Original Article

Antioxidant and Antidiabetic Activity of *Berberis lycium* : The Possibilities of TLC-MS Bioautography and *in silico* Study

Harjeet Singh[1,*], Nardev Singh[2]

[1]Regional Ayurveda Research Institute, Jhansi; CCRAS, Ministry of AYUSH, Government of INDIA.
[2]School of Pharmaceutical Sciences, Graphic Era University, Dehradun, INDIA.

ABSTRACT

Background: Since ancient times, the traditional system of medicine has been practiced and developed an array of defense strategies to manage various ailments. About 60-80 percentof people from different parts of the world rely on herbal medicine for their health care. In the present study, a combined method using TLC mass spectrometry bioautography (TLC-MS bioautography) combined with DPPH and α-amylase activities was utilized to compare antioxidant and antidiabetic activities in an aqueous extract of *B. lycium*. **Materials and Methods:** In this paper, an aqueous extract of *B. lycium* was prepared, developed TLC and performed TLC-MS bioautographic to identify the antioxidant and antidiabetic bioactive metabolites. **Results:** TLC scans were performed at 254 and 366 nm, respectively and obtained results revealed there is a total of 11 and 10 metabolites present in *B. lycium*. Further, TLC-MS Bioautographic studies isolate a total of two lead compounds such as berberine and palmitine through a chemico-biological detection system. Out of two, berberine showed antioxidant and antidiabetic activity while palmitine only shows antidiabetic activity. **Conclusion:** TLC-MS bioautographic screening could be an excellent technique to assess the antioxidant and antidiabetic activity of herbal products and the results achieved will be very useful for promoting their utilization and development of new therapeutic regimens.

Key words: *Berberis lycium,* TLC-bioautography, Antioxidant, Antidiabetic, *in silico*.

INTRODUCTION

Since ancient times medicinal plants are an enormous source of bioactive compounds with health-promoting activities. A traditional medicinal system like Ayurveda, Unani and Chinese is still promising and has been practiced for over 150 decades. They have established an assortment of defense strategies to manage oxidative stress and other disorders like diabetes. About 60-80 percent of people from different parts of the world rely on herbal medicine for their health care.[1] Several research and meta-analysis articles have been published upon n-number of medicinal plants that possess specific action on various ailments such antioxidant, antidiabetic, anti-inflammatory, respiratory etc.[2]

Diabetes is a metabolic disorder which is acknowledged by its most common characteristic of diabetes such as hyperglycemia. It is mostly categorized into two main types i.e. type I diabetes mellitus which is associated with the deficiency of insulin production while in type II diabetes mellitus insulin secretion and tissue resistance occur mostly which caused the least active function of insulin. Although it can not be denied that most of the diseases are mainly accompanied by the diet pattern or lifestyle of the human being. The disturbed diet schedule or excess production even the least consumption of calories leads to obesity and a modern sedentary lifestyle.[3]

Submission Date: 22-09-2020;
Revision Date: 26-10-2021;
Accepted Date: 25-11-2021.

DOI: 10.5530/ijper.56.1.24
Correspondence:
Harjeet Singh,
Regional Ayurveda Research Institute, Jhansi; CCRAS, Ministry of AYUSH, Government of INDIA.
E-mail: harjeet_cops@yahoo.com

www.ijper.org

The transience associated with diabetes is primarily due to the amplified threat of several difficulties of this illness. Diabetes is one of the most common complications accompanied by mortality in the body system such as nephropathy, hypertension and retinopathy other cardiac-associated disorders. Taking all these into consideration, researchers are being more prompted to develop a new molecule with the least side effect and economic with do not only potentiate the diabetic therapy but also decrease the severe mortalities associated with the diabetes mellitus.[4]

