Profiling of interactions between diabetic enzymes and natural polyphenols from spices and edible herbs – An in-silico approach

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ABSTRACT

Background: Diabetes mellitus (DM) is one of the most prevalent public health concerns globally. Natural products and its derived active compounds may be achievable alternatives for the treatment of type 2 diabetes. Therefore, there is a need to explore natural agents to replace the current standard drugs with anti-diabetic functional food. Current study aimed to screen edible herbs and spices for their antidiabetic activity and docked their selected polyphenols with enzymes associated with diabetes to understand their interaction mechanism and mode of inhibition of respective enzymes. **Methods:** Alpha amylase and alpha glucosidase inhibition percentage of ethanolic extracts of *Cinnamomum zeylanicum, Cinnamomum tamala, Amomum subulatum, Trigonella foenum graecum, Mentha piperita, Coriandrum sativum, Lactuca sativa, and Brassica oleraceavar italic* was calculated. Pyrx virtual screening tool was used to dock the selected polyphenols with alpha amylase and human lysosomal alpha glucosidase to examine their binding affinities. **Results:** Highest inhibitory potential for alpha glucosidase was observed in case of *A. subulatum* with IC50 Value 372 µg/ml. However, *Cinnamomum zeylanicum* and *Cinnamomum tamala* displayed highest inhibition against Alpha amylase with IC50 68.75 µg/ml. Among selected bioactive compounds, Cinnamtanin B1 and Trigonelloside C exhibited the greatest affinity for Alpha amylase and Alpha glucosidase with lowest Kd (Dissociation constant) Values of -10.2 and -9.1 respectively. Cinnamtannin B1 interacts with aspartate300 at the active site of alpha amylase (serving as antagonist) while it serves as allosteric inhibitor for alpha glucosidase by binding non-competitively on sites other than active site. **Conclusion:** Such bioactive polyphenols in studied plant extracts may be used as anti-diabetic drugs as they bind competitively at active site of enzyme.

Key words: Edible herbs**,** Antidiabetic, polyphenols, molecular docking, alpha glucosidase, alpha amylase.

1. INTRODUCTION

Diabetes mellitus is considered as most common chronic disorder that does not only refer to an elevated blood glucose level but also can lead to severe complications like cardiovascular diseases, retinopathy, diabetic neuropathy etc. [1]. Number of diabetes cases have been increasing every year with a rough estimate of death of more than 151 million people per year and it is reported that its cases may reach to 693 million cases by 2045 [2]. Diabetes mellitus results in hyperglycemia

either due to insulin absence (Type 1) or resistance to available insulin (Type 2)[3]. It could be managed by inhibiting the enzymes involved in carbohydrate metabolism and blood glucose regulation. Activity of various enzymes such as alpha amylase, alpha glucosidase, glycogen synthase, gluco-kinase can be inhibited to control Type 2 diabetes. Alpha amylases carry out the hydrolysis of long chain carbohydrates i-e starch into shorter oligonucleotides such as maltose while alpha glucosidase converts starch and disaccharides to glucose[4]. In common, α-glucosidase and alpha amylase inhibitors hinder the digestion and absorption of carbohydrates in the small intestine, lessening postprandial hyperglycemia [5].

Conventional drug therapy for diabetes (e.g. acarbose) is found to be laborious because of severe side effects such as flatulence, diarrhea and abdominal pain etc.[6]. Moreover, most of these drugs cannot be taken as a single dose and recommended to use for entire life for chronic patients [3]. Thus, these limitations have prompted the discovery of management therapy in the form of cost effective medicinal plants with anti-diabetic potentials which have less side effects [7].

Plant bioactive compounds have been reported to restrain the activity of digestive enzymes such as pepsin, trypsin, lipase, alpha-glucosidase and amylase [8]. Depending on the structure, the phenolics react with proteins/enzymes and alter various properties of carbohydrates such as the molecular weight, solubility and in vitro digestibility [7]. Edible plants used as a common kitchen spice and herbs offer a very cost effective diet plan and therapy for diabetic patients as they are rich in such bioactive compounds. For example, C*innamomum zeylanicum* (*C. zelanicum*) and *Cinnamomum tamala* (*C. tamala*) possess polyphenols i-e cinnamic acid [9], kaempferol quercetin, rutin [10] that are reported to activate insulin receptor kinase, enhance glucose uptake thus provide effective substitute of conventional diabetic drugs. Likewise *Amomum subulatum (A.subulatum)* , *Trigonella foenum grae*cum (*T.F. graecum*) and *Coriandrum sativum (C. sativum)* extracts have been shown to have amylase inhibitory potential due to presence of phenols and flavonoids [11, 12]. Antidiabetic activity of such plant based bioactive compounds is well documented but there is less data available on type of bioactive compound and its mode of inhibition/interaction with a particular diabetic enzyme. Hence, medicinal plants should be assessed for antidiabetic potential and the mechanism of action should be focused to discover novel antidiabetic agents.

