SHORT COMMUNICATION

Promalabaricone B from *Myristica fatua* Houtt. seeds demonstrate antidiabetic potential by modulating glucose uptake via the upregulation of AMPK in L6 myotubes

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ABSTRACT

Promalabaricone B (PMB), an acylphenol was isolated from dichloromethane-soluble extract of the seeds of *Myristica fatua* Houtt. PMB exhibited significant inhibitory activity on \(\alpha\)-glucosidase enzyme. The molecular docking and dynamics studies of PMB with human maltase-glucoamylase were performed. PMB exhibited an enhanced glucose uptake in L6 myotubes with 46.3\% in 2.5 \(\mu\)M. Encouraged with these results; we investigated the molecular mechanism of PMB through the upregulation of AMPK. The results revealed that PMB promoted the glucose uptake in myocytes by stimulating the translocation and expression of GLUT4. From this, it is clear that PMB can acts as a potential therapeutic option for diabetes treatment, and its hypoglycaemic effect may be mediated by AMPK upregulation and induction of GLUT4 translocation.

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1. Introduction

*Myristica fatua* Houtt. var. magnifica (Bedd.) Sinclair (*M. fatua*) is the wild relative of nutmeg (*Myristica fragrans*), with restricted distribution in the freshwater swamps of Southern Western Ghats, India. Recently, our group reported the antidiabetic potential of phytochemicals from the stem bark of *M. fatua* (Prabha et al. 2018). In 2016, Pandey et al. reported the LC-MS screening of the different fruit parts of *M. fatua* and its in vitro antiproliferative activity (Pandey et al. 2016). Beside these, there is no detailed phytochemical and pharmacological evaluation of the seeds of *M. fatua*. However, several research groups have reported the phytochemical and pharmacological relevance of some other species of Myristicaceae (Asadhawut and Wanrudee 2018; Sajin et al. 2018; Somenath et al. 2018). Incidentally, in the present study, we carried out the phytochemical investigation of the seeds of *M. fatua* and resulted in the isolation of six major compounds. Among the isolates, the antidiabetic potential of promalabaricone B (PMB) has never been investigated. Hence, we did a detailed investigation on antidiabetic potential and their underlying molecular mechanisms of PMB, leading to improved glucose uptake in L6 skeletal muscle cells.

2. Results and discussion

2.1. Isolation and characterization

The purification of dichloromethane extract of the dried and milled seeds of *M. fatua* led to the isolation of 6 major compounds namely; trimyrisitin (1), 1-(2, 6-dihydroxy-phenyl)tetrade-can-1-one (2), malabaricone A (3), malabaricone B (4), malabaricone C (5) and promalabaricone B (6) (Pham et al. 2000). All the compounds were isolated
from the seeds of *M. fatua* for the first time. The structures of the compounds are shown in Figure 1 and the NMR and HRMS spectra of PMB are depicted in the supplementary information (Figure S1–S7).

### 2.2. Effect of PMB on digestive enzymes

One of the therapeutic methods for alleviating T2DM is to reduce the glucose absorption from the intestine through the inhibition of carbohydrate hydrolyzing enzymes such as α-amylase and α-glucosidase. Sivasothy and coworkers reported a potent α-glucosidase inhibitor, giganteone D from the stem bark of *Myristica cinnamomea* King (Sivasothy et al. 2016). In the limelight of this report, PMB was tested for their inhibitory effects against porcine pancreatic α-amylase and α-glucosidase from *Saccharomyces cerevisiae*. PMB exhibited moderate inhibitory activity on α-amylase with IC50 value of 82.00 ± 1.23 μM, the positive control acarbose displayed IC50 value of 8.20 ± 1.23 μM (p < 0.01). The PMB exhibited significant α-glucosidase inhibitory activity with IC50 value of 32.70 ± 0.47 μM (p < 0.01). The standard α-glucosidase inhibitor showed IC50 value of 52.04 ± 0.9 μM.