Oxidative stress is seen as another lethal complication that is induced due to the high production of free radicals. In oxidative stress, the intrinsic strength of the body to scavenge the free radicals decrease or even terminated up to a high level. Predominately reactive oxygen species (ROS) are the main sources that are responsible for the induction of oxidative stress. Several pieces of research have an emphasis on free radicals and claimed that oxidative stress is responsible for many complications such as cancer, atherosclerosis, inflammation, diabetes, and senescence. The major defense against free radicals is found from natural resources especially from medicinal plants as they are highly associated with the natural compounds which play a major role as antioxidants. Antioxidant compounds are highly enriched with polyphenols which havethe ability to scavenge the free radicals and strengthen the immune system against oxidative stress.[5] Natural antioxidants have worldwide popularity which is consumed as nutritional or medicinal supplements even in some tribal areas as food supplements. Moreover, considering the facts of natural antioxidants, many pharmaceutical industries are highly associated with the production of many health supplements for the well beings of the body system against oxidative stress or even associated disorders such as anticarcinogenic, antidiabetic, and anti-Alzheimer activities. In the current era of the medicinal system, natural antioxidants are gaining worldwide popularity for natural antioxidants to prevent oxidative stress-induced disease or even to decrease senescence-related disorders.

Berberis lycium is one of the important medicinal plants belonging to the family Berberidaceae. Himalayan region is highly engaged with the popularity of this plant and it is an evergreen shrub. It is used for the treatment of many disorders and because of that, it is gaining wider acceptance for its medicinal value especially in the Indian system of medicine such as liver disorders, skin diseases, abdominal disorders, cough, ophthalmic and many more.[6] Moreover, the therapeutic values for this plant are mainly associated such as hepatoprotective, hypoglycemic, hyperlipidaemic, antipyretic and anticarcinogenic properties. The various parts of *B. lycium* such as roots, stem, leaves, and fruit contain bioactive compounds like tannins, saponins, alkaloids, anthocyanins, berberine, berbamine, chinabine, baluchistanamine and sindamine.[7]

A number of chromatographic and spectroscopic analytical approaches are highly associated with quality-based standardization of herbal medicine or their derived products, which include planner chromatography, liquid chromatography, and gas chromatography. The approaches for scientific validation of herbal medicine or their derived products generate scientific evidence to validate their quality, safety, and efficacy.[8]

TLC-MS bioautographic hyphenated approaches have been frequently used for qualitative estimation of antidiabetic and antioxidants compounds through combination with α-amylase and DPPH free radicalbioassay. DPPH assays to assess the antioxidant capacity of natural compounds even plant matrix. Moreover, in various reports, it has been declared that oxidative stress is not only associated with many complications such as oxidative stress-induced inflammatory stress but also it is functioned to resist the activity of insulin and decrease the glucose uptakes by the muscle. Although, it has been declared that the antioxidant regimen combined with other therapeutic regimenspotentiates the excellent therapeutic approach to prevent the various complications associated with oxidative stress.[9] The *α*-amylase is accountable for the breakdown of starch in the gut and then glucose was released and finally reached to blood circulation. While inhibitors of amylase decrease the breakdown of starch in the gut and consequently decrease its absorption.[10] Hence, their preventivefunction can decrease the relief of glucose from saccharidesand ultimately lower the post-prandial uptake of glucose in the patient associated with diabetes. *Berberislycium* was carefully chosen for the current study. We aim to evaluate the antioxidant and anti-diabetic bioactive metabolites in aqueous extracts of *B. lyciumby* using hyphenated techniques TLC-MS bioautography.

MATERIALS AND METHODS

Collection and identification of plants

Fresh part of *Berberis lycium* was obtained from the local market Khari Bawli, New Delhi, and taxonomic identification was confirmed from National Medicinal Plant Board, New Delhi.

Preparation of extract

Berberis lycium

100 g of the drug was transferred in a 1000 ml flask containing 500 ml water and boiled at low temperature for 5-6 hr with gentle shaking to avoid direct heat to extracts. Further, the resultant extracts were filtered to the impurities and collected the useful filtrate. The semisolid extract was collected in a wide mouth bottle and is called rasont. Moreover, the semisolid rasont was kept in an air-tight container in a dark place for further analysis.

Estimation of total phenolic content

The Folin-Ciocalteu reagent was utilized for the assessment of the content of total phenolic in the samples.[11] Various concentrations of samples and standard gallic acid were used for estimation of total phenol content. Phenolic content is presented as mg of standard equivalent/gm of samples.

Estimation of total flavonoid content

The content of total flavonoids was determined througha colorimetric approach using aluminium chloride. Quercetin was utilized to plot standard calibration curves. Sample aliquot was prepared by mixing of drug extract and aqueous $AlCl_3$ (20% w/v). The spectrophotometric record was observed at 425 nm against a blank.[12] The total flavonoid content in the aqueous extract of *B. lycium* was calculated and data were presented as mg of standard equivalent/ gm of samples.

