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EVALUATION OF STANDARDIZATION AND ANTIGLYCATION ACTIVITY OF LEAF PART OF BARLERIA PRIONITIS L.

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ABSTRACT
The number of deaths due to stroke among diabetic patients escalated from 52,397 to 114,092. Similarly, fatalities from ischemic heart disease (IHD) in individuals with diabetes rose from 35,351 to 76,974, while deaths from chronic kidney disease in this population increased from 29,061 to 63,279. Overall, the mortality rate attributable to diabetes and its complications surged by 117% over a span of 25 years, averaging an annual increase of 4.7%. Increased glucose concentrations produce advanced glycation end products (AGEs). The reactions associated with protein glycation that lead to an overproduction of AGEs are primarily responsible for a range of complications related to diabetes. The potential of alternative medicine derived from plants can be explored. Phytochemicals found in Barleria species include iridoids, kaempferol, ferulic acid, and caffeic acid. Barleria prionitis L., a plant of medicinal significance, is classified within the Acanthaceae family. Objective: The study investigated standardization and antiglycation activity of crude dry extract of Barleria prionitis L. Method: The principle of antiglycation is the inhibition of the reaction of the formation of AGEs which the products of the glycation reaction between protein and glucose, and are measured using a UV-vis spectrophotometer. Result: The results of the standardization indicate that EEBL has a water soluble compound content of 40.45 ± 0.56%, an ethanol soluble compound content of 15.08 ± 0.74%. This is indicated the amount of compounds that dissolve in water compared to ethanol. The number of dissolved compounds reflects the quantity of secondary metabolite compounds that have been solubilized in the solvent. A water content of 7.508 ± 0.399%, a total ash content of 9.41 ± 0.67%, an acid insoluble ash content of 2.17 ± 0.03%, and an extract density of 0.913 ± 0.015 g/mL. Additionally, EEBL was found to contain flavonoids, alkaloids, tannins, saponins, and triterpenoids. Antiglycation activity results obtained IC50 values of 177.02 ± 0.80 mg/L. EEBL contains secondary metabolite compounds such as flavonoids, phenolics, and terpenes, that can act as free radical scavengers. The mechanism of polyphenol antioxidant protection is associated with its ability to bind proteins, thereby preventing the formation of AGE. Conclusions: This study provides the results of the antiglycation activity of standardized EEBL. These findings offer valuable insights for further investigation of the in vivo antidiabetic properties, as well as data that may contribute to the development of new herbal medicines and dietary supplement formulations utilizing the crude extract.

Keywords: age; antiglycation; barleria prionitis; standardization

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INTRODUCTION

Diabetes is one of the non-communicable diseases characterized by hyperglycemia, this disease is a major problem in terms of prevalence and mortality in the world and Indonesia (Sheleme et al., 2020). The International Diabetes Federation (IDF) predicts that diabetes will be suffered by 643 million people globally in 2030, this number will increase to 783 million in 2045 (Kumar et al., 2024). The estimated prevalence of diabetes in Indonesia is projected to rise from 9.19% in 2020, affecting approximately 18.69 million individuals, to 16.09% by 2045, impacting around 40.7 million people. The complications associated with diabetes contribute significantly to increased rates of morbidity and mortality. Specifically, the number

of deaths due to stroke among diabetic patients escalated from 52,397 to 114,092 during this timeframe. Similarly, fatalities from ischemic heart disease (IHD) in individuals with diabetes rose from 35,351 to 76,974, while deaths from chronic kidney disease in this population increased from 29,061 to 63,279. Overall, the mortality rate attributable to diabetes and its complications surged by 117% over a span of 25 years, averaging an annual increase of 4.7% (Wahidin et al., 2024).

