ABSTRACT

Two bioactive compounds caffeic and sinapic acid were isolated from the fruit of the *Piper mullesua* Buch–Ham ex D Don using bioassay guided approach. These compounds were isolated from water fraction using column chromatography followed by semi preparative HPLC. These compounds showed very potent anti-diabetic and antioxidant activities. The molecular docking was carried out to predict the mode of interaction of the isolated compounds with $\alpha$-glucosidase. The *in vitro* $\alpha$-glucosidase inhibitory activity of caffeic and sinapic acid was determined, and their IC$_{50}$ values were found 0.67 and 0.82 $\mu$g/ml, respectively. A QSAR equation was generated with an $R^2$ value of 84.81%, which is suitable enough for predicting the IC$_{50}$ values of test molecules. The aforementioned finding confirms the isolated compounds show very significant anti-diabetic potential which is supported by the molecular docking and QSAR study. So, it has ample scope for drug development with further *in vivo* and clinical study.
1. Introduction

Bioactive compounds present in medicinal plants that have anti-diabetic potential are now a days mostly used to develop therapeutic anti-diabetic drugs. The epigallocatechin gallate, hyperin, rosmarinic acid are some of the natural compounds-based inhibitors of $\alpha$-glucosidase reported previously (Yin et al. 2014; Chanu et al. 2018). The phenolic compounds are the most widely distributed antioxidants present in plants and these compounds reduce the risk of diabetes (Famuyiwa et al. 2019).

From the literature, it was found that the researcher carried out the studies on phytocemical and its biological properties on *Piper* species (Parra Amin et al. 2019; Atiya et al. 2020; 2020; Li et al. 2020; Noshita et al. 2020). North-Eastern India has suitable habitat for the growth of the *Piper* species (Gajurel et al. 2001). *Piper mullesua* Buch–Ham ex D Don (Syn: *Piper peepuloides* Roxb.) also known as hill pepper, which grows under cover of dense vegetation bearing fruits having medicinal properties. The fruit of the plant is used to treat colds, coughs and control the sugar level as per traditional knowledge (Parmar et al. 1997). Accordingly, it was considered for evaluation of anti-diabetic properties and isolate $\alpha$-glucosidase inhibitors. The aim of this study was to identify and isolate the active constituents of *Piper mullesua* Buch–Ham ex D Don through a bioactivity guided approach and evaluation of their anti-diabetic and antioxidant potential by different *in vitro* and *in silico* methods. Molecular docking and QSAR analysis were carried out to understand the interaction of bioactive molecules and $\alpha$-glucosidase enzyme accordingly. The molecular docking and QSAR analysis for predict the $\alpha$-glucosidase inhibitors has not been done for the plant species so far. This is the novelty of this study.

2. Results and discussions

The bioactive molecules were isolated using a bioassay-guided approach. Initially, the fruit of the plant was extracted in methanol and the $\alpha$-glucosidase assay was carried
out in methanol extract and the IC\textsubscript{50} value was found to be 4.58 ± 0.23 μg/ml which is very promising for further study. The methanol extract was fractionated then in ethyl acetate, n-butanol, and water. The IC\textsubscript{50} value was found 10.219 ± 0.37 and 7.897 ± 0.18 μg/ml for n-butanol and water extract respectively. The ethyl acetate part is inactive in the α-glucosidase assay. The four sub-fractionated parts from the water fraction i.e., WF-1, WF-2, WF-3, and WF-4 were also subjected for this inhibitory analysis. The IC\textsubscript{50} value was found 5.89 ± 0.08, 2.106 ± 0.54, 0.704 ± 0.0.16, and 2.89 ± 0.35 μg/ml in WF-1, WF-2, WF-3, and WF-4, respectively. The caffeic acid and sinapic acid which was isolated from the WF-3 subfraction using semi-preparative HPLC (Figure 1). The HPLC chromatogram of the compounds is shown in Figure S1 (Supplementary materials). The presence of the compounds was further confirmed through HRMS spectral analysis having molecular ion peak at \textit{m}/\textit{z} 180.1135 and 224.2052 for caffeic and sinapic acid, respectively, given in faction WF-3 (Figure S2) (Supplementary materials). It is supported by reported information (Jeong et al. 2011; Van et al. 2016). Table 1 shows the different IC\textsubscript{50} values of the prepared extract and subfractions along with the isolated compounds.