HPTLC analysis

Pre-coated TLC plates were simply cut into 10×10 cm (Merck, Germany) by pressing against a hard surface. Then the prepared solution was centrifuged and collects the supernatant and then applied on an activated TLC plate using Camag Linomat-5 applicator. The applied plate of *B. lycium* was developed in the solvent containing n-butanol: acetic acid: water; 4: 1: 5 v/v/v and further plates were transferred in the development chamber. After 40 min the developed plate was removed gently, air dried, and scanned at 254 and 366 nm, respectively. Same TLC plates were used for TLC-MS bioautographic analysis.

In vitro DPPH assay

The DPPH free radical activities of the *B. lycium* were determined by the spectrophotometric method for the presence of DPPH as a free radical. Briefly, the extract was mixed with DPPH solution and keptin a dark place at optimum temperature for 1/2 hr. Finally, the UV was measured at 517 nm. The following equation has been used to examine the percentage inhibition (PI) activity against control (contain only methanol instead of samples).[13] Ascorbic acid was utilized as a reference compound for the comparison.

$$PI = (1\text{-}A_{sample}/A_{control}) \times 100 \quad (1)$$

Detection of antioxidant compounds using TLC-bioautography

The antioxidant bioactive compound in *B. lyceum* extract was identified by utilizing bioautography. After running the sample on TLC plates using Camag Linomat-V applicator. Saturated the TLC plates in the suitable solvent for the respective plant samples in a twin glass HPTLC chamber. Then remove the TLC plates from the solvent chamber and air-dried. After air dried activated plates and sprayed with 5mM DPPH solution. After few minutes, the yellowish or cream color appeared against purple backgrounds, antioxidant bioactive compounds.[9]

Detection of anti-diabetic compounds using TLC-bioautography

Alpha-amylase

The alpha-amylase working solution was prepared in the normal buffer. Further, prepared the starch and iodine solution and kept them at 37°C for further analysis. The dine solution of iodine was kept in a dark place. The *B. lycium* sample was applied on TLC silica G_{60} F_{254} plates, placed in the development chamber for action. After completion of activation, plates were removed, air-dried. Thereafter enzyme solutions were sprayed and kept in a desiccator for 1.5 h. After, 1.5 hr, the plate was removed from the desiccator and treated with the solution of starch, kept for 15 min for the completion of enzyme-substrate reaction, finally the plate was dried and sprayed within the solution of iodine. The inhibitors of α-amylase were visible on the plate.[10]

Mass Spectrometry analysis of metabolites identified on TLC

Areas corresponding to the DPPH, alpha-amylase inhibition zones on a bioautogram were marked on the duplicate chromatogram and scraped the encircled zones with a specially designed scraper and dissolved in methanol. Prepared sample solutions were filtered using a 0.22μm syringe. Both positive and negative ionization mode was utilized to ensure the bioactive compounds present on TLC plates. Chromatographic separation of metabolites was done on the C_{18} columnusing methanol: water: formic acid (8:2:0.1% v/v) as mobile phase, cone gas, and the temperature were set to 500 L/h, 50 L/h, and 100°C, respectively.[14] Mass

spectrometric compounds were tentatively ensured based on their *m/z* value from the mass data bank.

In silico analysis

Initial step in the *in silico* drug designing procedure is the finding and choosing of there levant drug target or receptor. To study the nature of the interaction, binding modes and selectivity of amylase and glucosidase receptors with quantified secondary metabolites of aqueous extract of *B. lycium*, and molecular docking was performed with Autodock 4.2. The crystal structure of the enzyme wasretrieved from the RCSB protein data bank. The ligands namely berberine and palmitine for *B. lycium,* were downloaded as a 3D structure SDF file from PubChem (http://pubchem.ncbi.nlm.nih.gov/) and optimized using Ligands Input in the AD 4.2. The target enzyme was prepared by the addition of all polar hydrogen atoms with the AutoDock Tools and merging its nonpolar hydrogen atoms along with the removal of the water molecule and other heteroatoms to the target enzyme, a necessary process for the computation. Three-dimensional affinity grids (126 × 126 × 126Å) were considered for each atom.[15] The optimized ligand molecules were docked with refined diabetes receptors using autodock 4.2. The docking results were analyzed using PyMOL molecular graphics visualization tool. The autodock was used for preparing files in the form of PDBQT from PDB files and then docking the ligands to enzymes on the actual site using autodock 4.2. After completion of docking searches, the best conformation was selected with the lesser binding energy. The ligand-protein complex includes hydrogen bonds were analyzed using PyMOL Molecular Graphics Visualizer 1.7.4.5.

