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In-vitro Pharmacological Evaluation of Anti-diabetic Activity of Roots Extract of *Doronicum Hookeri*

Pratima Chourasiya[1], Rekha Gour[2]

[1]PG Student, Swami Vivekanand College of Pharmacy, Indore (M. P.), India

[2]Professor, Swami Vivekanand College of Pharmacy, Indore (M. P.), India

Abstract: ***In recent years, there has been a growing interest in exploring medicinal plants for their potential to yield more effective anti-diabetic drugs compared to conventional oral hypoglycemic medications. The current study focuses on Doronicum hookeri, a traditional medicinal plant, and investigated its in-vitro anti-diabetic properties through alpha-amylase inhibition assay, glucose uptake assay, and lipase inhibition assay. Among the various extracts, the methanolic roots extract of Doronicum hookeri exhibited the highest amylase inhibition with the lowest IC50 value (0.151). In contrast, the aqueous extracts from both plants demonstrated the most significant inhibition of glucose movement across the membrane, while the methanolic extract displayed the least inhibition. Additionally, the methanolic extract of Doronicum hookeri exhibited the strongest lipase inhibition with the lowest IC50 value (38.2). These findings suggest that Doronicum hookeri holds promise as a potential candidate for diabetes treatment.***

Keywords: Amylase, Hypoglycemic, *Doronicum hookeri, In-vitro*

1. Introduction

Diabetes Mellitus, often referred to as diabetes, is a complex and progressive chronic health condition influenced by a combination of genetic and environmental factors [1-2]. Certain populations, such as American Indians and Alaska Natives, have a higher genetic predisposition to diabetes. The World Health Organization's global survey has reported a significant increase in the number of adults living with diabetes, which has risen from 422 million in 1980 to an estimated 693 million by 2045 [3]. The rise is particularly notable in Africa and Asia [5, 6], and diabetes is a lifelong condition characterized by elevated blood sugar levels.

Diabetes mellitus is primarily categorized into Type 1 and Type 2 diabetes. Type 1 diabetes is insulin-dependent, where the body fails to produce insulin, necessitating insulin injections for patients [7] Type 2 diabetes, on the other hand, is insulin-independent, where cells become resistant to insulin and cannot efficiently utilize blood glucose for energy. Type 2 diabetes is the most prevalent form, accounting for approximately 90% of diabetes cases. Notably, pre-diabetes and Type 2 diabetes are increasingly affecting children and young adults [8, 9].

Plants have long been used as a source of medicinal treatments, offering a diverse range of remedies for various ailments. It is estimated that approximately 80% of the global population relies on traditional medicine and herbal products for their healthcare needs. In many developing countries, diabetic individuals often combine conventional and traditional medicine [10,11,12]. Medicinal plants encompass a wide variety of herbs, shrubs, and trees, with different parts of these plants, including leaves, stems, bark, and fruits, being used for treating various diseases [13]. Countries like India and China have been actively researching medicinal plants, with a specific focus on diabetes since 1995. These plants are known to store bioactive components, with leaves containing around 20% of these compounds compared to other herbs. The global diabetes burden is expected to reach alarming proportions, with an estimated 493 million adults suffering from diabetes by 2030 [14].

Traditional medicinal plants have played a crucial role in diabetes management across different cultures worldwide. However, despite significant progress in diabetes treatment over the past three decades, there is no perfect solution. Conventional therapies have drawbacks, such as drug resistance, side effects, and toxicity. For instance, sulfonylureas lose 44% of their potency in patients after six years of treatment. Furthermore, glucose-lowering drugs often cannot effectively regulate hyperlipidemia [15]. Consequently, many therapies now recommend the use of medicinal plants [16]. Most plants contain compounds like carotenoids, flavonoids, terpenoids, alkaloids, and glycosides, which often exhibit anti-diabetic properties [17].

Doronicum hookeri, a lesser-known member of the Asteraceae family, is a significant but often overlooked component of traditional medicine. This plant boasts a wide array of properties, including its positive effects on the heart, nervous system, liver, as well as its antioxidant, anti-fungal, and antibacterial characteristics. In Hindi, it goes by the name "leopard's bane," while in Urdu, it's known as "toos,""tarang," and "aqrabi." While there is limited literary documentation about *Doronicum hookeri*, there is some experimental evidence supporting its antibacterial, anti-fungal, antioxidant, and cardio-protective effects, as well as its hepatoprotective and antioxidant properties. Further research is warranted to explore the various phytoconstituents and medicinal qualities present in this plant. The standardization and pharmacological analysis of dried *Doronicum hookeri* rhizomes remains an underexplored area in current literature [18-22].

