"ASSESSMENT OF HYPOGLYCEMIC EFFECT OF *HIPPOPHAE RHAMNOIDES* AND *BOERRHAVIA DIFFUSA* AGAINST STREPTOZOTOCIN INDUCED DIABETES IN RATS"

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ABSTRACT

Objective: To assess the hypoglycemic effect of ethanolic extract of *Hippophae rhamnoides* and *Boerrhavia diffusa* in rats.

Methods: The ethanolic extract of berries and aerial parts of plants, *Hippophae rhamnoides* and *Boerrhavia diffusa* respectively were collected, shade dried, prepared and evaluated for phytochemical constituents using standard methodology. The hypoglycemic effect of the combination plant extract was then evaluated with the help of rat models by taking Glibenclamide as a standard drug against different doses of *Hippophae Boerrhavia* Ethanolic Extract (HBEE) by using Oral Glucose Tolerance Test (OGTT) and Streptozotocin Induced Diabetes method.

Results: The present study assessed the phytochemical analysis, acute toxicity test and hypoglycemic effect of HBEE. Phytochemical analysis revealed the presence of saponins, flavonoids, terpenoids, glycosides, steroids, proteins, amino acids, phenols and tannins. The ethanolic extract of the plants showed hypoglycemic effect in dose dependent manner in both OGTT and Streptozotocin Induced Diabetes method. The HBEE has significantly shown hypoglycemic effect with $p<0.05$, 0.01 and 0.005 at the dose of 125, 250 and 500 mg/kg, respectively in both the models i.e. OGTT and Streptozotocin Induced diabetes method.

Conclusion: The results of present study revealed that the ethanolic extract of *Hippophae rhamnoides* and *Boerrhavia diffusa* contain high content of phytochemicals and possess significant ($p<0.05$) hypoglycemic effect.

Keywords: *Hippophae rhamnoides, Boerrhavia diffusa,* Oral Glucose Tolerance Test, Streptozotocin, Glibenclamide, Phytochemicals, Hypoglycemia, plasma glucose levels, Oral glucose.

INTRODUCTION

In recent years, there has been a significant surge in the use of herbal medicines[1], with these remedies becoming increasingly popular in both developed and developing nations. This growing interest is largely due to their natural origin and generally lower risk of side effects[2]. A large number of traditional treatments currently in practice are sourced from medicinal plants, natural minerals, and organic substances.[3]

The World Health Organization (WHO) has identified approximately 21,000 plant species globally that are used for medicinal purposes[4]. Of these, about 2,500 species are found in India, with around 150 being widely used for commercial purposes[5]. India is recognized as the leading producer of medicinal herbs and is often referred to as the "botanical garden of the world."[6]

Herbal drugs, also known as phytomedicines, are medicinal products derived from plants and their extracts. They have been used for centuries across various traditional systems of medicine, such as Ayurveda, Traditional Chinese Medicine, and Unani. In recent times, the demand for herbal remedies has grown significantly due to

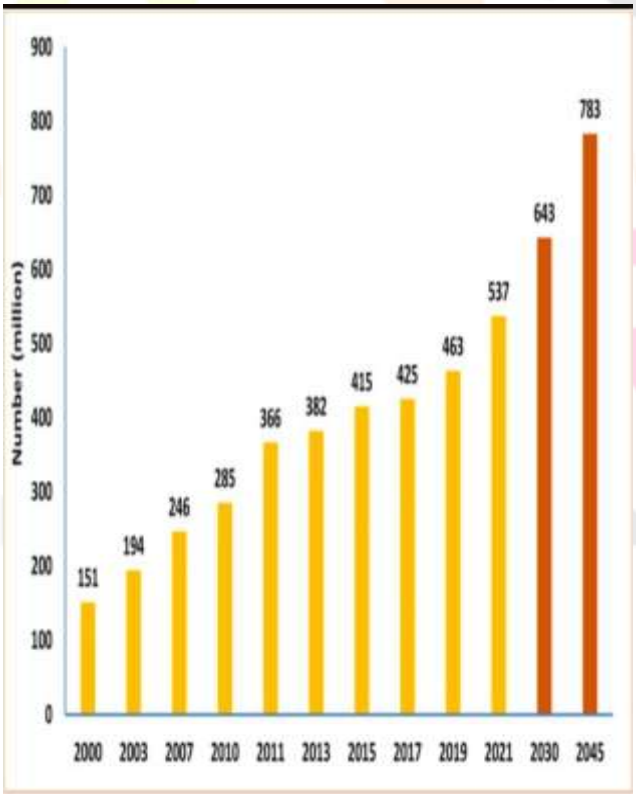
increased awareness of their natural origin, relatively low side effects, and holistic approach to health. These plant-based medicines are used to prevent, treat, and manage a wide range of health conditions. With the rising interest in natural and sustainable healthcare, herbal drugs continue to play an important role in modern therapeutic practices.