RESULTS

The main objective of the study was to identify active metabolites present in aqueous extract of *B. lycium* to identify the bioactive compounds as antioxidant and antidiabetic activities. Keeping in view, a developed method of TLC-MS bioautography was performed. The respective resultant extract was filtered and completely evaporated to dryness. After successive dryness, the percentage yield was recorded as 12.5% for *B. lycium*.

Total Phenolic and Flavonoid Content

In this study, the number of total polyphenols for *B. lyceum* was estimated from the determined calibration curve of the standard molecule as gallic acid (R^2= 0.9969) and quercetin (R^2= 0.9921). The outcomes revealed that the content of phenols and flavonoids was found to be 29.38 and 18.36 mg of gallic acid and quercetin respectively in an aqueous extract of *B. lycium*. The standard curve of gallic acid and quercetin were depicted in Figure 1.

HPTLC fingerprint analysis

Plants containa variety of bioactive phytoconstituents which possess the biological activities against several dysfunctions and are attributed for their principal actions. In such cases, finger print profiling of the plant extracts is very convenient and broadly used for its qualitative and quantitative estimation. In this analysis, different metabolites possess at different retardation times which revealed individual constituents at single retardation time. The HPTLC finger printing of *B. lyceum* was established on a TLC plate and visualized at 254 and 366. HPTLC finger printing showed a maximum number of compounds viz. 11 and 10 at 254 and 366 nm, respectively (Table 1 and Figure 2).

Table 1: Data pertaining to HPTLC fingerprint of aqueous extract of *B. lycium* at 254 and 366 nm.

S. No	Retention Time	Percentage Area	
		At 254	At 366
1.	0.04	+	-
2.	0.07	+	+
3.	0.13	-	+
4.	0.17	+	+
5.	0.24	+	+
6.	0.27	+	+
7.	0.31	+	-
8.	0.36	+	+
9.	0.41	+	+
10.	0.46	-	+
11.	0.50	+	-
12.	0.55	+	+
13.	0.70	-	+
14.	0.78	+	-
15.	**Total (14)**	**11**	**10**

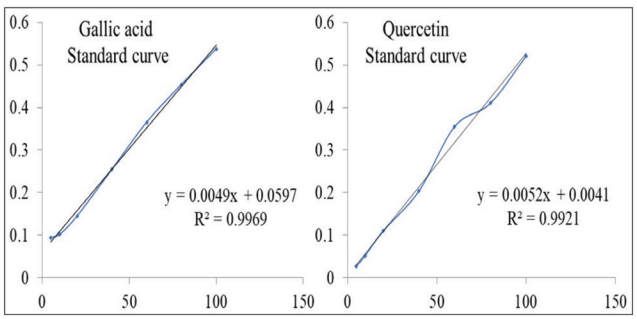


Figure 1: Standard calibration curve of gallic acid and quercetin.

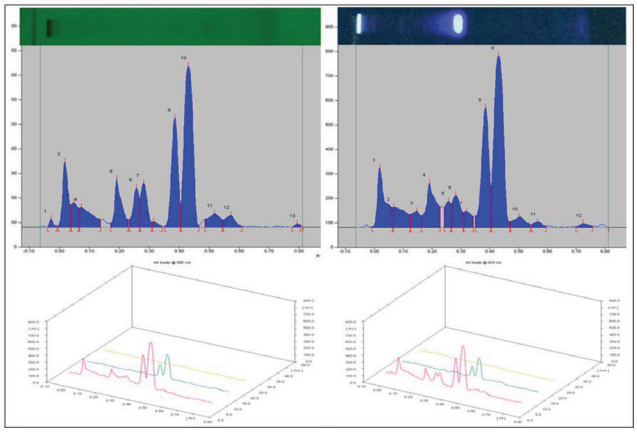


Figure 2: TLC photography and chromatogram of aqueous extract of *B. lycium* at 254 and 366 nm.