Consequently, this study undertook an *in-vitro* investigation of the anti-diabetic properties of dried *Doronicum hookeri* rhizomes. Future research should build upon this data to conduct more comprehensive pharmacological assessments of this plant.

2. Materials and Methods

2.1 Plant Material

In this research, the rhizome of the plant was sourced from a traditional herbal market in Indore, India. Once obtained, the process of gathering plant materials started with air-drying the rhizomes. These dried rhizomes were subsequently ground into a powder, sifted through a 60# mesh screen, and securely stored in airtight containers for future investigations.

2.2 Extraction Methods

This process, which entails the continuous extraction of solids using a high-temperature solvent (typically an organic solvent like methanol), is commonly referred to as Soxhlet extraction or soxhelation. The apparatus employed for this purpose consists of specialized glass reflux equipment, comprising an extraction chamber, a round-bottom flask, and a condenser. To initiate the process, 14 grams of powdered medication are placed within a Soxhlet extractor along with a filter paper thimble. Positioned beneath the extractor chamber, there is a round-bottom flask (RBF) containing the solvent, and a condenser is affixed to it. As the solvent in the RBF reaches its boiling point, the resultant vapor ascends through the side tubes towards the condenser, where it undergoes condensation. The condensed liquid then accumulates in the extraction chamber before dripping into the thimble holding the medication. The extractor tube is filled with the condensed vapors and connected to the solvent. The solvent is subsequently transported through a siphon tube back into the flask (RBF), where the extract condenses. To obtain a high-quality extract, it is essential to complete a minimum of 25 cycles [23]

3. *In-vitro* Anti-diabetic Assays.

3.1 Amylase Inhibition Assay

The assessment of amylase inhibition activity followed a specific protocol with slight adaptations [24] A starch solution (500 mg/25 ml) was prepared by dissolving it in 0.4 M NaOH and then heated at 100°C for 5 minutes. The volume was adjusted to 100 ml by adding distilled water while maintaining a pH of 7. The plant extract was dissolved in acetate buffer, and various concentrations were prepared. The pH of the acetate buffer was adjusted to 6.5. In a micro well plate, 20 µl of the substrate was combined with 10 µl of the sample, followed by the addition of 10 µl of α-amylase solution (50 µg/ml). The mixture was then incubated at room temperature for 15 minutes. To halt the reaction, 40 µl of 0.1 M HCl was added, followed by the introduction of 100 µl of a 1 mM iodine solution. The optical density was measured at 650 nm. Amylase inhibitory activity was assessed using the following formula: Amylase inhibitory activity (%) = {1 - (OD2-OD1) / (OD4-OD3) X100}.

3.2 Lipase Inhibition Assay

The evaluation of lipase inhibition activity followed a specific protocol with some modifications. [25] A substrate solution was created in 9 ml of 0.1 M TES buffer (pH 7.0) by dissolving lecithin (10 mg), sodium cholate (5 mg), and glycerol trioleate (80 mg). Different concentrations of plant extracts were prepared in 0.1 M TES buffer. In microplate wells, 20 µl of the sample and substrate solutions were combined, and 10 µl of lipase solution (20 µg/ml) was added. The mixture was incubated for 30 minutes at 37°C, and the optical density was measured at 550 nm using a microplate reader. Lipase inhibitory activity (percent) was calculated as follows: Lipase Inhibition (%) = {1 - (OD2-OD1)/(OD4-OD3)X100}.

3.3 Glucose Uptake Assay

The glucose uptake assay was conducted according to a described method with some adjustments. Methanolic and aqueous extracts (100 mg/ml) of the plant were prepared. The experiment involved using a one-sided sealed dialysis tube (12,000 MW, Himedia), into which 1 ml of 22 mM D-glucose in 0.15 M NaCl and 1 ml of the extract (100 mg/ml) or a control (water) were introduced. The opposite end of the tube was sealed, and the membrane was placed in a beaker containing 45 ml of 0.15 M NaCl. The beaker was then positioned in an orbital shaking incubator at 37°C with a speed of 100 rotations per minute. The movement of glucose into the solution was recorded every 30 minutes. This experiment was conducted in triplicate, and the observations were monitored over a three-hour period.

3.4 Statistical Analysis

All experiments were performed in triplicate for each sample, and the results were expressed as mean ± SD. IC50 values were determined through linear regression.

4. Results and Discussion

Phytochemical Screening Test

The phytochemical screening test revealed the presence of Phenols, Flavonoids, Alkaloids, Terpenoids, and glycosides in our plant. Amino acids were found in *Doronicum hookeri* (Table 1).

