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RAPID COMMUNICATION

Anti-diabetic and anti-urease inhibition potential of *Amomum dealbatum* Roxb. seeds through a bioassay-guided approach

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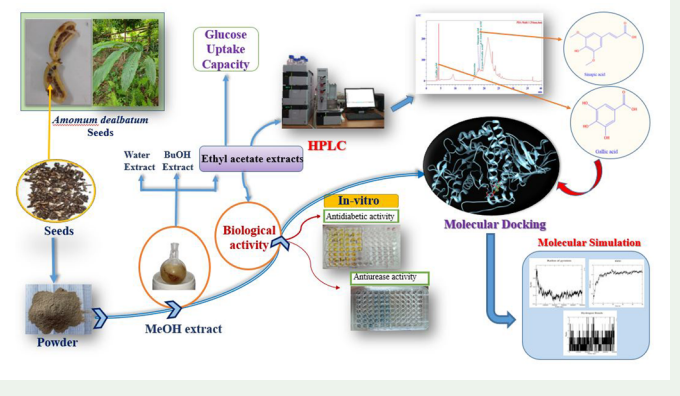
ABSTRACT

Using HPLC-PDA and HRMS analysis, five compounds *p*-coumaric acid, sinapic acid, quercetin, *trans*-ferulic and gallic acid were identified in seeds of *Amomum dealbatum* Roxb. The GC-MS analysis identified 1-dodecanol, phenol, 3,5-bis(1,1-dimethylethyl), Oleic Acid and 1-Heptacosanol which possess anti-diabetic properpties. A bioassay-guided technique was used to determine the degree of inhibition that *A. dealbatum* seeds crude methanol extract and its most active sub-fraction had against the α-glucosidase and *Helicobacter pylori* urease enzymes. In the Rat L6 myoblast cell line, glucose absorption through the GLUT4 transporter of most active subfraction (EASF80) was examined. According to a molecular docking investigation, these compounds strongly interacted with the GLUT4 transporter, *H pylori* and α-glucosidase enzyme. Sinapic acid interacted most strongly with the *H. pylori* urease enzyme while gallic acid interacted with both the α-glucosidase enzyme and the GLUT4 transporter. Additionally, a molecular docking simulation study was carried out to recognise the stability of the complexes.



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1. Introduction

Diabetes Mellitus (DM) is a metabolic disorder that are brought on by chronic hyperglycaemia (high blood glucose level). It has become a serious epidemic during the past few years. The International Diabetes Federation (IDF) predicts that by 2045, 783 million adults worldwide will have diabetes, with one in ten having the condition as per the literature report (International Diabetes Federation 2021). Over time, persistent hyperglycaemia can damage several organs, including the kidneys, blood vessels, eyes, heart, etc. (Canivell and Gomis 2014). About 90–95% of cases of diabetes are due to type 2 diabetes mellitus (T2DM) which is associated with insulinemia and pancreatic beta cell impairment resulting in defective insulin secretion causing high glucose content in the blood (Gilbert and Pratley 2015). Insulin is required for glucose uptake into the cell, it activates the GLUT4 intracellular protein vesicles to translocate to the plasma membrane and facilitate glucose uptake through the GLUT4 transporter (Merz and Thurmond 2020). α-Glucosidase enzyme is present in the epithelium of the small intestine, breaking down starch and disaccharides into glucose for absorption into the bloodstream (Assefa et al. 2020). Inhibiting the α-glucosidase enzyme in the intestine delays the rate of hydrolytic cleavage of oligosaccharides to monosaccharides. Therefore, blocking this enzyme might lessen the postprandial rise in blood glucose that causes T2DM (Kumar et al. 2011). Several drugs are used to treat T2DM, such as gliptins, biguanides, sulfonylureas, and thiazolidine derivatives. However, prolonged use of these medications can lead to negative side effects like acute pancreatitis, headache, lactic acidosis, vomiting, weight gain, hypoglycaemia, and fluid retention (Chaudhury et al. 2017). *Helicobacter pylori*, a gram-negative spiral bacterium that mainly infects the epithelial lining of the stomach, (Hooi et al. 2017) is the main reason behind chronic gastritis and gastric cancer (Malfertheiner et al. 2007). *H. pylori* is responsive to antibiotics; however, it has been reported that 15% of the patients undergoing drug treatment encounter therapeutic failure (Marcus et al. 2016). Ethnomedicinal plants are now being aggressively explored due to their rich therapeutic phytochemical contents such as phenolics, flavonoids, tannins, saponins, and so on (Eid and Haddad 2014). Natural products are gaining popularity as an alternative to current therapies for T2DM and *H. pylori-borne* disorders due to their promising results and minimal adverse effects.