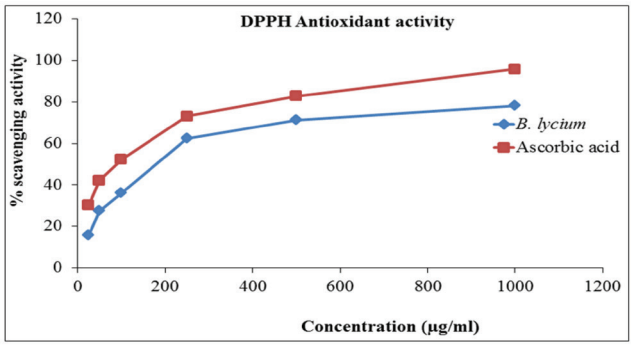


Figure 3: DPPH scavenging activity of *B. lycium* with ascorbic acid.

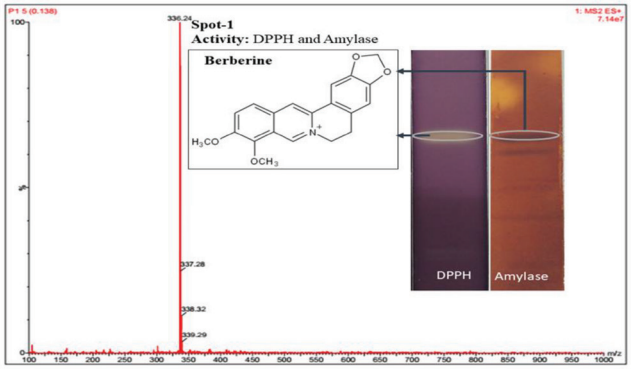


Figure 4: MS bioautogram of *B. lycium*: spot 1: DPPH and amylase.

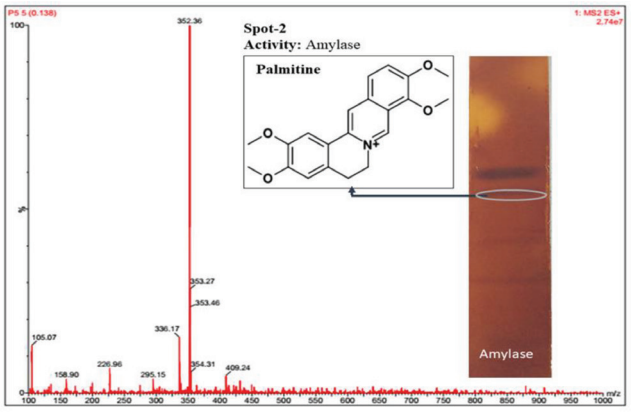


Figure 5: MS bioautogram of *B. lycium*: spot 2: amylase.

DPPH radical scavenging assay

The DPPH free radical scavenging potential of the sample was determined successively and the outcomes revealed that results are shown in Figure 3 as compared with known antioxidant compounds ascorbic acid. From the analysis of Figure 3, we can conclude that the scavenging effects of *B. lycium* show excellent scavenging activity as compared to ascorbic acid. The antioxidant activity of *B. lycium* was found in a dose-dependent manner. When the dose increases the activity increases accordingly.

HPTLC-Bioautography assay of DPPH and alpha-amylase

Antidiabetic and antioxidant compounds accompanied with *B. lycium* extract were alienated on TLC plate using the established solvent system and estimated for their identified metabolites retard at specific place of stationary phase.

For screening of potential scavenging compounds from *B. lycium* extract, a direct bioautographic assay (TLC-DPPH) was established.[16] After separation metabolites on the stationary phase of TLC plates, the compounds which showed potential scavenging activity was considered as antioxidant compounds. Bioactive bands isolated from TLC plate after treatment with DPPH and perform the mass spectrometry analysis. Results from mass spectra revealed berberine (*m/z*= 336.36) shows the antioxidant activity in the case of *B. lyceum* (Figure 4). Mass spectrometry of the scraped bioautogram of the TLC, revealed there are two bioactive compounds from *B. lyceum* showed α-amylase inhibitory compounds like berberine (*m/z*= 336.36) and palmitine (*m/z*=342.40) (Figure 5).