Numerous herbal treatments have been proposed for managing diabetes and its associated complications. These remedies primarily rely on medicinal plants, which serve as the key components in various therapeutic formulations.

Diabetes mellitus is among the most prevalent endocrine metabolic disorders and is a major contributor to global morbidity and mortality. Its complications are both microvascular—such as retinopathy, neuropathy, and nephropathy[7]—and macrovascular, including cardiovascular diseases like heart attacks, strokes, and peripheral vascular disorder. The human body is equipped with both enzymatic and non-enzymatic antioxidant defense systems that help limit the formation of reactive oxygen species (ROS), which are implicated in the pathogenesis of many chronic diseases, including diabetes.

Diabetes is a chronic health condition characterized by the body's inability to properly regulate blood sugar levels. This may result from either insufficient insulin activity or an impaired response to insulin, even when it is present in adequate amounts. Insulin is a hormone composed of polypeptides and is produced by the beta cells within the islets of Langerhans in the pancreas. Its primary functions include maintaining normal blood glucose levels, facilitating the absorption of glucose by cells, and supporting glucose metabolism.

According to recent estimates, approximately 240 million individuals worldwide are living with undiagnosed diabetes, with nearly 50% of adults affected by the disease unaware of their condition [8](International Diabetes Federation [IDF], 2021). The prevalence of diabetes is expected to increase significantly, with estimates indicating that by 2030, 643 million people (11.3%) will be affected, and by 2045[9], this number could rise to 783 million (12.2%) (IDF, 2021). Figure 1 illustrates the upward trend in the global number of individuals aged 20-79 years living with DM



TYPES OF DIABETES MELLITUS

Type 1 Diabetes

Type 1 diabetes is a chronic autoimmune condition characterized by the destruction of pancreatic beta cells, resulting in a complete deficiency of insulin and persistent hyperglycemia .It requires lifelong insulin therapy to manage blood glucose levels and prevent serious complications, such as cardiovascular disease, renal impairment, stroke, and vision loss.[10]

Type 2 Diabetes

Type 2 diabetes is a widespread metabolic disorder that is caused due to impaired insulin secretion and the body's resistance to insulin action. Several risk factors contribute to its development, including obesity, sedentary lifestyle, poor dietary habits, hypertriglyceridemia, family history, aging, and psychological stress.[11]

Gestational Diabetes

Gestational diabetes mellitus (GDM) occurs during pregnancy, typically in the second or third trimester, and affects women without a prior history of diabetes. Risk factors include maternal obesity, advanced age, and genetic predisposition. GDM is associated with future risk of type 2 diabetes and cardiovascular complications for both the mother and the child.[12]

Punarnava, scientifically known as *Boerhavia diffusa*, belonging to Family Nyctagynaceae is a well-known medicinal herb used in traditional medicine systems for centuries. The name "Punarnava" means "that which rejuvenates the body." Found widely across India, especially in tropical and subtropical regions, the plant is used for its diuretic, anti-inflammatory, anti-spasmodic, anti-fibrotic, and hepatoprotective properties. Various studies have identified the following major constituents in *Boerhavia diffusa* as Punarnavine – an alkaloid, Boeravinone A–F as Flavonoids (known for anti-inflammatory and anticancer activity), Steroids and Saponins, Lignans and Glycoproteins, Triterpenoids and Phenolic compounds.

