
3	8.33	3.71	354	$[M + Na]^+$	10-Shogaol
4	8.58	7.34	356	$[M\text{-}H_2O + Na+H]^+$	10-Gingerol
5	9.35	3.55	428	$[2M + Na]^+$	Ar-Curcumene
6	10.37	5.14	326	$[M\text{-}CH_3 + 2Na]^+$	6-Zingiberene
7	12.02	6.74	601	$[2M + Na]^+$	6-Gingerdione
8	12.21	17.42	919	$[3M\text{-}H_2O + 3Na]^+$	6-Gingerol

TABLE 3 HPLC profile of D-pinitol identified in MPT (Microwave assisted Polyphenolic extract of Tamil Nadu variety) ginger

S.No	Compound/plant extracts	Retention Time (Min)	Peak Area	Area %
1	D-pinitol (Standard)	10.198	287,752	100
2	MPT	10.00	141,410	15.3

intoxicated mice and they could potentially reduce the oxidative stress by elevating the level of antioxidant enzyme such as catalase, superoxide dismutase. Considering the safety profile, nutritive value including the phenolics, mineral contents, protein and fiber content of ginger extract, it can be utilized in the functional food production (Srivastava et al., 2019).

3.9 | HPLC analysis

We identified D-pinitol through HPLC analysis at the retention time 10.1 min with 15.3% area (Table 3 and Supplementary Figure S2). The quantitative analysis reveals that 14.7 µg of D-pinitol is present in MPT extract. It was calculated from the peak area of MPT extract at 1mg/ml in comparison with the standard solution (Y = 10094x–7680, $R^2 = 0.986$) (Supplementary Figure S3). Recently, Ran et al. (2019) reported that dry ginger decoctions could normalize the metabolites levels of glycerol, D-pinitol in the cold asthma affected rats, but no proof is available for the actual presence of D-pinitol metabolite in ginger, which was investigated through HPLC analysis. It showed insulin stimulated glucose uptake activity of 1.37 ± 0.32 fold at 25 µM concentration than at the basal level (1.11 ± 0.25 fold) with the same cell line. There are supporting reports regarding the antidiabetic potential of D-pinitol that might have contributed to the hypoglycemic action of ginger. Gao et al. (2015) reported that D-pinitol exhibited insulin stimulated hypoglycemic activity via PI3K/Akt pathways in streptozocin (STZ)-induced diabetic rats. D-pinitol concentration is found to be lesser here due to the sugar like nature of the compound that might not be totally extracted using different extraction techniques (Rafiee et al., 2011). Yet, this study remains new in primarily exploring the presence of D-pinitol that could have contributed to the antidiabetic activity of ginger and needs detailed affirmative studies.

4 | CONCLUSION

The present study demonstrates the comparative antidiabetic study of polyphenol rich ginger cultivars, Odisha (MPO) and Tamil Nadu (MPT). MPT possessed better antidiabetic and antioxidant activities and effectively inhibited the α-amylase and α-glucosidase enzymes and AGEP formation. It increased the insulin stimulated GLUT4 protein expression up to 1.4 fold even at a low dose of 6.25 µg/ml of concentration. GC-ESI/MS and HPLC analysis revealed the presence of active compounds such as gingerol, shogaol, and D-pinitol, which is a new finding. Consumption of phytocompounds rich ginger either in the dried form or decoction can be beneficial to health which is of therapeutic and dietary importance. The study indicates that MPT ginger variety is a potential adjuvant antidiabetic therapy that needs further studies to strengthen our findings.

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CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization; Data curation; Funding acquisition; Project administration; Supervision; Writing-review & editing: Periyasamy. *Conceptualization; Data curation; Formal analysis; Methodology; Resources; Validation; Visualization; Writing-original draft*: Venkateswaran. *Conceptualization; Formal analysis; Investigation; Methodology; Validation; Writing-review & editing*: Jayabal. *Conceptualization; Data curation; Formal analysis; Supervision; Writing-review & editing*: Hemaiswarya. *Investigation; Supervision; Writing-review & editing*: Murugesan. *Investigation; Validation*: Enkateswara. *Conceptualization; Data curation; Investigation; Methodology; Supervision; Validation; Writing-review & editing*: Doble.

ORCID

Meenakshi Venkateswaran https://orcid.org/0000-0002-7972-2510

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