Docking interaction analysis

Simulated analysis of biomolecule collections mightenable the search for innovative lead constituents that is appropriate for additional new drug discovery study. In this study *in silico* computational docking studies were carried according to hyphenated bioautographic results to find out the enhancing activity of the bioactive compounds present in the extract of *B. lycium* on AD 4.2 using PyMol visualizer. We got the binding

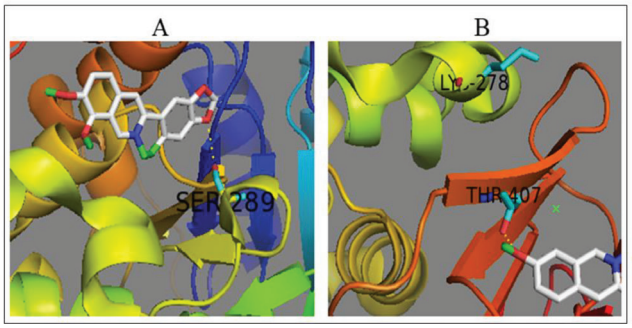


Figure 6: Docked pose of A) berberine, B) palmitinewith α-amylase using PyMOL molecular graphics system 1.7.4.2.

Table 2: The interaction energy analysis of four ligands with that of α-amylase using PyMOL molecular graphics system 1.7.4.2.

Proteins	Ligands	Modes	Binding Energy	Interacting Residue
α-amylase	Berberine	Pose 1 and 3	-7.3, -7.1	SER 289, ALA 307
	Palmitine	Pose 5 and 6	-5.7, -5.7	LYS 271, THR 407

interaction of ligands such as berberine, and palmitine with the receptor alpha-amylase. Results come out from docking studies revealed several amino acids of alpha-amylase residue strongly bind to mentioned bioactive metabolites. The inhibition of alpha-amylase was considered based on choosing new small molecule drugs. Berberine and palmitine bind with amylase by interacting with several amino acids at different positions such as berberine SER 289, ALA 307, for palmitine LYS 271, THR 407, respectively Figure 6 and (Table 2).

DISCUSSION

B. lyceum is augmented with enriched polyphenolic compounds which are mostly accountable for the antioxidant as well as antidiabetic activity of the extract. The bioactive potential of the plant extract against oxidative stress might be due to the occurrence of the free hydroxyl group in flavonoids. The free hydroxyl group is responsible to scavenge the free radicals which potentiate excellent antioxidant activity. Phenols and flavonoids (ArOH) are acknowledged to decrease the rates of oxidative stress by relocating the H atom to ROO* radicals.[17] Through this action of phenols and flavonoids inhibited the construction of ROS or by the disturbing proliferation of the ROS and play essential roles in ROS metabolism and escaping of abandoned oxidation of important bioactive constituents in the biological system. Conjectured relative between diabetes, ROS, and inflammation, the potential for phenolics and flavonoids protected the body against free radicals. Therefore, biologically and pharmacologically believe that phenolic and flavonoids rich foods may reduce the risk of diabetes.[18]

The quality control studies of medicinal plants play a significant role in the biological activity. Therefore, HPTLC analysis was performed to identify the number of secondary metabolites present in extracts. This is one of the best techniques to set the quality of natural products.

The outcomes suggested that the plant matrix comprises phenols and flavonoids that are accomplished of contributing hydrogen to a ROS to scavenge the probable damage. Moreover, polyphenols inhibited the generation of ROS, and significantly enhanced the antioxidant defenses which are straight complicated in the construction of ROS. Phenolic compounds of natural origins fall into several categories. Among these, flavonoids are the chief which has essential to the body. Studies on flavonoids and their associated compounds have revealed bioactivities such as anti-inflammatory, antibacterial, antiviral, anticancer, and anti-allergic activities, and many more.[19]

Herbal medicine seems to possess most metabolites, extensively used as a folk therapeutic regimen for the management of various disorders. We determined and compared the docking scores of compounds presented in the *B. lycium* with diabetic receptors using an automated docking model. Alpha-amylase receptors represent the forefront of recent research and major advances have been made in understanding the molecular function of diabetes and its related disorders.[20]

Summarily, a strong therapeutic target is presumed to be the generation of reactive oxygen species in the body. Natural compounds contain several bioactive metabolites which inhibited free radicle generation in the body as well as enhance the defense mechanism of the body.[21] Amylase breaks the starch into saccharides and then releases the glucose and engrossed into the blood circulation. The inhibition of enzyme amylase directly decreases the collapse of starch into the gastrointestinal tract and succeeding glucose absorption. These deals decrease post-prandial hyperglycemia levels. Moreover, inhibitors of these enzymes such as amylase are of therapeutic interest in type 2 diabetes by inhibiting the breakdown and absorption of starch and carbohydrates from the gastrointestinal tract. The scoring function of molecular computational docking is a basic fundamental component. Successful and easy application examples showed that computational approaches have

one of the powerful approaches to screen hits/leads from a huge database and design novel small molecules. However, the realistic binding between bioactive molcules and receptors still relies on experimental technology.