The adverse effects of persistently elevated blood glucose levels in various parts of the body of people with diabetes cause complex reactions of cell damage in response to high glucose levels. One of them is the formation of advanced glycation end products (AGEs) (Singh et al., 2014). Continuously elevated glucose levels can form covalent bonds with plasma proteins through a non-enzymatic process known as glycation. Glycation reactions that cause excessive AGEs formation are the main cause of various complications of diabetes (Vlassara et al., 2014). Patients with type 2 diabetes mellitus have a higher risk of experiencing microvascular and macrovascular complications compared to the non-diabetic population (Blahova et al., 2021). As people with diabetes age, they become susceptible to various complications (Tomic et al., 2022). A number of synthetic drugs used in the treatment of diabetes currently have the main objective of reducing postprandial hyperglycemia by inhibiting the activity of digestive enzymes or by stopping glucose absorption in the intestine (Sagandira et al., 2021). However, these drugs are expensive and have side effects, which limit their use. Therefore, it is necessary to develop alternative drugs from natural resources such as plants with fewer side effects and are easily available (Bindu et al., 2019).

Alternative drugs from plants can be utilized because of the content of single phytochemicals or the synergistic effects of several phytochemical compounds (Ali et al., 2017). Phytochemical compounds from Barleria species are iridoids, kemferol, and ferulic acid, caffeic acid (Singh et al., 2023). Flavonoid content in ethanol extract of Barleria prionitis leaves is 44.23±2.82 mg RE/g (Ranade et al., 2016). Parts of the Barleria prionitis L. have been studied pharmacologically for antimicrobial and hepatoprotective (Tuliballi et al., 2013), anticancer (Panchal et al., 2018), gastroprotective (Choudhary et al., 2014), and antidiabetic (Reema et al., 2010). In addition, the ethanol extract and water extract of this plant have antioxidant activity as indicated by the IC50 values of 65.58 µg/ml and 77.40 µg/ml, respectively (Ranade et al., 2016). Antioxidants have an important function in protecting the human body from excessive ROS (Zehiroglu et al., 2019). Hyperglycemia induces a state characterized by increased biosynthesis of reactive oxygen species (ROS) (González et al., 2023). Therefore, compounds in plants that have antioxidant properties provide an opportunity to be developed as antidiabetic agents.

Previous studies have shown that methanol extract of Barleria prionitis leaves has the potential as an antidiabetic based on inhibition of α-amylase and α-glucosidase enzymes in vitro (Kato et al., 2020). Inhibitors of these enzymes help maintain cell function and lower blood glucose levels (Gong et al., 2020). However, the use of methanol is not recommended because it is very toxic and impractical for use in pharmaceutical products, so this study used 70% ethanol which lower toxicity. Based on previous studies, ethanol extract of Barleria prionitis leaves with soxhlet extraction techniques can attract more secondary metabolite compounds and is more effective in decrease glucose levels compared to maceration extraction techniques (Utami et al., 2023), so this study continues previous studies by conducting specific and non-specific standardization of ethanol extracts carried out to ensure the quality of the extract (Syukri et al., 2020). Antiglycation testing was also carried out in vitro as an initial investigation into the prevention of complications in diabetic. The purpose

of this study was to test the antiglycation activity and standardization of landep leaf extract with parameters in the form of water or ethanol soluble compounds, determination of water content, density of extract, determination of ash content, determination of acid-insoluble ash content, heavy metal contamination (Hg, As, Pb, and Cd).

METHOD

Sample preparation

The identification of *Barleria prionitis* L. samples was conducted at the Tawangmagu Traditional Health Service Functional Implementation Unit (UPF Yankestrad Tawangmangu) through the comparison of morphological traits. The leaf of *Barleria prionitis* were selected and thoroughly washed, followed by drying the samples in an oven at 50°C. Subsequently, the dried leaves were ground to a smaller size and sifted using a mesh size of 40.

Extraction

600 grams of *Barleria prionitis* leaf powder was put into a soxhlet apparatus and 70% ethanol was added in a ratio of 1:10 at a temperature of 50°C. The extract was concentrated with a rotary evaporator at a temperature of 50°C and continued with a water bath until a thick extract was obtained extract ethanol of *Barleria prionitis* (EEBL).