Amomum dealbatum Roxb. is a hardy perennial herb that grows on damp, humus-rich soils on hill slopes in North-Eastern India, Thailand, and Southwestern China. It has a thick rhizome that can reach a height of 3 m (Sajem and Gosai 2006). This species is mostly found in the Nyishi belt of Arunachal Pradesh locally known as 'Talang or Lakchung packang'. They consume its various organs, including stems and flowers as boiled vegetables and its seeds are eaten raw as a delicacy. (Lamxay and Newman 2012) reported using the bark of the plant as an antiseptic while (Dalisay et al. 2018) used the rhizome extract to treat abscesses, rheumatism, and arthritis. In its Leaf flavonoids, alkaloids and tannins were identified by Hanifa et al. (2021). The antioxidant activity of essential oil from rhizomes was investigated by Mohanty et al. (2023). Myricetin, gallic acid and quercetin-3-*O*-galactoside were identified from the flower (Chelleng et al. 2023) who also mentioned the flower's potent anti-diabetic properties with IC_{50} values of 5.385 µg/mL. There hasn't been any reporting on the phytochemical

screening of its seeds yet. It is delicate, juicy, dark brown and spherical in shape. Therefore, in this preliminary study we made an effort to explore the potential of *A. dealbatum* Roxb. seeds (ADS) for glucose uptake capacity, anti-diabetic, and anti-urease through *in-vitro* analysis. Bioactive and phytochemical compounds were identified by HPLC and GC-MS. *In-silico* docking analysis was employed to predict the binding patterns with GLUT4, α-glucosidase and urease enzyme structures.

2. Results and discussion

The crude methanol extract of ADS yielded 31.25% w/w, and its highest active sub-fraction (EASF80) yielded 1.5% w/w. The Preliminary screening analysis of methanol extract indicates the presence of alkaloids, flavonoids, phenols, carbohydrates, saponins, tannins and anthocyanins (Table S1, Supplementary Material (SM)). The GC-MS analysis of most active sub-fraction EASF80 results revealed 1-dodecanol, phenol, 3,5-bis(1,1-dimethylethyl), Oleic Acid and 1-Heptacosanol, etc. which possess anti-diabetic and other bioactivities (Table S2, SM). The sub-fraction, EASF80 inhibited α-glucosidase and urease enzymes with IC_{50} values of 5.32 µg/mL (437.46 µg/mL for acarbose) and 86.74 µg/mL (39.09 µg/mL for thiourea), respectively (Table S3, SM). The bioactive compounds of the most active sub-fraction, EASF80 were identified using HPLC-PDA analysis using a C-18 analytical column. The detection wavelength was set at 254 nm. The five potential bioactive compounds appeared at retention time sinapic acid (18.21 min), quercetin (16.67 min), *trans*-ferulic acid (19.34 min), gallic acid (3.49 min) and *p*-coumaric acid (18.41 min) (Figure 1) and (Table S4, Figure S2, SM). (Table S5, SM) shows the occurrence of these compounds in the same species. The concentrations of these compounds were found to be 0.039, 0.101, 0.052, 0.0003, and 0.3850 mg/mL, respectively and the calibration curves are in (Table S6, Figure S5, SM). HRMS analysis was used to confirm the identified compounds through their mass fragments. EASF80's mass spectra showed the precursor peaks at $m/z = 225.137$ of sinapic acid (Molecular weight = 224), $m/z = 302.210$ of quercetin (M.W. = 302), $m/z = 195.169$ of *trans*-ferulic acid (M.W. = 194), $m/z = 107.083$ of gallic acid (M.W. = 170), and $m/z = 165.103$ of *p*-coumaric acid (M.W. = 164) as shown in (Figure S4, SM).

Incubation of Rat L6 myoblast cell line with EASF80 and EAF extracts resulted in a significant ($p < 0.05$) and concentration-dependent amelioration of glucose utilisation and GLUT4 translocation compared to the control. The concentration of 20 µg/mL was significantly ($p < 0.05$) higher than that of the 5 and 10 µg/mL of the extracts, control and FFA (0.75 mM) (Figure S5, SM).

The identified compounds i.e. sinapic, *trans*-ferulic, gallic, *p*-coumaric acid and quercetin were well subjected to molecular docking with the human GLUT4 transporter, α-glucosidase, and *H. pylori* urease enzymes based on inhibition assay results. Gallic acid formed two H-bonds with human GLUT4 transporter having a binding energy of −170.857 kcal/mol, whereas *p*-coumaric and *trans*-ferulic acid shared one H-bond each with binding energies of −105.867 and −121.68 kcal/mol, respectively (Table S7, Figure S8, SM). Gallic acid formed seven H-bonds with α-glucosidase with interaction energy of −27.2538 kcal/mol, quercetin with −27.236 kcal/mol formed three H-bonds, while sinapic, *trans*-ferulic, and *p*-coumaric acid shared only one H-bond having interaction energies of −14.1661, −24.4939, and −21.1729 kcal/mol, respectively.