Hippophae rhamnoides, Sea buckthorn belonging to family Elganaceae typically grows in **high-altitude, arid, and semi-arid regions,** especially in **cold deserts and mountainous areas. It contains Vitamin C** in very high amounts, supporting immunity and tissue repair, **Vitamin E** and carotenoids, which help protect cells from damage, **Flavonoids** such as quercetin and kaempferol, known for their anti-inflammatory and antioxidant effects, **Omega fatty acids** including omega-3, 6, 7, and 9, especially omega-7 (palmitoleic acid), which supports skin health, **Amino acids**, minerals, and phytosterols, contributing to the plant's overall therapeutic potential.

MATERIALS AND METHODS

Plant Material Collection and Authentication

The berries of *Hippophae rhamnoides* and leaves of *Boerrhavia diffusa* were collected and authenticated by Department of Pharmacognosy, Shadan College of Pharmacy, Hyderabad based on standard botanical characteristics.

Preparation of Plant Extract

Fresh plant material of *Hippophae rhamnoides* and *Boerrhavia diffusa* was shade-dried for 15 days and coarsely powdered using a mechanical grinder. The powdered material was subjected to Soxhlet extraction using 95% ethanol for 24 hours. The ethanolic extract was then concentrated and dried using an electric water bath at 70 ºC followed by oven-drying at 30 ºC for two hours. The final extract was stored in a refrigerator[13]. The extraction yielded 7.5% of the initial plant material.

Preliminary Phytochemical Screening

The preliminary phytochemical screening of *Hippophae Boerrhavia* Ethanolic Extract (HBEE) was subjected to standard qualitative tests to detect the presence of bioactive compounds such as steroids, flavonoids, glycosides, tannins, phenols, oils, saponins and fats. The screening was done by utilizing standard procedures[14].

Test for alkaloids:

Wagner's test: To 1-2 ml of concentrate 1 to 2 drops of wagner's reagent was included. Reddish brown coloured precipitate is obtained, affirms the presence of alkaloids.

Test for Carbohydrates:

Molisch's Test: 2 drops of Molisch's reagent were added to 2 mL of extract followed by 1 mL of concentrated sulfuric acid along the test tube wall. A purple ring at the interface confirmed the presence of carbohydrates.

Fehling's Test: 1 mL each of Fehling's A and B solutions were mixed and boiled with 2 mL of the extract. Formation of a brick-red precipitate indicated reducing sugars.

Legal's Test: Sodium nitroprusside and sodium hydroxide were added to the extract. A pink to red color indicated the presence of keto groups found in certain sugars.

Borntrager's Test: Negative result (No pink color appeared), indicating absence of anthraquinone glycosides.

Test for Fixed Oils and Fats:

Filter Paper Test: A drop of extract was placed on filter paper and left to dry. Permanent greasy spot indicated presence of fixed oils.

Saponification Test: 2 mL of extract was heated with ethanol and KOH. Formation of soap upon addition of water confirmed the presence of fats and oils.

Test for Proteins and Free Amino Acids:

Millon's Test: Upon addition of Millon's reagent and heating, a red color appeared, confirming tyrosine-containing proteins.

Biuret Test: No violet color appeared upon addition of copper sulfate and sodium hydroxide, indicating a negative result.

Ninhydrin Test: A purple-blue color developed upon heating the extract with Ninhydrin, confirming free amino acids.

Test for Tannins and Phenolic Compounds:

Ferric Chloride Test: Addition of ferric chloride solution to extract produced a blue-black coloration, confirming phenolic compounds.

Lead Acetate Test: No precipitate formed, indicating a negative result for certain tannins.

Test for Phytosterols:

Salkowski Test: Extract was treated with chloroform and concentrated sulfuric acid. A reddish-brown color at the interface indicated phytosterols.

Liebermann-Burchard Test: Addition of acetic anhydride and sulfuric acid resulted in a green-blue color, confirming presence of sterols.

Test for Gums and Mucilages:

No viscous or gelatinous precipitate was observed upon addition of alcohol to aqueous extract, indicating absence.

Test for Flavonoids:

Shinoda Test: Magnesium turnings and concentrated hydrochloric acid were added to the extract. Formation of pink to crimson red coloration confirmed flavonoids.

Alkaline Reagent Test: Addition of sodium hydroxide resulted in yellow color, which disappeared upon addition of dilute acid.