### 2.3. Antiglycation property

It is well recognized that advanced glycated end products and their derivatives are involved in the pathogenesis of several diabetic complications (Adrover et al. 2014). A plethora of natural products and their modified motifs have showed promising antiglycation property. Exhilarated with these observations, we checked the protein glycation property of PMB and the compound exhibited moderate antiglycation property with IC50 value of 227.26 ± 0.80 μM (p < 0.01). Here, the positive control, ascorbic acid showed IC50 value of 155.38 ± 0.55 μM (p < 0.01).
2.4. Molecular simulation studies

In detailed pharmacokinetic studies, PMB exhibited significant ADME/T properties (Table S1) and satisfy Lipinski Rule of Five; which demonstrates that PMB could act as a promising drug candidate. In human pancreatic α-amylase (4GQQ), PMB exhibits G-/D-score of −2.87 kcal/mol while in the case of human maltase glucoamylase (2QMJ, C-terminal and 3TOP, N-terminal), it displayed G-/D-score of −4.78 and −6.81 kcal/mol respectively. The PMB interacted strongly with 3TOP via hydrogen bonding and aren-arene interactions (Figure S8A).

The root mean square deviation (RMSD) of molecular dynamics simulation of PMB-3TOP complex shows that the protein and the ligand are fluctuated from the initial binding mode around 4.5 Å and attains a stable conformation at the end of the trajectory (Figure S8B). The P-L histogram (Figure S8B) shows that there is a strong H-bonded interaction between the p-hydroxy phenolic group and ASP 1279 which lasts for 92% of interaction time whereas the H-bond between enolizable hydroxyl and ARG 1371 lasts for 106% and the acyl group and TRP 1369 lasts for 62% of the simulation time. In addition, H-bond and hydrophobic interactions are the major binding forces which hold the ligand inside the binding site during the simulation time.

2.5. 2-NBDG uptake is upregulated by promalabaricone B

Cells were treated with different concentration of PMB ranging from 2.5 μM to 100 μM and after 24 h treatment, it was found that PMB was less than 20% toxic up to 2.5 μM concentration (Figure S9A). This concentration was taken for further studies. The effect of PMB on glucose uptake was assessed in differentiated skeletal myotubes. From flow cytometry analysis, we found that PMB has a remarkable potential to increase glucose uptake in cells to an extent of 46.3%, which was higher than that of positive control, metformin (35.2%, P < 0.05) (Figure S9B). The results suggest the possibility that PMB can upregulate downstream biological pathways of glucose uptake that’s by revealing its therapeutical potential.

2.6. GLUT4 expression was upregulated by promalabaricone B

Glucose uptake in myocytes was achieved via glucose transporters. To gain further insight into the mechanisms, GLUT4 upregulation in L6 myotubes were examined by immunoblot analysis. Results (Figure S10A) confirmed that PMB can unregulate GLUT4 expression in cells.

2.7. AMPK signalling was upregulated by PMB

AMPK is believed to be a target for diabetes treatment. To elucidate the mechanism by which PMB exerts its antidiabetic potential, involvement of AMPK signalling in PMB treated myotubes were examined by immunoblot analysis. Results (Figure S10B) confirmed that PMB has the potential to upregulate AMPK pathway, showing its antidiabetic activity. Based on the report by McGee et al., increased AMPK activity is associated with increased GLUT4 gene expression (McGee et al. 2008). Our results also
support the finding that increased intrinsic activity of GLUT4 could potentially induce the enhancement of glucose uptake after AMPK upregulation in L6 myocytes. This study provides the promising activity of PMB as an AMPK activator, so as to target the metabolic syndrome through the downstream molecular pathways. Altogether, the antihyperglycaemic activity of PMB on skeletal muscles through the upregulation of AMPK pathway and thus enhancing glucose uptake by the upregulation of GLUT4 gives evidence for its novel therapeutic role in diabetes management. Moreover, the present study explores the unidentified activity of PMB as an AMPK activator. The physiological action of PMB highlights the need to explore its activities in an in vivo system.

3. Conclusion

In summary, the phytochemical investigation of the seeds of *M. fatua* led to the isolation of six major compounds, including PMB with significant inhibitory activity on α-glucosidase enzyme. The molecular docking and dynamics simulation studies revealed that PMB effectively binds the pocket of N-terminal human maltase glucoamylase (3TOP). In further experiments, PMB stimulated the glucose uptake in skeletal muscle cells by enhancing the translocation and expression of GLUT4. Our results clearly indicate that PMB is a potential therapeutic option for diabetes treatment, and its hypoglycaemic effect may be mediated by the main mechanism that includes AMPK upregulation and induction of GLUT4 translocation.

Disclosure statement

The authors confirm that this article content has no conflict of interest.

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