Standarization of crude extract of *Barleria prionitis*

Water or ethanol soluble compounds

Weighed ±2.5 g of EEBL, stirred for 1 hour with 100 mL of 95% ethanol or aquadest-chloroform (1:1), leaved for 18 hours. Then filter, took 20 mL of filtrate, put it in a shallow flat-bottomed dish that has been tared and then evaporated until dry in a water bath at 80ºC, the residue is heated at 105ºC until the weight remains constant. The content of ethanol soluble extract was calculated as a percentage of extract dissolved in ethanol to the material that has been dried in air.

Determination of Water Content

1 gram of EEBL was placed in a cup that has been weighed. The sample was dried at a temperature of 105ºC until a constant weight is obtained.

Density of extract

The pycnometer was clean and dry. EEBL is diluted 5% using water. The water extract was put into the calibrated pycnometer, wiped with tissue, and weighed. The density of extract of the extract obtained is the result of dividing the density of the EEBL by the density of water in the pycnometer at a temperature of 25°C.

Determination of Ash Content

Weigh 1 gram of EEBL in a pre-weighed porcelain cup. Ignite the sample until all charcoal was consumed, then allow it to cool in a desiccator before weighing.

Determination of Acid-Insoluble Ash Content

The obtained ash, add 25 ml of dilute HCl and boiled for 5 minutes. Collect the insoluble residue by filtering through filter paper, then ignite the filter paper until a constant weight is achieved, and record the weight.

Heavy metal contamination (Hg, As, Pb, and Cd)

A quantity of 1 gram of EEBL was measured and combined with 10 mL of concentrated HNO_3, 10 mL of aquadest, and 5 mL of $HClO_4$. The mixture was then heated using a heating mantle until it reached a thick consistency. Subsequently, the thick extract was filtered through Whatman paper no. 41 and transferred into a 50 mL volumetric flask. The sample was subsequently analyzed using Atomic Absorption Spectroscopy (AAS).

In Vitro Antiglycation Activity Assay

Hemoglobin preparation

The preparation of blood hemolysate was conducted through the process of hypotonic lysis. Initially, red blood cells with a NaCl solution 0.9% in a 1:1 ratio were mixtured to three times. Subsequently, these cells are combined with a red blood cell suspension in a 0.01M phosphate buffer at pH 7.4, along with CCl_4 in a ratio of 1:2:0.5. The resulting hemolysate was then centrifuged for 15 minutes at ambient temperature, and the hemoglobin fraction is collected from the upper layer.

Antiglycation test

In the antiglycation assessment, a mixture is prepared consisting of 1 mL of hemoglobin solution, 5 µL of gentamicin, and 2 mL of EEBL at varying concentrations (75, 100, 125, 150, and 175 mg/L). Following this, 2% glucose in a 0.01M phosphate buffer (pH 7.4) as much as 1 mL was added, and the mixture is incubated in the dark condition at room temperature. The concentration of glycosylated hemoglobin was measured after a 24-hour incubation period using a visible spectrophotometer set to wavelength 443 nm. A similar procedure was applied to the positive control group using gliclazide (10, 20, 30, 40, 50, 60 mg/L) and similiar procedure was applied for blank solution without EEBL or gliclazide

Data analysis

The efficacy of in vitro inhibitors of glycated hemoglobin is quantified by determining the IC50 value. This value is derived from the linear regression equation $Y = bx + a$, which describes the relationship between the concentration of the test solution and the percentage reduction in glucose levels, with the Y variable being substituted with the value of 50.