Test for glycosides:

Keller-Killiani test:

To 2ml concentrate, glacial acetic acid, 1 drop of 5% ferric chloride and concentrated sulphuric acid was included. There was no reddish brown coloured shade shows up at the intersection of two fluids layers and does not seem blue green, indicates absence of glycosides.

Experimental Animals

Wistar albino rats of either sex weighing between 150-200 g were housed in colonial cages and kept in standard laboratory environmental conditions; temperature 25±2°C, 12 h of light: 12 h of dark cycle and 50±5% of Relative humidity with free access to food and water ad libitum.[50] The animals were adapted to the laboratory conditions before testing. Each group consists of six animals (n=6). Each of the test was performed in the light time period (80:16h). The investigations were conducted as per the standards provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India. All animal experiments were approved by Institutional Animal Ethics Committee (IAEC) of the Shadan Institute of Medical Sciences.

Acute Toxicity Study

The acute toxicity profile of the extract was evaluated in accordance with OECD guideline 423 (acute toxic class method). Experimental animals were observed individually for 14 days for any signs of toxicity such as changes in skin, behavior, mortality, tremors, convulsions, salivation, diarrhea, lethargy, sleep, or coma.[14]

EVALUATION OF ANTI-DIABETIC ACTIVITY OF HBEE BY USING INVIVO MODELS

Blood Glucose Measurement

Blood glucose levels were assessed using a portable glucometer on days 0, 1, 7, and 14. Blood samples were collected via tail vein puncture, and a drop of blood was analyzed using glucose oxidase-peroxidase strips.

Oral Glucose Tolerance Test (OGTT)

The OGTT was conducted in overnight-fasted rats (18 hours). The animals were divided into five groups (n = 6) and administered either the vehicle, two different doses of the extract, or Glibenclamide (10 mg/kg). Thirty minutes post-treatment, glucose (2 g/kg) was administered orally. Blood samples were taken at 0, 30, 60, 90, and 120 minutes to evaluate blood glucose levels.[15]

Streptozotocin (STZ)-Induced Hyperglycemia Model[16,17]

Diabetes was induced with a single i.p. dose of Streptozotocin (50 mg/kg), dissolved in 0.1 M citrate buffer (pH 4.5), in overnight-fasted rats. After seven days, animals with fasting glucose levels exceeding 200 mg/dL were included in the study and grouped as follows:

* Group 1: Normal control (0.5% Tween 80 in distilled water)

* Group 2: Diabetic rats received STZ (50 mg/kg)

* Group 3: Diabetic rats treated orally with Glibenclamide (10 mg/kg)

* Group 4: Diabetic rats treated orally with HBEE (125 mg/kg)

* Group 5: Diabetic rats treated orally with HBEE (250 mg/kg)

* Group 6: Diabetic rats treated orally with HBEE (500 mg/kg)

Statistical Analysis

The statistical analysis was done by using GraphPad Prism software version 8.0. The data is represented as the mean plus or minus the standard error of the mean. The one-way analysis of variance (ANOVA) and Dunnett's multiple comparison assay are statistically significant at p-values of 0.05, 0.01, and 0.001 respectively in comparison to the control group.

RESULTS

PHYTOCHEMICAL TESTS	RESULTS (COMPOUND)
1)Carbohydrates	+
a) Molish's Test	+
b) Fehling's Test	+
c) Legal's Test	+
d) Brontrager's Test	-
2)Fixed Oils and Fats	+
a) Filter paper Test	+
b) Saponification Test	+
3)Proteins and Free Amino acids	+
a) Millon's Test	+
b) Burett Test	-
c) Ninhydrin Test	+
4)Tannins and Phenolic Compounds	+
a) Ferric chloride Test	+
b)Lead Acetate Test	-
5)Phytosterols	+
a)Salkowski Test	+
b)Libermann Burchard Test	+
6)Gums and Mucilages	-
7)Flavonoids	+
a)Shinoda Test	+

Note: + indicates presence of phytoconstituents and - indicates absence of phytoconstituents