Therefore, our study presents an in vitro and in silico approach towards identifying the antidiabetic mechanism of action of most common reported bioactive compounds from edible plants. In this contribution, we selected eight plants (i-e. *C. zeylanicum, C. tamala, A. subulatum, T. F. graecum, Mentha piperita (M. piperita), C. sativum, Lactuca sativa (L. sativa), and Brassica oleracea var italic (B. oleracaea*) and evaluated their inhibition against alpha amylase and alpha glucosidase. Most common reported phytochemicals were selected from plants showing highest in vitro antidiabetic potential and were docked with alpha amylase and alpha glucosidase to comprehend the underlying antidiabetic mechanism of selected compounds. To the best of our knowledge, there is no report on docking studies of selected plants/ polyphenols for identifying their anti-diabetic mechanism of action.

2. MATERIALS AND METHODS

Ethanolic extracts of Common kitchen spices (such as *C.zeylanicum C.tamala, A. subulatum and T.F. graecum*) and herbs (*C. sativum, L. sativa, M. piperita and B. oleracaea*) were prepared. Alpha amylase and Alpha glucosidase inhibition of selected plant extracts was observed using previously reported method [13] [14] with some modifications. Molecular docking was performed to predict the antidiabetic mechanisms of reported phytochemicals. 3D structures of antidiabetic enzymes i-e Alpha amylase and human lysosomal alpha glucosidase were obtained with PDB IDs 1HNY and 5KZX, respectively. Structure of ligands (i-e: Asparasaponin, luteolin 7-O glucoside, Trigonelloside C, Cinnamtannin B1, Kaempferol 3-O glucoside, Menthol, Rosmarinic acid, Cinnamaldehyde, 1-8 Cineol and alpha terpinyl acetate) were acquired from pubchem with PubChem IDs 75528903, 45933934, 441899, 475277,582102, 1254, 528179,637511,2758 and 11037 respectively). Pyrx -virtual screening tool was used to perform docking, binding affinities were saved as CSV files and Discovery studio was used to visualize the final protein–ligand interactions [15]. Detailed methodology is provided in supplementary information.

3. RESULTS

3.1 Alpha amylase inhibition assay

Results of alpha amylase assay exhibited a wide range of inhibition percentage of plant extracts ranging from 29.3% to 79.5% (Fig.1). Highest inhibitory potential was observed in case of *A. subulatum* followed by *C. tamala*, *A. subulatum* displayed the lowest IC50 (372 µg/ml) while *C. sativum* exhibited highest IC50 (3082µg/ml) (Table 1).

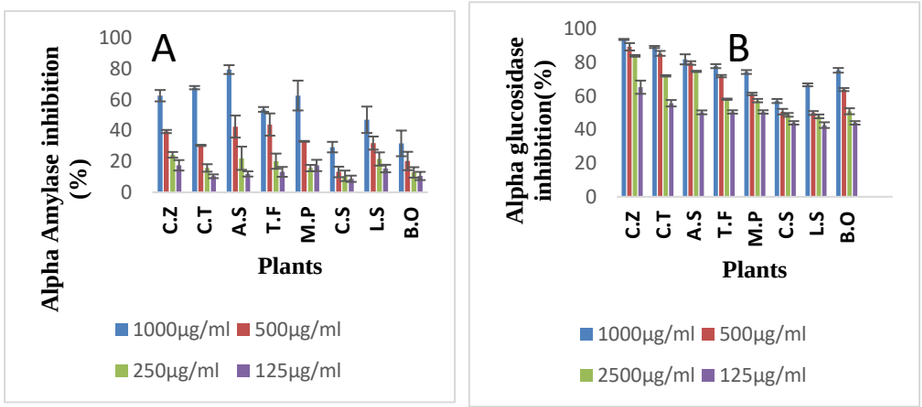

Figure 1. A). Alpha amylase inhibition assay B). Alpha glucosidase inhibition assay