CONCLUSION

The aqueous extract of *B. lyceum* had remarkable antioxidant and antidiabetic activities. TLC-MS bioautographic data revealed bioactive metabolites present in *B. lyceum* possess excellent antidiabetic and antioxidant activities. Overall, the studies illustrate that *B. lyceum* extract has huge potential to be developed into an antidiabetic drug. Thus, it could be further explored for its possible application in diabetes.

ACKNOWLEDGEMENT

The author would like to acknowledge all the members of BNPL to assist me in my research work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

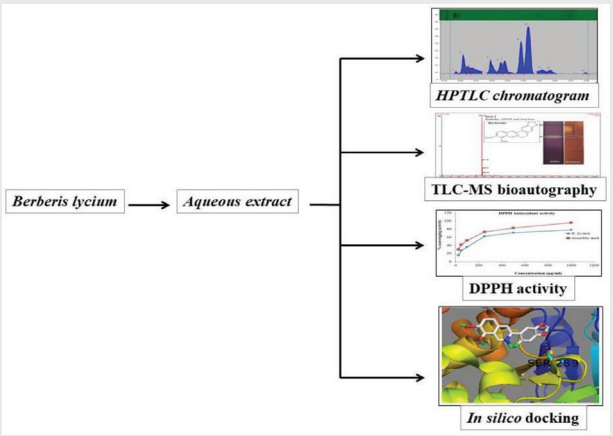
ABBREVIATIONS

ROS: reactive oxygen species; **GIT:** gastrointestinal tract; **DPPH:** 2,2-Diphenyl-1-picrylhydrazyl radical; **LC:** Liquid chromatography; **GC:**gas chromatography; **MS:** mass spectrometry; **TLC:** thin layer chromatography; **HPLC:** high-performance liquid chromatography; **HPTLC:** high-performance thin layer chromatography; **pNPG:**(4-Nitrophenyl-β-D- glucopyranoside).