Further, the isolated compounds were confirmed by their \textsuperscript{1}H and \textsuperscript{13}C-NMR spectra. The \textsuperscript{1}H NMR spectra clearly suggest the structure of isolated compounds which was given in Figure S3 (A–B) validated by \textsuperscript{13}C-NMR spectra in Supplementary materials Figure S4 (A–B). The NMR data are reported in section 2 in supplementary materials. The reported information is supported by previous data (Jeong et al. 2011; Van et al. 2016).

The calibration curves of both the compounds in Figure S5 showed good linear regressions. Table S1 gave the linear range and content of the isolated compounds.

![Figure 1. Structure of (A) caffeic acid (B) sinapic acid.](image-url)
The sinapic acid and caffeic acid content were found to be 0.0601 mg/L and 0.0239 mg/L, respectively. It was reported that the caffeic acid derivative like rosmarinic acid has shown significant results in *in-vivo* assay (Chanu et al. 2018). So, it has ample scope for further in vivo study.

To study the interaction of caffeic and sinapic acid with α-glucosidase, molecular docking studies were carried using the CDOCKER module of Biovia Discovery Studio 2019 (DS). The structure of α-glucosidase was retrieved from RCSB-Protein Data Bank, where it was complexed with standard inhibitor acarbose. The site where acarbose was bound to α-glucosidase was chosen as the active site for screening the binding efficacy of caffeic and sinapic acid to α-glucosidase. The structure of caffeic, sinapic acid, and standard inhibitor (positive control) were minimized using the full energy minimization module of DS. Molecular CDocker interaction energies of caffeic and sinapic acid with α-glucosidase (PDB ID: 5NN8) are $-25.9692$ kcal/mol and $-28.4307$ kcal/mol respectively (Table S2). The caffeic acid forms four conventional H-bonds with the residues ASP282, MET519, LEU677, and ARG600 and one Pi–Pi T shaped interaction with TRP481, while sinapic acid also forms four conventional H-bonds with the residues ASP282, ASP518, ARG600, and LEU677 and one Pi–Pi T shaped interaction with TRP481. Upon comparing the interactions of these compounds with that of the standard inhibitor acarbose, it was found that the CDocker interaction energy is $-26.7226$ kcal/mol. It forms four conventional H-bonds with ASP282, ASP404, ARG600, ASP616, and HIS674.

The generated QSAR model shows a significant coefficient of determination $R^2 = 84.81\%$. Using this model, pIC$_{50}$ of caffeic acid, sinapic acid and acarbose was found to be 5.87325, 5.88645, and 6.03058, respectively. Therefore, it has been observed that both the compounds show significant inhibition of the enzyme and good agreement with experimental information. The 2D and 3D interactions from the docking results are given in Supplementary materials Figures 6S–7S. The generated QSAR plot is shown in Figure 8S, which is significantly capable of predicting the pIC$_{50}$ of the test set molecules.

The plant based natural antioxidant compounds can protect β-cells from reactive oxygen species (ROS) which can prevent diabetes. Therefore, an alternative for the management of obesity and diabetes are mainly medicinal plants which help to maintain low blood glucose as well as boost body antioxidant system and insulin regulation (Patel et al. 2012).

The antioxidant activity of the extracts was analyzed through three different methods, that is, DPPH, ABTS, and FRAP (Table S3). The WF-3 has the highest scavenging activity confirmed by all three assays. The isolated compounds, that is, caffeic and sinapic acid from WF-3 may possibly be the active antioxidant components as these have the lowest IC$_{50}$ values. The predominant DPPH scavenging activity was found which may be due to presence of the both isolated caffeic and sinapic acid. The isolated molecules may interact with DPPH scavengers to be produced a more stable intermediate or product than other scavengers produced in ABTS and FRAP assay (Chelleng et al. 2021).