C.Z= *Cinnamomum zeylanicum* C.T= *Cinnamomum tamala* A.S= *Amomum subulatum* T.F= *Trigonella foenum graecum*

M.P= *Mentha piperita* C.S= *Coriandrum sativum* L.S= *Lactuca sativa* B.O= *Brassica oleracaea*

3.2 Alpha glucosidase inhibition assay

Alpha glucosidase inhibitory potential of ethanolic plant extracts was assessed and expressed as percentage inhibition (Fig.1). Highest inhibitory potential was observed in case of *Cinnamomum* species i-e C. *zeylanicum* and C. *tamala* followed by *A. subulatum. C. zeylanicum* displayed the lowest IC50 (68.75) while *C. sativum* exhibited highest IC50 (342.5) (Table 1).

Table 1. IC50 Values of selected plant extracts against alpha amylase and alpha glucosidase

S.NO	Plant	IC50 (µg/ml)	
		Alpha amylase	Alpha glucosidase
1	*C. Zeylanicum*	672	68.7
2	*C. tamala*	517	97.9
3	*A. subulatum*	372	100.7
4	*T.F.graecum*	686	127.7
5	*M. piperita*	504	130.9
6	*C. sativum*	3082	342.5
7	*L. sativum*	1211	298.4
8	*B. var. oleraceae*	2961	201.9
9	Acarbose	157.1	16

3.3 Molecular Docking

Docking was performed to explore the anti-diabetic mechanism of action of selected plant's phytochemicals against two anti-diabetic enzymes i-e alpha amylase and alpha glucosidase. Major reported Polyphenols such as 1-8 cineol and alpha terpinyl from *A. subulatum, Cinnamaldehyde,* Cinnamtannin B1 and Kaempherol 3-o-glucoside from *C. Zeylanicum and C.tamala* respectively, Asparasaponin-1, luteolin 7-o-glucoside and trigonellloside C from *T. F. graecum* and Menthol and rosmarinic acid from *M. piperita* were selected for docking against alpha amylase and alpha glucosidase

Among selected bioactive compounds, Cinnamtanin B1 and Trigonelloside C exhibited the greatest affinity for Alpha amylase and Alpha glucosidase with lowest Kd (Dissociation constant) Values of -10.2 and -9.1 respectively.

Lower K_d values represent better binding affinity of ligand to its target. Binding affinity of bioactive compounds with Alpha amylase was found to be increased in following order (Table 2): Cinnamtannin B1› Asparasaponin 1› Trigonelloside C› Luteolin 7-o-glucoside ›Kaempherol 3-o-glucoside › Rosmarinic acid › Alpha-terpinyl acetate › Cinnamaldehyde › Menthol ›1-8-cineole.

As per docking results in fig 2 (3d interactions) and 3 (2d interactions), Cinnamtanin B1 formed hydrogen bond with Histidine 305, glutamate 233, Asparatate 197 of alpha amylase. While Asaprasaponin 1 formed hydrogen bonds with Glycine 238, Lysine 200, Aspartate 197 and isoleucine 235 of alpha amylase. Trigonelloside C was found to interact with alpha glucosidase by forming hydrogen bonds with glutamate 866, Histidine 717 and Arginine 608,870 (Fig. 3). In terms of binding affinity scores, Trigonelloside C, Asparasaponin 1, Luteolin 7-o-glucoside and cinnamtannin B1 exhibited lowest Kd values and thus greater affinity to inhibit both enzymes (Table 2).