REFERENCES

1. Pandey MM, Rastogi S, Rawat AKS. Indian traditional ayurvedic system of medicine and nutritional supplementation. Evid Based Complement Alternat Med. 2013;2013:376327. doi: 10.1155/2013/376327, PMID 23864888.
2. Ekor M. The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol. 2014;4:177. doi: 10.3389/fphar.2013.00177, PMID 24454289.
3. Mahler RJ, Adler ML. Clinical review 102: Type 2 diabetes mellitus: Update on diagnosis, pathophysiology, and treatment. J Clin Endocrinol Metab. 1999;84(4):1165-71. doi: 10.1210/jcem.84.4.5612, PMID 10199747.
4. American Diabetes Association. Standards of medical care in diabetes. Diabetes Care. 2004;27;Suppl 1:S15-35. doi: 10.2337/diacare.27.2007.s15. PMID 14693923.
5. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007;39(1):44-84. doi: 10.1016/j.biocel.2006.07.001, PMID 16978905.
6. Sood P, Modgil R, Sood M. *Berberis lyciuma* Medicinal Plant with Immense Value. Indian J Pharm Biol Res. 2012;1(1):9.
7. Ahmed S, Shuaib M, Ali K, Ali S, Hussain F. Evaluation of different parts of *Berberis lycium* and their biological activities: a review. Pure Appl Biol. 2017;6(3):897-907.
8. Valle DL, Puzon JJ, Cabrera EC, Rivera WL. Thin Layer Chromatography-Bioautography and Gas Chromatography-Mass Spectrometry of Antimicrobial Leaf Extracts from Philippine *Piper betle* L. against Multidrug-Resistant Bacteria. Evid Based Complement Alternat Med. 2016;2016:4976791. doi: 10.1155/2016/4976791. PMID 27478476.
9. Fahim M, Ibrahim M, Zahiruddin S, Parveen R, Khan W, Ahmad S, *et al*. TLC-bioautography identification and GC-MS analysis of antimicrobial and antioxidant active compounds in *Musa paradisiaca* L. fruit pulp essential oil. Phytochem Anal. 2019;30(3):332-45. doi: 10.1002/pca.2816, PMID 30609101.
10. Dewanjee S, Gangopadhyay M, Bhattacharya N, Khanra R, Dua TK. Bioautography and its scope in the fi eld of natural product chemistry. J Pharm Anal. 2015;5(2):75-84. doi: 10.1016/j.jpha.2014.06.002, PMID 29403918.
11. Khan W, Parveen R, Chester K, Parveen S, Ahmad S. Hypoglycemic potential of aqueous extract of *Moringa oleifera* leaf and *in vivo* GC-MS metabolomics. Front Pharmacol. 2017;8:577. doi: 10.3389/fphar.2017.00577, PMID 28955221.
12. Amoussa AMO, Sanni A, Lagnika L. Antioxidant activity and total phenolic, flavonoid and flavonol contents of the bark extracts of *Acacia ataxacantha*. J Pharmacogn Phytochem. 2015;4(2):172-8.
13. Chester K, Paliwal S, Khan W, Ahmad S. UPLC-ESI-MS/MS and HPTLC method for quantitative estimation of cytotoxic glycosides and aglycone in bioactivity guided fractions of *Solanum nigrum* L. Front Pharmacol. 2017;8:434. doi: 10.3389/fphar.2017.00434, PMID 28729835.
14. Kanama SK, Viljoen AM, Kamatou GPP, Chen W, Sandasi M, Adhami HR, *et al*. Simultaneous quantification of anthrones and chromones in *Aloe ferox* ('Cape aloes') using UHPLC-MS. Phytochem Lett. 2015;13:85-90. doi: 10.1016/j.phytol.2015.04.025.
15. Ferreira LG, Dos Santos RN, Oliva G, Andricopulo AD. Molecular docking and structure-based drug design strategies. Molecules. 2015;20(7):13384-421. doi: 10.3390/molecules200713384, PMID 26205061.
16. Gu L, Wu T, Wang Z. TLC bioautography-guided isolation of antioxidants from fruit of *Perilla frutescens* var. acuta. LWT Food Sci Technol. 2009;42(1):131-6. doi: 10.1016/j.lwt.2008.04.006.
17. Brewer MS. Natural Antioxidants: Sources, Compounds, Mechanisms of Action, and Potential Applications. Compr Rev Food Sci Food Saf. 2011;10(4):221-47. doi: 10.1111/j.1541-4337.2011.00156.x.
18. Vinayagam R, Xu B. Antidiabetic properties of dietary flavonoids: A cellular mechanism review. Nutr Metab (Lond). 2015;12:60. doi: 10.1186/s12986-015-0057-7. PMID 26705405.
19. Saeed N, Khan MR, Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L BMC Complement Altern Med. 2012;12(1):221. doi: 10.1186/1472-6882-12-221, PMID 23153304.
20. Tundis R, Loizzo MR, Menichini F. Natural products as alpha-amylase and alpha-glucosidase inhibitors and their hypoglycaemic potential in the treatment of diabetes: An update. Mini Rev Med Chem. 2010;10(4):315-31. doi: 10.2174/138955710791331007, PMID 20470247.
21. Phaniendra A, Jestadi DB, Periyasamy L. Free radicals: Properties, sources, targets, and their implication in various diseases. Indian J Clin Biochem. 2015;30(1):11-26. doi: 10.1007/s12291-014-0446-0, PMID 25646037.

PICTORIAL ABSTRACT



SUMMARY

In this study, extraction, and evaluation of aqueous extracts of *Berberis lycium* for its antioxidant potential by DPPH method. HPTLC phytochemical screening revealed a number of metabolites present in the extracts. Further, by using TLC-MS bioautography, antioxidant and antidiabetic leads were also identified. Therefore, it can be summarized that *Berberis lycium* may prevent oxidative stress and diabetes.

Cite this article: Singh H, Singh N. Antioxidant and Antidiabetic Activity of *Berberis lycium*: The Possibilities of TLC-MS Bioautography and in silico Study. Indian J of Pharmaceutical Education and Research. 2022;56(1):207-14.