RESULTS

Spesific Paramerer

Table 1.
The Result of Specific Parameter Standardization of EEBL

Assay	Result
Organoleptic	Shape: Thick extract Color: dark Green Taster: bitter Odor: distinctive odor
Water Soluble Compound	40.45 ± 0.56%
Ethanol Soluble Compound	15.08 ± 0.74%
Phytochemistry Content	flavonoids, alkaloids, tannins, saponins, and triterpenoids

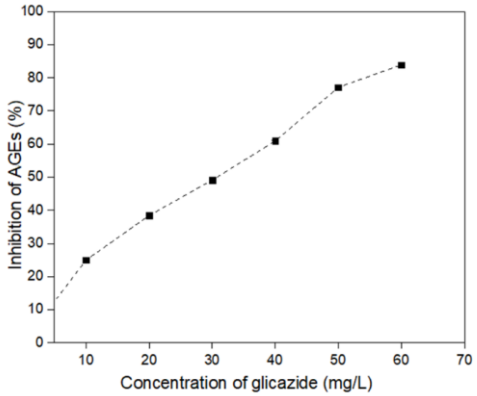
Non-Specific Parameters

Table 2.
The Result of Non Specific Parameter Standardization of EEBL

Parameter	Result
Water content	6.59 ± 0.56%
Ash content	7.41 ± 0.67%
Acid insoluble ash	2.17 ± 0.03%
As	<0.218 mg/Kg
Pb	0.427 ± 0.051 mg/Kg
Cd	<0.029 mg/Kg
Hg	<0.023 mg/Kg
Density of Extract	0.913 ± 0.015 g/mL

Antiglycation activity

The inhibition of AGEs by gliclazide could be seen in Figure 1. The inhibition of AGEs by EEBL could be seen in Figure 2.



Figue 1. The inhibition of AGEs by gliclazide

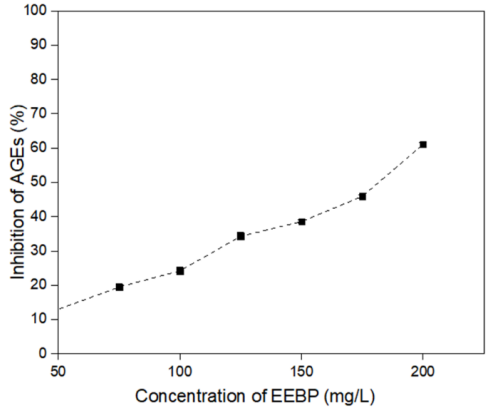


Figure 2. The inhibition of AGEs by EEBL

Table 3.
The Value of IC_{50} Gliclazide and EEBL

Sample	IC_{50} (mg/L)
Gliclazide	30.24 ± 0.62
EEBL	177.02 ± 0.80

DISCUSSION

Sample preparation

The identification of *Barleria prionitis* leaf was conducted to ensure the accurate classification of the plant, thereby eliminating any potential sampling errors in the research. The results of the identification confirmed that the species is indeed *Barleria prionitis*. The leaf powder of *Barleria prionitis* was subjected to extraction using the maceration method. This extraction aimed to extraced the chemical compounds present in the sample by utilizing a solvent with suitable polarity, specifically 70% ethanol, which effective in attracting a polar range of chemical compounds. Soxhlet extraction is a technique employed to extract the powdered sample under controlled temperature conditions with a continuous supply of solvent, facilitating the extraction of multiple components due to the regulated heat and the introduction of fresh solvent (Utami et al., 2023).

Standardization of Extract

Standardized extracts refer to high-quality extracts utilized as raw materials in the production of herbal medicines. These extracts contain specific levels of compounds and have successfully undergone quality control assessments throughout the processes of cultivation, harvesting, and the manufacturing of herbal products (Patnala & Kanfer, 2021).

Spesific Parameters

The organoleptic analysis of EEBL includes observations of shape, odor, color, and taste. This observation aims to provide initial recognition information on EEBL. The organoleptic examination of EEBL showed results in the form of thick extract consistency, dark green in color, bitter taste and has an atypical distictive odor in Table 1.Analysis of dissolved compounds in water or ethanol solvents aims to predict the amount of active compound content according to its polarity. Based on the results obtained, it showed that the compounds contained in EEBL are more polar. In this research, it was found that the content of soluble compounds in ethanol was 15.08 ± 0.74% and water soluble compound was 40.45 ± 0.56%. This is indicated the amount of compounds that dissolve in water compared to ethanol. The number of dissolved compounds reflects the quantity of secondary metabolite compounds that have been solubilized in the solvent (Patil et al., 2023).