Table 2. Binding affinities of plant bioactive compounds with alpha amylase and alpha glucosidase in terms of Kd values using molecular docking

S.No	Plants	Polyphenols	Alpha amylase	Alpha glucosidase
			Binding Energy	
1	*A. subulatum*	Alpha-terpinyl acetate	-6.4	-5.7
		1-8-cineole	-5.2	-4.6
2	*C. tamala*	Cinnamtannin B1	-10.2	-8.8
		Kaempherol 3-o-glucoside	-7.9	-7.8
3	*C. zeylanicum*	Cinnamaldehyde	-5.7	-6.3
4	*M. Piperita*	Menthol	-5.5	-5.8
		Rosmarinic acid	-7.4	-7.9
5	*T.F. graecum*	Asparasaponin 1	-9.6	-8.5
		Luteolin 7-o-glucoside	-9	-8.5
		Trigonelloside C	-9.5	-9.1
6	Standard	Acarbose	-7.8	-9.65

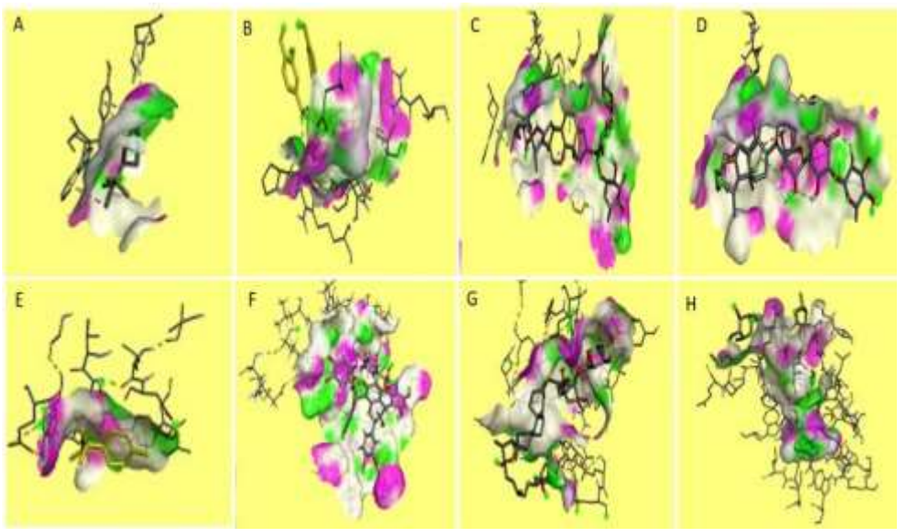

Figure 2. 3D interactions of plant bioacti ve compound binding modes with diabetic enzymes. Alpha amylase docked with A). Alpha terpinyl acetate B). Cinnamtannin B1 C). Trigonelloside D). Asparasaponin 1
Alpha glucosidase docked with E). Alpha terpinyl acetate F). Cinnamtannin B1 G). Trigonelloside H). Asparasaponin 1