Acute Toxicity Study

Response	Type	Result
Behavioural	Alertness	+
	Stereotypy	-
	Irritability	+
	Touch	-
	Pain	-
	Spontaneous Activity	- +
	Grooming	-
	Restlessness	+
Neurological	Righting Reflex	+
	Limp Tone Grip Strength	+ +
	Twitching	-
	Abdominal Tone	- -
	Pinna Reflex	+
	Cornea Reflex	+
	Strab's Tail	+
	Tremors	-
	Convulsions	-
Autonomic	Writhing	+
	Defecation	-
	Urination	-

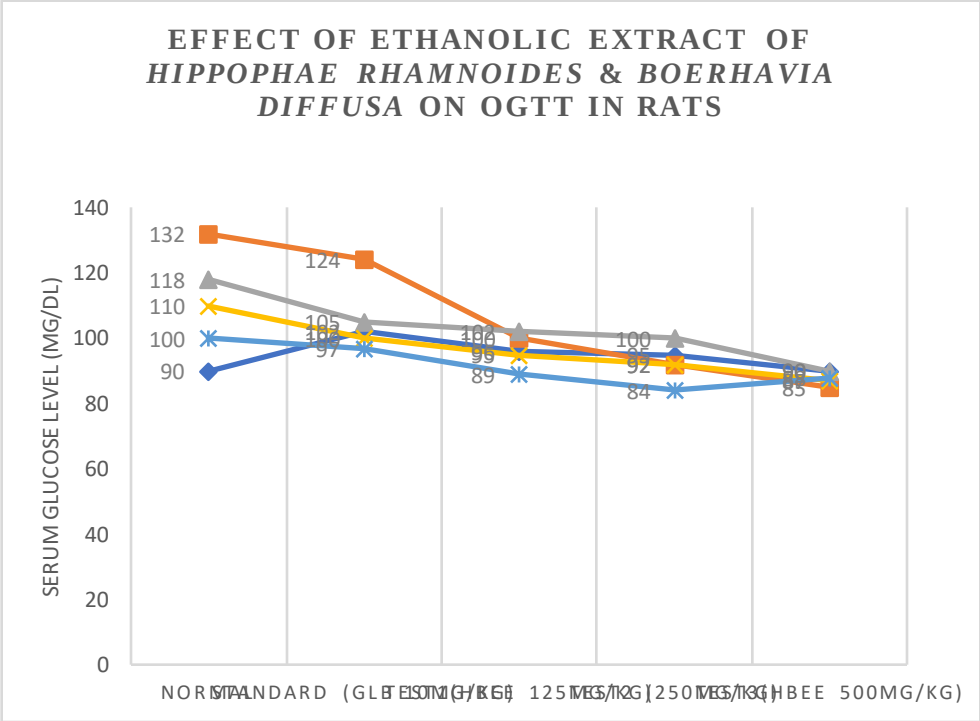
	Piloerection	-
	Heart Rate	+
	Respiration	+
	Pupil Size	-
	Skin Colour	-

n indicates normal, + indicates Increased/ present, - indicates Decreased /Absent

Effect of ethanolic extract of *Hippophae rhamnoides* & *Boerhavia diffusa* on OGTT in rats

Groups	Treatment	0 h	2 h	4 h	6 h	12 h
I	**Normal Control**	95.333±0.43*	99.30±0.077*	97.22±0.04**	93.19±0.033**	90.14±0.04***
II	**Diabetic Control (Glucose 2mg/kg)**	415.88±1.882	447.53±1.577	451.333±1.333	452.65±1.577	474.03±1.29
III	**Glibenclamide (10mg/kg)**	445.86±2.133	437.33±0.433**	426.667±0.28**	424.36±0.233***	418.30±0.53*
IV	**Test Dose-1(*HBEE*-125 mg/kg)**	424.16±2.36	422.30±0.92*	421.70±0.63**	419.70±0.577***	419.60±0.48**
V	**Test Dose-2(*HBEE*250 mg/kg)**	425.20±2.177	425.50±0.833*	424.333±0.633**	421.53±0.363***	419.36±0.642**
VI	**Test Dose-3 (*HBEE*500 mg/kg)**	415.32±1.577	415.33±0.633*	410.667±0.333**	408.66±0.333***	406.38±0.58**

The data is shown as mean ± SEM and is statistically different from session 1 for each group at *P< 0.05, **P < 0.01 and ***P < 0.001.