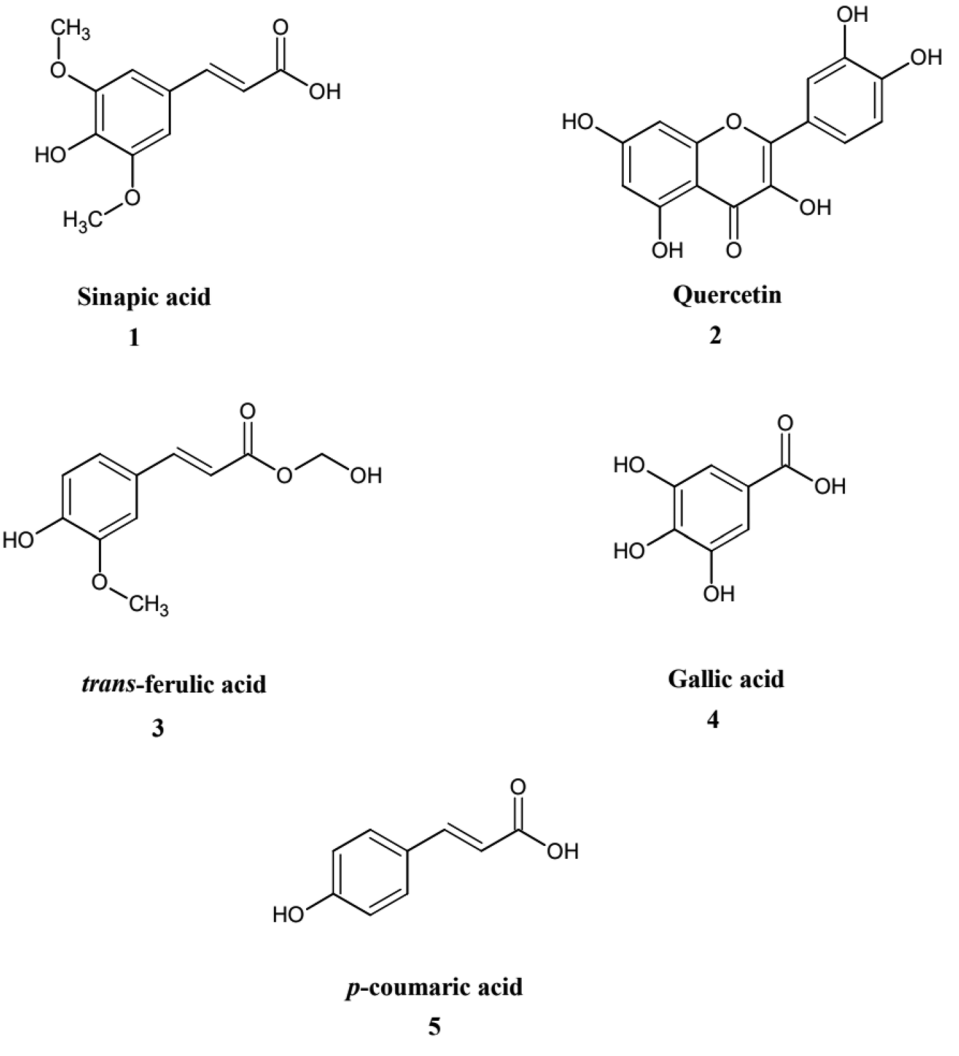

Figure 1. Chemical structure of **(1)** sinapic acid **(2)** quercetin **(3)** *trans*-ferulic acid **(4)** gallic acid **(5)** *p*-coumaric acid.

Out of all, gallic acid was found to be the most significant one against α-glucosidase (Table S8, Figure S9, SM). Docking analysis of *H. pylori* urease revealed that sinapic acid had shown the best protein-ligand interaction by forming five H-bonds with −22.0667 kcal/mol binding energy (Table S9, Figure S10, SM). Molecular dynamics simulations (MDS) were performed for gallic acid with the α-glucosidase for 50 ns while for sinapic acid with *H. pylori* urease. The RMSD plots of gallic acid with α-glucosidase showed stability after 7.5 ns ranging below 0.4 nm and sinapic acid with urease showed stability after 15 ns and ranging below 2 nm (Figure S12, S15, SM).

3. Experimental

See Supplementary Materials.

4. Conclusion

In the present study, the inhibition potential of different extracts of ADS against α-glucosidase and *H. pylori* urease and its glucose uptake capacity were evaluated. Five compounds viz., sinapic, *p*-coumaric, *trans*-ferulic, gallic acid and quercetin were identified from ADS as potential inhibitors. Detailed computational and structural insights confirmed considerable interaction between the target proteins and the compounds, verified by simulation studies. From our study, it can be concluded that ADS, due to its good inhibitory action against both anti-diabetic and anti-urease activity, can be further explored as a new natural drug candidate.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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