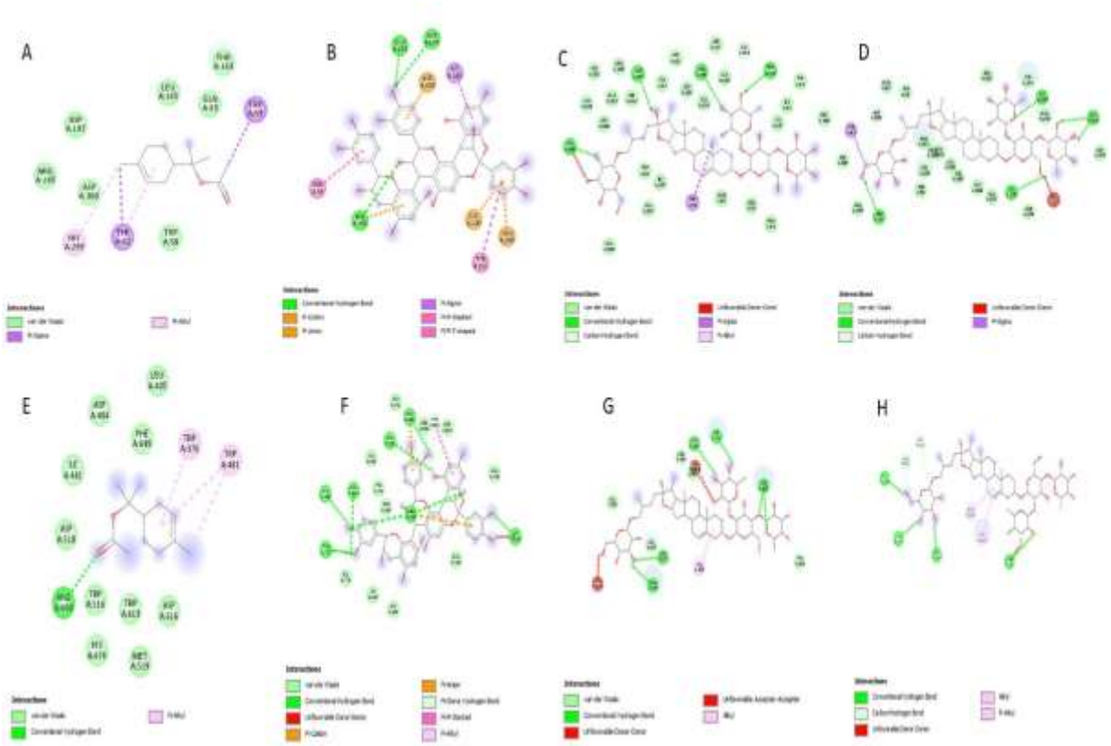

Figure 3. 2D interactions of plant bioactive compound with diabetic enzymes. Alpha amylase docked with A). Alpha terpinyl acetate B). Cinnamtannin B1 C). Trigonelloside D). Asparasaponin 1 Alpha glucosidase docked with E). Alpha terpinyl acetate F). Cinnamtannin B1 G). Trigonelloside H). Asparasaponin 1

4. Discussion

Several secondary metabolites (phenols, flavonoids and alkaloids) from medicinal plants have been found to have a hypoglycemic effect [2]. Recently, a database (DiaNat-DB) was constructed for integrated molecular info on anti-diabetic bioactive compounds from plants[16]. However, still there is need to explore the anti-diabetic mechanism of plant bioactive compounds that might be helpful to design better anti-diabetic drugs. In this regard, alpha amylase and alpha glucosidase inhibition of common kitchen spices was observed and the plants exhibiting ≥ 50% inhibition were examined for their antidiabetic mechanism via docking of their reported polyphenols with diabetes associated enzymes.

Alpha amylase inhibition helps in slowing down the hydrolysis rate of Inhibition of α-1, 4-glcosidic bonds leading to slow release of glucose into the blood stream thus controlling diabetes. Results of alpha amylase inhibition assay indicated that *A. subulatum and C. tamala* showed highest inhibition against this enzyme (i-e 79% and 67% respectively). IC5O values of *A. subulatum* (372 µg/ml) was found to be lower than its ethyl acetate and water extract IC50 (i-e 2000 and 800 µg/ml respectively) as reported by a previous author [17]. *C. tamala* IC50 (517µg/ml) in our study was not in agreement to a recent research on methanolic extract of *C. tamala* young leaves where it displayed lowest IC50 (i-e 2.76 µg/ml) [11]. It may be because of difference in solvent system, experimental conditions and collection source.

Alpha glucosidase, found in brush border of small intestine, is known to convert disaccharides into glucose. Significant alpha glucosidase inhibition was seen in case of *C. Zeylanicum* and *C. tamala* (with IC50 of 68.7 and 97.9µg/ml). This inhibition effect was found to be only 4.25 fold less than standard drug acarbose (i-e 16µg/ml). It is desirable because studies have reported that high inhibitory effect of acarbose may lead to abdominal discomfort due to excessive delay in starch hydrolysis in gastrointestinal tract [18].

Common reported bioactive compounds in selected plant extracts were picked out from literature survey. Major active constituent of *C. zelaynicum* is Cinnamic acid, Cinnamaldehyde and Cinnamtannin B1 while methyle eugnol, Phellandrene, kaempferol are also found in its ethanolic extract[19]. *C. tamala* also contains Cinnamtannin B1 and kaempferol 3-O glucoside[20]. However, 1-8 cineole and alpha terpinyle acetate and alpha terpinol were found to be main compounds in *A.subulatum* [17]. *T.F graecum* ethanolic extract contains *Asparasaponin,* luteolin 7-o-glucoside and trigonellloside C [12] [21] while *M. piperita* extract possesses rosmarinic acid and menthol [22] as main constituents. In silico interactions of rosmarinic acid, luteolin 7-o-glucoside, menthol, eugnol, terpinol, Cinnamic acid, kaempferol 3-o glucoside with alpha amylase and alpha glucosidase [23, 24] have already been explored. However, to the best of our knowledge, there is no single report on docking interactions of Cinnamtannin B1, Asparasaponin 1, Trigonelloside C and alpha terpinyl acetate. Therefore, only these interactions will be discussed in this manuscript.