Effect of ethanolic extract of *Hippophae rhamnoides* & *Boerhavia diffusa* on Streptozotocin induced diabetes in rats

Groups	Treatment	Day 0	Day 1	Day 7	Day 14
I	**Normal Control**	185.10±0.043*	185.15±0.047*	178.24±0.04**	195.19±0.003**
II	**Diabetic Control (Glucose 2mg/kg)**	203.88±1.18	202.73±1.377	200.33±1.33	191.65±1.37
III	**Glibenclamide (10mg/kg)**	187.36±1.43	191.53±1.433**	188.87±0.88**	202.39±0.33***
IV	**Test Dose-1(*HBEE*-125 mg/kg)**	182.46±1.36	178.60±1.92*	175.32±0.63**	196.97±0.77***
V	**Test Dose-2(*HBEE*250 mg/kg)**	195.20±1.77	198.35±0.73*	196.333±0.533**	202.53±0.363***
VI	**Test Dose-3 (500 mg/kg)**	185.32±1.577	186.33±0.69*	186.37±0.43**	204.26±0.43***

The data is shown as mean ± SEM and is statistically different from session 1 for each group at *P< 0.05, **P < 0.01 and ***P < 0.001.

DISCUSSION

Diabetes mellitus is among the most prevalent endocrine metabolic disorders and is a major contributor to global morbidity and mortality. Its complications are both microvascular—such as retinopathy, neuropathy, and nephropathy—and macrovascular, including cardiovascular diseases like heart attacks, strokes, and peripheral vascular disorder. The human body is equipped with both enzymatic and non-enzymatic antioxidant defense systems that help limit the formation of reactive oxygen species (ROS), which are implicated in the pathogenesis of many chronic diseases, including diabetes. It is characterized by the body's inability to properly regulate blood sugar levels which may result from either insufficient insulin activity or an impaired response to insulin, even when it is present in adequate amounts. Insulin is a hormone composed of polypeptides and is produced by the beta cells within the islets of Langerhans in the pancreas.[18] Its primary function is to maintain normal blood glucose levels, facilitate the absorption of glucose by cells, and support glucose metabolism. Since the synthetic drugs are known to produce numerous side effects, more focus and significance is being given to the medicines obtained from natural origin. The phytoconstituents present in the plant extracts have antioxidants and this antioxidant activity of it is due to presence of secondary active constituents like phytosterols, alkaloids, tannins and flavonoids. The current study utilized berries and leaves of the plants viz., *Hippophae rhamnoides* and *Boerrhavia diffusa* which were then shade dried and soaked in soxhlet in ethanol by soxhlet extraction. The *Hippophae Boerrhavia* Ethanolic Extract (HBEE) was then obtained after distillation and subjected to phytochemical screening and acute toxicity study. The extract was confirmed to be safe at a dose of 125,250 and 500 mg/kg body weight. Thus, 125, 250 and 500 mg/kg p.o test extracts were utilized for assessing the hypoglycemic effect. Streptozotocin was administered to all the animal groups for inducing the symptoms of Hyperglycemia as it destroys insulin producing cells of islets of Langerhans[19] while Glibenclamide was used as a standard drug. Both the plants exhibit antioxidant property and hence showed antihyperglycemic effect which was assessed by using different models such as Oral Glucose Tolerance Test (OGTT) and Streptozotocin induced Hyperglycemia in rats. It was found that test dose 2 (250mg/kg) and test dose 3 (500mg/kg) showed significant result when compared to normal with $P<0.05$, $P<0.01$ respectively thus signifying antihyperglycemic activity.

CONCLUSION

The present study was done to assess the hypoglycemic effect of HBEE. In this all the animals were given Streptozotocin which releases reactive oxygen species thus destroying the β cells of Islets of Langerhans which produce insulin and maintain the blood glucose levels. After carrying out the experiment in animals through different models viz., OGTT and Streptozotocin induced hyperglycemia it was observed that test groups with dose 125 and 250 mg/kg body weight showed significant reduction in blood glucose level when compared with the normal group. This potential effect against the increased blood glucose levels of the test extract can be due to the presence of phytoconstituents such as flavonoids, tannins, alkaloids, etc. which possess anti-oxidant characteristic and hence protect the β cells from getting destroyed. Thus, the study concludes that HBEE has potential effect in lowering the blood glucose levels.