### 3. Conclusion

The two potential anti-diabetic and antioxidant agents, that is, caffeic and sinapic acid were isolated from the fruit of *Piper mullesua* Buch–Ham ex D Don by bioassay guided
approach. The IC$_{50}$ value clearly revealed that the responsible compounds show very significant $\alpha$-glucosidase enzyme inhibitory properties. Molecular docking and QSAR studies of the isolated compounds revealed the possible interaction with $\alpha$-glucosidase enzyme inhibitors. Molecular docking studies of the isolated compounds result in a good agreement with the in-vitro analysis data that suggests that both caffeic and sinapic acid inhibits the enzyme significantly. So, there is ample scope of in-vivo and clinical study for developing pharmaceutical products for health benefit.

Disclosure statement
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References
Atiya A, Salim MA, Sinha BN, Ranjan Lal U. 2020. Two new anticancer phenolic derivatives from leaves of Piper betle Linn. Nat Prod Res. 1–9. https://doi.org/10.1080/14786419.2020.1762186
Atiya A, Singha BN, Lal UR. 2020. The new ether derivative of phenylpropanoid and bioactivity was investigated from the leaves of Piper betle L. Nat Prod Res. 34(5):638–645.
Chanu MB, Labala RK, Yunus Y, C JC, K SK, Sahoo D, Singh OJ, Shakya A, Thongam B. 2018. Bioassay guided isolation of $\alpha$-glucosidase inhibitory compound, in vivo postprandial anti hyperglycemia and docking study of the isolated compound from the leaves of the methanolic extract of Quercus serrata. Biosci Biotech Res Comm. 11(4):647–657.
Chelleng N, Hazarika M, Kalita AJ, Guha AK, Tamuly C. 2021. Effect of solvent on antioxidant activity of Zanthoxylum oxyphyllum edgew and its DFT study cur. Bioactive Comp. :17.
Famuyiwa SO, Sanusi K, Faloye KO, Yilmaz Y, Ceylan Ü. 2019. Antioxidant and antidiabetic: activities: is there any link between them. New J Chem. 43(34):13326–13329. ?
Gajurel PR, Rethy P, Kumar Y. 2001. A new species of Piper (Piperaceae) from Arunachal Pradesh, North-Eastern India. Bot J Linnean Soc. 137(4):417–419.
Jeong C-H, Jeong HR, Choi GN, Kim D-O, Lee U, Heo HJ. 2011. Neuroprotective and anti-oxidant effects of caffeic acid isolated from Erigeron annuus leaf. Chin Med. 6:25.
Li D, Wang R, Cheng X, Yang J, Yang Y, Qu H, Li S, Lin S, Wei D, Bai Y, et al. 2020. Chemical constituents from the fruits of Piper longum L. and their vascular relaxation effect on rat mesenteric arteries. Nat Prod Res. 1–6. https://doi.org/10.1080/14786419.2020.1797726
Noshita T, Sato T, Iwayama T, Yamada Y, Ouchi H. 2020. The proposed structures of phenolic compounds isolated from *Piper betle* L. differ from those of the compounds obtained by total synthesis. Nat Prod Res. 1–7. https://doi.org/10.1080/14786419.2020.1739038

Parmar VS, Jain SC, Bisht KS, Jain R, Taneja P, Jha A, Tyagi OD, Prasad AK, Wengel J, Olsen CE, et al. 1997. Phytochemistry of genus *Piper*. Phytochem. 46(4):597–673.

Parra Amin JE, Cuca LE, González-Coloma A. 2019. Antifungal and phytotoxic activity of benzoic acid derivatives from inflorescences of *Piper cumanense*. Nat Prod Res. 35:1–9.

Patel DK, Kumar R, Laloo D, Hemalatha S. 2012. Diabetes mellitus: an overview on its pharmacological aspects and reported medicinal plants having antidiabetic activity. Asian Pac J Trop Biomed. 2(5):411–420.

Van SJ, Canalle LA, Smid J, Meuldijk J. 2016. Conversion of syringaldehyde to sinapinic acid through Knoevenagel-Doebner condensation. OJPC. 06(04):101–108.

Yin Z, Zhang W, Feng F, Zhang Y, Kang W. 2014. Glucosidase inhibitors isolated from medicinal plants. Food Sci Human Wellness. 3(3-4):136–174.