Xenobiotics can interact with enzymes in two ways either serving as agonist (behaving like natural ligand) or antagonist (i-e binding at active pocket of enzyme or binding at allosteric site or covalent interaction resulting in inhibition). As per docking results, Cinnamtanin B1 formed hydrogen bond with Histidine 305, glutamate 233, Asparatate 300 and 197 of alpha amylase. Previous reports indicate that ASP300, ASP 197 and GLU223 constitute the active site and acarbose also inhibits alpha amylase by occupying this active site [25]. Therefore, it can be concluded that Cinnamtannin B1 interacts with a binding affinity of -10 with ASP300 at active site of alpha amylase and serve as competitive inhibitor of acrabose which just exhibited a binding affinity of -7.8 for alpha amylase. However, Cinnamtannin B1 serve as allosteric inhibitor for alpha glucosidase inhibition as it binds non-competitively on sites other than active site i-e THR 771, LEU 769, ALA 820, LYS 348 etc. Therefore, Considerable alpha amylase inhibition and significant alpha glucosidase inhibition by Cinnamomum species is predicted to occur due to antagonist behavior of Cinnamtannin B1. Asparasaponin 1 forms hydrogen bond with ASP 197 found in A-domain of Alpha amylase catalytic site thus serve as competitive inhibitor (Fig 2). Trigoneloside C and Luteolin 7-o-glucoside also formed hydrogen bonds with Asparatate 197 and histidine 201(part of alpha amylase B domain) respectively (Fig 2). In a recent study, Beta-caryophyllene and Acarbose showed binding affinity of -6.9 and -7.3[25] which is higher than binding affinities of Cinnamtannin B1, Trigonelloside C and asparasaponin-1 obtained in our study. Therefore, *T.f.graecum* and *C.tamala* compounds may be used as anti-diabetic drugs to replace Acarbose as they also bind competitively at same active pocket of enzyme.

Reported residues for active pocket of alpha glucosidase are TRP 1355, PHE 1559, ASP 1526, HIS 1584, ASP 1279, TYR 1251, ARG 1510, TRP 1369, TRP 1523, TRP 1418, and PRO 1159. Trigonelloside C was found to make hydrogen bonds with glutamate 866, Histidine 717 and Arginine 608,870 of alpha glucosidase thus serving as antagonist. While luteolin 7-o-glucoside displayed a carbon hydrogen bond with Aspartate 518 which is considered as most important carboxylate in active site of alpha glucosidase [25]. Docking results indicate that these selected bioactive compounds bind with catalytic site of alpha glucosidase by hydrophobic forces and hydrogen bonding, thus block the enzyme reaction with its substrate and slow down the release of

glucose in blood stream. However, it still needs further molecular docking stimulations to confirm this reaction.

5. Conclusion

The present study revealed that how edible herbs and spices such as C. zeylanicum, *A. subulatum*, *C. tamala* and *T.F. graecum* may exert their antidiabetic action through polyphenols such as Cinnamtannin B1, Asparasaponin 1, Trigonelloside C and alpha terpinyl acetate. These polyphenols serve as either competitive or allosteric inhibitors of carbohydrate digesting enzymes and exhibit strong binding affinity to these enzymes which is comparable to standard drug acarbose. Therefore, the tested polyphenols can be used either alone or in synergy with antidiabetic drugs to reduce their side effects and to develop safe and effective therapeutic strategy for diabetes.