CONFLICT OF INTEREST

All authors approve the final manuscript and declare that there are no conflicts of interests.

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REFERENCES

1) Ekor, M. (2014). The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Front Pharmacol*, 4, 177. https://doi.org/10.3389/fphar.2013.00177
2) Tilburt, J.C., & Kaptchuk, T.J. (2008). Herbal medicine research and global health: An ethical analysis. *Bull World Health Organ*, 86(8), 594–599. https://doi.org/10.2471/BLT.07.042820

3) Gurib-Fakim, A. (2006). Medicinal plants: Traditions of yesterday and drugs of tomorrow. *Mol Aspects Med*, 27(1), 1–93. https://doi.org/10.1016/j.mam.2005.07.008
4) Patwardhan, B., Warude, D., Pushpangadan, P., & Bhatt, N. (2005). Ayurveda and traditional Chinese medicine: A comparative overview. *Evid Based Complement Alternat Med*, 2(4), 465–473. https://doi.org/10.1093/ecam/neh140
5) Modak, M., et al. (2007). Indian herbs and herbal drugs used for the treatment of diabetes. *J Clin Biochem Nutr*, 40(3), 163–173. https://doi.org/10.3164/jcbn.40.163
6) Grover, J.K., Yadav, S., & Vats, V. (2002). Medicinal plants of India with anti-diabetic potential. *J Ethnopharmacol*, 81(1), 81–100. https://doi.org/10.1016/S0378-8741(02)00059-4.
7) Heinrich, M., et al. (2012). *Fundamentals of Pharmacognosy and Phytotherapy* (2nd ed.). Elsevier Health Sciences.Chattopadhyay, R.R. (2003). A comparative evaluation of some blood sugar lowering agents of plant origin. *J Ethnopharmacol*, 84(1), 105–108. https://doi.org/10.1016/S0378-8741(02)00215-2
8) Gupta, R., et al. (2012). Hypoglycemic and antidiabetic effect of *Punica granatum* in rats. *J Ethnopharmacol*, 141(3), 831–836. https://doi.org/10.1016/j.jep.2012.03.036
9) Shetty, A.K., et al. (2005). Antidiabetic properties of *Aegle marmelos* in streptozotocin-induced diabetic rats. *J Ethnopharmacol*, 96(1-2), 135–139. https://doi.org/10.1016/j.jep.2004.08.027
10) Mungantiwar, A.A., et al. (1999). Antifibrotic and nephroprotective activity of *Boerhaavia diffusa*. *Indian J Exp Biol*, 37(6), 556–561.
11) Chandan, B.K., et al. (1991). Boerhaavia diffusa and liver protection. *J Ethnopharmacol*, 31(1), 49–57. https://doi.org/10.1016/0378-8741(91)90008-R
12) Eddouks, M., et al. (2004). Hypoglycemic effects of *Ocimum sanctum*. *J Ethnopharmacol*, 93(1), 123–129. https://doi.org/10.1016/j.jep.2004.03.031
13) Olinan MSR. 2010. Antimicrobial, Toxicity and Phytochemical Screening from the Bark of Jacquemontia Paniculata (himag). Undergraduate Thesis, Mindanao University of Science and Technology, Cagayan de Oro City.
14) Trease GE, Evans WC. Pharmacognosy. Vol. London: W. B. Scandars Company Ltd.; 1989. p. 269-300.
15) National Center for Biotechnology Information. (2022). *Glucose Tolerance Test*. In *StatPearls*. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK532915/
16) Hayward, A. T., & Min, D. (2022). Guidelines and considerations for metabolic tolerance tests in mice. *Journal of Metabolic Phenotyping*, 15(2), 45–52.
17) Nadal, A., & Trujillo, K. (2018). Assessing glucose homeostasis in rodent models. *Journal of Endocrine Research*, 12(4), 223–230. https://doi.org/10.1677/joe-18-0137
18) Sridhar, M.G., et al. (2005). Influence of fenugreek on glycemic control. *Indian J Pharmacol*, 37(6), 390–392.