Non-Specific Parameters

Determination of water content aims to determine the water residue after drying. The results obtained were 6.59 ± 0.56%, this meets the quality requirements where the water content in the extract should not be more than 10% (Departemen Kesehatan RI, 2022). High water content could cause microbes to grow in the extract and result in decreased extract stability. Density of extract testing aims to describe the purity of a substance whose density of extract is determined. The density of extract of EEBL is 0.913 ± 0.015 g/mL. The density of extract results are attached in Table 2. Total ash content analysis aims to determine the amount of minerals contained in EEBL. The total ash content obtained was 9.41 ± 0.67%. The acid-insoluble ash content describes the presence of metal or mineral contamination that was insoluble in acid from soil or sand. The ash content of an extract indicates the purity and cleanliness of a product processing process. The results of the acid-insoluble ash content were 2.17 ± 0.03%. The heavy metal contamination is As, Cd, and Hg have concentration below limit of detection AAS, while Pb was 0.427 ± 0.051 mg/Kg.

Heavy metals can lead to various health-related pathologies. They have the potential to interact directly with DNA, resulting in numerous DNA lesions, which include both strand breaks and cross-linking with proteins (Witkowska et al., 2021). The presence of heavy metals in herbal medicines could be attributed some factors. The first factor is the inconsistent exposure to environmental pollutants, which arise from industrial activities, as well as contaminated soil or air (Meng et al., 2022). The physicochemical characteristics of the soil, such as pH, temperature, redox potential, cation exchange capacity, and organic matter content, can affect the availability of metals to plants. The second factor involves the inherent phytological traits of medicinal plants, where indicators of heavy metal toxicity may include reduced biomass, root length, and shoot length (Wan et al., 2024). The third factor is the potential for contamination of herbal plants during various stages of production and agricultural practices (Pant et al., 2021), which encompasses growing, harvesting, transportation, processing, and storage, often due to pesticide residues, chemical fertilizers, and irrigation with substandard water. For instance, cadmium and lead could infiltrate the soil through fertilizer impurities, emissions from non-ferrous smelters, lead mining activities, sewage sludge application, and the burning of fossil fuels (Angon et al., 2024). Moreover,

fumigants containing heavy metals may be utilized to deter pests such as rats and to combat mildew. The fourth factor is that plant uptake represents a significant pathway for dietary exposure to heavy metals present in the soil, and the considerable variability in metal concentrations found in analyzed herbs can be linked to differences in plant morphology (Luo et al., 2021).

Antiglycation Activity

Glycation, also known as the Maillard reaction, is a spontaneous and naturally occurring process characterized by a complex network of non-enzymatic reactions. This process begins with the interaction of the carbonyl group from a reducing sugar with a free amino group (Younus & Anwar, 2016). Typically, this involves the amino group of lysine residues or the α-amino group, resulting in the formation of an adduct known as a Schiff base. Subsequently, these Schiff bases undergo Amadori rearrangement, leading to a series of additional rearrangements and cyclizations that yield a diverse array of compounds collectively referred to as AGEs (Aragno & Mastrocola, 2017). Gliclazide serves as a positive control in the evaluation of antiglycation agents due to its ability to diminish platelet adhesion, aggregation, and hyperactivity, while simultaneously enhancing fibrinolysis. These effects, believed to operate independently of its hypoglycemic properties, may render gliclazide beneficial in preventing the progression of diabetic microangiopathy (Landman et al., 2014). The IC_{50} value of gliclazide is 30.24 ± 0.62 mg/L, however synthetic compounds are recognized as potent antiglycating agents or effective inhibitors of AGE formation, they may also lead to serious adverse effects. Consequently, there has been a growing interest in identifying natural phytochemicals derived from plants that can effectively inhibit glycation while exhibiting fewer side effects. The IC_{50} value of EEBL is177.02 ± 0.80 mg/L. EEBL contains secondary metabolite compounds such as flavonoids, phenolics, and terpenes, that can act as free radical scavengers. The mechanism of polyphenol antioxidant protection is associated with its ability to bind proteins, thereby preventing the formation of AGE (Leonardo et al., 2021), so that EEBL has the ability as an antiglycation. Naturally occurring phytochemicals and products are generally considered safer for human consumption compared to their synthetic counterparts, being relatively non-toxic, cost-effective, and available in forms suitable for ingestion (Gaston et al., 2020).