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Conflicts of Interest

The authors affirm no conflicts of interest

References

1. Faselis, C.; Katsimardou, A.; Imprialos, K.; Deligkaris, P.; Kallistratos, M.; Dimitriadis, K., Microvascular complications of type 2 diabetes mellitus. *Current vascular pharmacology* **2020,** 18, (2), 117-124. https://doi.org/10.2174/1570161117666190502103733.
2. Salehi, B.; Ata, A.; V. Anil Kumar, N.; Sharopov, F.; Ramírez-Alarcón, K.; Ruiz-Ortega, A.; Abdulmajid Ayatollahi, S.; Valere Tsouh Fokou, P.; Kobarfard, F.; Amiruddin Zakaria, Z., Antidiabetic potential of medicinal plants and their active components. *Biomolecules* **2019,** 9, (10), 551. https://doi.org/10.3390/biom9100551.
3. Unuofin, J. O.; Lebelo, S. L., Antioxidant effects and mechanisms of medicinal plants and their bioactive compounds for the prevention and treatment of type 2 diabetes: an updated review. *Oxidative medicine and cellular longevity* **2020**. https://doi.org/10.1155/2020/1356893.
4. Navarro, D. M.; Abelilla, J. J.; Stein, H. H., Structures and characteristics of carbohydrates in diets fed to pigs: a review. *Journal of Animal Science and Biotechnology* **2019,** 10, (1), 1-17. https://doi.org/10.1186/s40104-019-0345-6.
5. Isono, Y.; Watanabe, H.; Kumada, M.; Takara, T.; Iio, S.-i., Black tea decreases postprandial blood glucose levels in healthy humans and contains high-molecular-weight polyphenols that inhibit α-glucosidase and α-amylase in vitro: a randomized, double blind, placebo-controlled, crossover trial. *Functional Foods in Health and Disease* **2021,** 11, (5), 222-237. https://doi.org/10.31989/ffhd.v11i5.791.
6. Kato-Schwartz, C. G.; Corrêa, R. C. G.; de Souza Lima, D.; de Sá-Nakanishi, A. B.; de Almeida Gonçalves, G.; Seixas, F. A. V.; Haminiuk, C. W.; Barros, L.; Ferreira, I. C.; Bracht, A., Potential anti-diabetic properties of Merlot grape pomace extract: An in vitro, in silico and in vivo study of α-amylase and α-glucosidase inhibition. *Food Research International* **2020,** 137, 109462. https://doi.org/10.1016/j.foodres.2020.109462.
7. Gaonkar, V. P.; Hullatti, K., Indian Traditional medicinal plants as a source of potent Anti-diabetic agents: A Review. *Journal of diabetes & metabolic disorders* **2020,** 19, 1895-1908. https://doi.org/10.1007/s40200-020-00628-8.
8. Giuberti, G.; Rocchetti, G.; Lucini, L., Interactions between phenolic compounds, amylolytic enzymes and starch: An updated overview. *Current Opinion in Food Science* **2020,** 31, 102-113. https://doi.org/10.1016/j.cofs.2020.04.003.
9. Huang, Z.; Pang, D.; Liao, S.; Zou, Y.; Zhou, P.; Li, E.; Wang, W., Synergistic effects of cinnamaldehyde and cinnamic acid in cinnamon essential oil against S. pullorum. *Industrial Crops and Products* **2021,** 162, 113296. https://doi.org/10.1016/j.indcrop.2021.113296.
10. Sharma, V.; Rao, L. J. M., An overview on chemical composition, bioactivity and processing of leaves of Cinnamomum tamala. *Critical reviews in food science and nutrition* **2014,** 54, (4), 433-448. https://doi.org/10.1080/10408398.2011.587615.
11. Maharjan, R.; Thapa, P.; Khadayat, K.; Kalauni, S. K., Phytochemical Analysis and α-Amylase Inhibitory Activity of Young and Mature Leaves of Cinnamomum tamala. *Nepal Journal of Biotechnology* **2021,** 9, (2), 14-20. https://www.doi.org/10.54796/njb.v9i2.41909.

12. Pereira, A. S.; Banegas-Luna, A. J.; Peña-García, J.; Pérez-Sánchez, H.; Apostolides, Z., Evaluation of the anti-diabetic activity of some common herbs and spices: Providing new insights with inverse virtual screening. *Molecules* **2019,** 24, (22), 4030. https://doi.org/10.3390/molecules24224030.
13. Nazli, A.; Irshad Khan, M. Z.; Ahmed, M.; Akhtar, N.; Okla, M. K.; Al-Hashimi, A.; Al-Qahtani, W. H.; Abdelgawad, H.; Haq, I.-u.-. HPLC-DAD Based Polyphenolic Profiling and Evaluation of Pharmacological Attributes of Putranjiva roxburghii Wall. *Molecules* **2021,** 27, (1), 68. https://doi.org/10.3390/molecules27010068.
14. Naseem, S.; Ismail, H., In vitro and in vivo evaluations of antioxidative, anti-Alzheimer, antidiabetic and anticancer potentials of hydroponically and soil grown Lactuca sativa. *BMC Complementary Medicine and Therapies* **2022,** 22, (1), 30. https://doi.org/10.1186/s12906-022-03520-5.
15. Baig, M. W.; Nasir, B.; Waseem, D.; Majid, M.; Khan, M. Z. I.; Haq, I.-u., Withametelin: A biologically active withanolide in cancer, inflammation, pain and depression. *Saudi Pharmaceutical Journal* **2020,** 28, (12), 1526-1537. https://doi.org/10.1016/j.jsps.2020.09.021.
16. Madariaga-Mazón, A.; Naveja, J. J.; Medina-Franco, J. L.; Noriega-Colima, K. O.; Martinez-Mayorga, K., DiaNat-DB: a molecular database of antidiabetic compounds from medicinal plants. *RSC advances* **2021,** 11, (9), 5172-5178. https://doi.org/10.1039/D0RA10453A
17. Giri, B. R.; Baral, R.; Bhatt, H.; Khadka, A.; Tamrakar, R.; Timalsina, G.; Gyawali, R., Phytochemical screening, free-radical scavenging activity, in vitro alpha-amylase inhibitory activity, and in vivo hypoglycemic activity studies of several crude drug formulations based on selected medicinal plants of Nepal. *Pharmaceutical Chemistry Journal* **2023,** 56, (10), 1369-1378. https://doi.org/10.1007/s11094-023-02799-z.
18. Adefegha, S. A.; Oboh, G.; Omojokun, O. S.; Jimoh, T. O.; Oyeleye, S. I., In vitro antioxidant activities of African birch (Anogeissus leiocarpus) leaf and its effect on the α-amylase and α-glucosidase inhibitory properties of acarbose. *Journal of taibah university medical sciences* **2016,** 11, (3), 236-242. https://doi.org/10.1016/j.jtumed.2016.03.001.
19. Husain, I.; Ahmad, R.; Chandra, A.; Raza, S. T.; Shukla, Y.; Mahdi, F., Phytochemical characterization and biological activity evaluation of ethanolic extract of Cinnamomum zeylanicum. *Journal of ethnopharmacology* **2018,** 219, 110-116. https://doi.org/10.1016/j.jep.2018.02.001.
20. Medagama, A. B., The glycaemic outcomes of Cinnamon, a review of the experimental evidence and clinical trials. *Nutrition journal* **2015,** 14, 1-12. https://doi.org/10.1186/s12937-015-0098-9.
21. Wojdyło, A.; Oszmiański, J.; Czemerys, R., Antioxidant activity and phenolic compounds in 32 selected herbs. *Food chemistry* **2007,** 105, (3), 940-949. https://doi.org/10.1016/j.foodchem.2007.04.038.
22. Ćavar Zeljković, S.; Šišková, J.; Komzáková, K.; De Diego, N.; Kaffková, K.; Tarkowski, P., Phenolic compounds and biological activity of selected Mentha species. *Plants* **2021,** 10, (3), 550. https://doi.org/10.3390/plants10030550.
23. Meidinna, H. N.; Fatchiyah, F., The potential role of rosmarinic acid and sinensetin as α-amylase inhibitor: in silico study. *The Journal of Pure and Applied Chemistry Research* **2019,** 8, (1), 73-79. http://dx.doi.org/10.21776/ub.jpacr.2019.008.01.460.
24. Siddique, M. H.; Ashraf, A.; Hayat, S.; Aslam, B.; Fakhar-e-Alam, M.; Muzammil, S.; Atif, M.; Shahid, M.; Shafeeq, S.; Afzal, M., Antidiabetic and antioxidant potentials of Abelmoschus esculentus: In vitro combined with molecular docking approach. *Journal of Saudi Chemical Society* **2022,** 26, (2), 101418. https://doi.org/10.1016/j.jscs.2021.101418.
25. Liu, Y.; Kong, K. W.; Wu, D.-T.; Liu, H.-Y.; Li, H.-B.; Zhang, J.-R.; Gan, R.-Y., Pomegranate peel-derived punicalagin: Ultrasonic-assisted extraction, purification, and its α-glucosidase inhibitory mechanism. *Food chemistry* **2022,** 374, 131635. https://doi.org/10.1016/j.foodchem.2021.131635.