CONCLUSION

The results of the standardization indicate that EEBL has a water soluble compound content of 40.45 ± 0.56%, an ethanol soluble compound content of 15.08 ± 0.74%, a water content of 7.508 ± 0.399%, a total ash content of 9.41 ± 0.67%, an acid insoluble ash content of 2.17 ± 0.03%, and an extract density of 0.913 ± 0.015 g/mL. Additionally, EEBL was found to contain flavonoids, alkaloids, tannins, saponins, and triterpenoids and the IC50 value of EEBL is177.02 ± 0.80 mg/L.

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REFERENCES

Ali, M.Y., Paul, S., Tanvir, E.M., Hossen, M.S., Rumpa, N.N., Saha, M., Bhoumik, N.C., Aminul Islam, M., Hossain, M.S., Alam, N., Gan, S.H., Khalil, M.I. (2017). Antihyperglycemic, Antidiabetic, and Antioxidant Effects of Garcinia pedunculata in

Rats. Evid Based Complement Alternat Med.; 2979760. https://doi.org/10.1155/2017/2979760

Angon, P. B., Islam, M. S., Kc, S., Das, A., Anjum, N., Poudel, A., & Suchi, S. A. (2024). Sources, effects and present perspectives of heavy metals contamination: Soil, plants and human food chain. Heliyon, 10(7), e28357. https://doi.org/10.1016/j.heliyon.2024.e28357

Aragno, M., & Mastrocola, R. (2017). Dietary Sugars and Endogenous Formation of Advanced Glycation Endproducts: Emerging Mechanisms of Disease. Nutrients, 9(4), 385. https://doi.org/10.3390/nu9040385

Bindu, J., Narendhirakannan, R.T. (2019). Role of medicinal plants in the management of diabetes mellitus: a review. 3 Biotech. 9(1):4. https://doi.org/10.1007/s13205-018-1528-0

Blahova, J., Martiniakova, M., Babikova M, Kovacova V, Mondockova V, Omelka R. (2021). Pharmaceutical Drugs and Natural Therapeutic Products for the Treatment of Type 2 Diabetes Mellitus. Pharmaceuticals (Basel). 14(8):806.

Choudhary, M., Kumar, V., Singh, S. (2014). Gastro protective potential of chloroform leaves extract of Barleria prionitis Linn.: From traditional use to scientific approach. Adv. Chem. Biochem. Sci. 1, 1–11.

Departemen Kesehatan Republik Indonesia. (2022). Farmakope Herbal Indonesia. Suplemen I. Edisi II. Jakarta: Departemen Kesehatan Republik Indonesia.

Gaston, T. E., Mendrick, D. L., Paine, M. F., Roe, A. L., & Yeung, C. K. (2020). "Natural" is not synonymous with "Safe": Toxicity of natural products alone and in combination with pharmaceutical agents. Regulatory Toxicology and Pharmacology, 113. https://doi.org/10.1016/j.yrtph.2020.104642

Gong, L., Feng, D., Wang, T., Ren, Y., Liu, Y., Wang, J. (2020). Inhibitors of α-amylase and α-glucosidase: Potential linkage for whole cereal foods on prevention of hyperglycemia. Food Sci Nutr. 8(12):6320-6337. https://doi.org/10.1002/fsn3.1987

González, P., Lozano, P., Ros, G., Solano, F. (2023). Hyperglycemia and Oxidative Stress: An Integral, Updated and Critical Overview of Their Metabolic Interconnections. Int J Mol Sci. 24(11):9352. https://doi.org/10.3390/ijms24119352

Kato-Schwartz, C.G., Corrêa, R.C.G., de Souza Lima, D., de Sá-Nakanishi, A.B., de Almeida Gonçalves, G. (2020). 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 (Ottawa, Ont.). 137, 109462. https://doi.org/10.1016/j.foodres.2020.109462

Kumar, A., Gangwar, R., Zargar, A.A., Kumar, R., Sharma, A. (2024). Prevalence of Diabetes in India: A Review of IDF Diabetes Atlas 10th Edition. Curr Diabetes Rev. 20(1). https://doi.org/10.2174/1573399819666230413094200

Landman, G. W., de Bock, G. H., van Hateren, K. J., van Dijk, P. R., Groenier, K. H., Gans, R. O., Houweling, S. T., Bilo, H. J., & Kleefstra, N. (2014). Safety and efficacy of

gliclazide as treatment for type 2 diabetes: a systematic review and meta-analysis of randomized trials. PloS one, 9(2), e82880. https://doi.org/10.1371/journal.pone.0082880

Leonardo, C. F., María, P. L., Cristina, S. F., María, F. M., Ndumiso, M., Jessy, María P. B. (2021). The Antioxidant And Antiglycation Activities Of Selected Spices And Other Edible Plant Materials And Their Decay In Sugar-Protein Systems Under Thermal Stress, Food Chemistry. Volume 371, 131199. https://doi.org/10.1016/j.foodchem.2021.131199

Luo, L., Wang, B., Jiang, J., Fitzgerald, M., Huang, Q., Yu, Z., Li, H., Zhang, J., Wei, J., Yang, C., Zhang, H., Dong, L., & Chen, S. (2021). Heavy Metal Contaminations in Herbal Medicines: Determination, Comprehensive Risk Assessments, and Solutions. Frontiers in pharmacology, 11, 595335. https://doi.org/10.3389/fphar.2020.595335

Meng, C., Wang, P., Hao, Z., Gao, Z., Li, Q., Gao, H., Liu, Y., Li, Q., Wang, Q., & Feng, F. (2022). Ecological and health risk assessment of heavy metals in soil and Chinese herbal medicines. Environmental geochemistry and health, 44(3), 817–828. https://doi.org/10.1007/s10653-021-00978-z

Panchal, P, Meena S, Singh K, Sharma N. (2018). Anticancer and antimicrobial potential of Barleria prionitis leaves ethanol extract. Int. J. Pharm. Pharm. Sci. 10, 100.

Pant, P., Pandey, S., & Dall'Acqua, S. (2021). The influence of environmental conditions on secondary metabolites in medicinal plants: a literature review. Chemistry & Biodiversity, 18(11). https://doi.org/10.1002/cbdv.202100345

Patil, R.H., Patil, M.P., Maheshwari, V.L. (2023). Extraction and Isolation of Secondary Metabolites from Apocynaceae Plants. In: Apocynaceae Plants. Springer, Singapore.

Patnala, S., & Kanfer, I. (2021). Quality control, extraction methods, and standardization: Interface between traditional use and scientific investigation. In Elsevier eBooks (pp. 175–187). https://doi.org/10.1016/b978-0-12-815565-3.00006-0

Ranade, R., Jain A, Joshi N. (2016). Estimation of Phenolic Compounds By RP-HPLC And Antioxidant Activity in Leaf and Stem Extracts of Barleria Prionitis L. International Journal of Pharmaceutical Sciences and Research. 7(6), 2445. https://doi.org/10.13040/IJPSR

Reema, D, Pradeep BA. Study of the antidiabetic activity of Barleria prionitis Linn. (2010). Indian J. Pharmacol. 42, 70–73. https://doi.org/10.4103/0253-7613.64493

Sagandira, C.R., Khasipo A.Z., Sagandira MB, Watts P. (2021). An overview of the synthetic routes to essential oral anti-diabetes drugs, Tetrahedron. 96, 132378, https://doi.org/10.1016/j.tet.2021.132378.

Sheleme, T, Mamo G, Melaku T, Sahilu T. (2020). Prevalence, Patterns and Predictors of Chronic Complications of Diabetes Mellitus at a Large Referral Hospital in Ethiopia: A Prospective Observational Study. Diabetes Metab Syndr Obes. 13:4909-4918. https://doi.org/10.2147/DMSO.S281992

Singh, S, Kumar M, Dwivedi, S., Yadav, A., Sharma, S. (2023). Distribution profile of iridoid glycosides and phenolic compounds in two Barleria species and their correlation with

antioxidant and antibacterial activity. Frontiers in Plant Science. (13). https://doi.org/10.3389/fpls.2022.1076871

Singh, V.P., Bali, A., Singh, N., Jaggi, A.S. (2014). Advanced glycation end products and diabetic complications. Korean J Physiol Pharmacol. 18(1):1-14. https://doi.org/10.4196/kjpp.2014.18.1.1

Syukri, Y., Purwati R., Hazami, N., Anshory, Tahmid H, Fitria, A. (2020). Standardization of Specific and Non-Specific Parameters of Propolis Extract as Raw Material for Herbal Product. EKSAKTA: J. Sci. Data Anal. 20(1):36-43. https://journal.uii.ac.id/Eksakta/article/view/14354

Tomic, D., Shaw J.E., Magliano, D.J. (2022). The burden and risks of emerging complications of diabetes mellitus. Nat Rev Endocrinol. 18, 525–539. https://doi.org/10.1038/s41574-022-00690-7

Tulliballi, S., Seru G. (2013). Phytochemical investigation and evaluation of hepatoprotective and antimicrobial activities on the aerial parts of Barleria montana (Acanthaceae). Rasayan J. Chem. 6, 102–106.

Utami, N., Susilowati, S., Angelia, P.T., Paramesti, NA. (2023). Profil Senyawa Metabolit Sekunder Ekstrak Daun Barleria prionitis (Barleria prionitis L.) sebagai Kandidat Antidiabetes dengan Variasi Metode Ekstraksi. Jurnal Farmasetis. 12(4), 431-440. https://doi.org/10.32583/far.v12i4.1580

Vlassara, H., Uribarri, J. (2014). Advanced glycation end products (AGE) and diabetes: cause, effect, or both. Curr Diab Rep.14(1):453. https://doi.org/10.1007/s11892-013-0453-1

Wan, Y., Liu, J., Zhuang, Z., Wang, Q., & Li, H. (2024). Heavy Metals in Agricultural Soils: Sources, Influencing Factors, and Remediation Strategies. Toxics, 12(1), 63. https://doi.org/10.3390/toxics12010063

Wahidin, M., Achadi, A., Besral, B. (2024). Projection of diabetes morbidity and mortality till 2045 in Indonesia based on risk factors and NCD prevention and control programs. Sci Rep 14. https://doi.org/10.1038/s41598-024-54563-2

Witkowska, D., Słowik, J., & Chilicka, K. (2021). Heavy Metals and Human Health: Possible Exposure Pathways and the Competition for Protein Binding Sites. Molecules (Basel, Switzerland), 26(19), 6060. https://doi.org/10.3390/molecules26196060

Younus, H., & Anwar, S. (2016). Prevention of non-enzymatic glycosylation (glycation): Implication in the treatment of diabetic complication. International journal of health sciences, 10(2), 261–277.

Zehiroglu, C., Ozturk Sarikaya S.B. (2019). The importance of antioxidants and place in today's scientific and technological studies. J Food Sci Technol. 56(11):4757-4774. https://doi.org/10.1007/s13197-019-03952-x.