***In-vitro* Anti-diabetic Analysis**

Amylase Inhibition Assay

For many years, Ayurveda has explored and widely utilized various plant extracts known for their anti-diabetic properties. In this study, the anti-diabetic properties of two medicinal plants were examined using an *in-vitro* anti-diabetic assay. Alpha-amylase is responsible for postprandial glucose levels, and several plant extracts with alpha-amylase inhibitory activity are under investigation for their potential to reduce postprandial blood glucose levels. This represents a crucial and innovative therapeutic avenue for managing diabetes mellitus. The study's findings revealed that the methanolic extract of *Doronicum hookeri* exhibited IC50 value (0.169), as detailed in Table 2.

Lipase Inhibition Assay

Obesity is a significant contributing factor to increased rates of cardiovascular disease, non-alcoholic fatty liver, metabolic syndrome, and non-insulin-dependent diabetes. The hydrolysis of dietary lipids into fatty acids and 2-monoacylglycerol by pancreatic lipase is a crucial step before absorption in the intestines. Consequently, inhibiting digestive enzymes, specifically α-amylase and pancreatic lipase, may offer an effective approach to diabetes treatment. The study's results revealed that the methanolic extract of *Doronicum hookeri* has IC50 value (38.2) among all plant extracts, as indicated in Table 4. Thus, *Doronicum hookeri* is a potent plant for diabetes treatment.

Glucose Uptake Assay

Figure a present the results of the glucose diffusion assay, demonstrating significant inhibition of glucose activity by plant extract. According to the tables, the aqueous extracts of root showed the most substantial inhibition, while the methanolic extract exhibited the least inhibition, as detailed in Table 3.

Table 1: Phytochemicals analysis in different extracts

Phytochemical Test	Test Name	*Doronicum hookeri*
Glycoside	Keller Killiani test	+ve
Phenol	Ferric chloride test	+ve
Saponins	Froth test	+ve
Proteins	Ninhydrin test	+ve
Amino acids	Millon's test	-ve
Alkaloids	Mayer's test	+ve
Carbohydates	Benedict's test	+ve
Flavanoids	Lead acetate test	+ve

Table 2: IC50 value of Amylase and Lipase Inhibition assay

Plant	Extract Type	Amylase assay IC50 (μg mL^{-1})	Lipase assay IC50 (μg mL^{-1})
Doronicum hookeri	Methanolic	0.169	38.2
	Aqueous	0.151	98.3

IC50 = half maximal inhibitory concentration.

Table 3: Effect of methanolic extract of *Doronicum hookeri* on diffusion of glucose out of a dialysis membrane over 180 minutes

Time (Minutes)	Control Mean±SEM*	*Doronicum hookeri* methanolic Mean±SEM*	Relative Movement %**	*Doronicum hookeri* Aqueous, Mean±SEM*	Relative Movement %**
30	0.003±0.0004	0.00±0.0002	65.5	0.002±0.0001	70
60	0.058±0.0022	0.0031±0.0005	70.50	0.0030±0.00020	70.50
90	0.082±0.0019	0.101±0.0014	78.51	0.080±0.0024	74.00
120	0.100±0.0024	0.123±0.0015	80.48	0.105±0.0030	76.96
150	0.118±0.0027	0.096±0.0014	82.80	0.090±0.0018	85.48
180	0.122±0.0035	0.109±0.0020	87.37	0.102±0.0024	92.44

5. Discussion

Diabetes has now become the third leading cause of human mortality, following cancer and cardiovascular diseases, due to its high prevalence, morbidity, and mortality [26]. The chronic elevation of blood sugar levels in diabetes leads to long-term damage, including inflammation and organ damage. [27] Herbal medicines and plant-based compounds, known for their low or negligible toxicity and absence of side effects, have emerged as significant therapeutic options for diabetes treatment worldwide. Previous research has predominantly focused on the hypoglycemic effects of medicinal plants in diabetes management. The key bioactive components derived from medicinal plants, such as flavonoids, tannins, phenolics, and alkaloids, have been identified as pivotal contributors to their anti-diabetic properties. Many studies have confirmed the effectiveness of these compounds in the context of anti-diabetic treatments [28]

The study's findings indicated that methanol extracts contain a higher concentration of active compounds compared to aqueous extracts and demonstrate robust antioxidant and anti-diabetic activities. Preliminary phytochemical screening tests have been instrumental in identifying pharmacologically active components. The results from this study showed the presence of alkaloids, carbohydrates, flavonoids, phenols, saponins, and proteins. This aligns with previous reports on the composition of *Psidium guajava* and *Ficus benghalensis* extracts [29]. Numerous studies have highlighted the antioxidant and anti-diabetic potential of phytochemicals, particularly phenolics and flavonoid compounds found in various herbs [30]. Phenols and flavonoids have historically been recognized as two key phytochemical classes that significantly contribute to the antioxidant capabilities of plants. Polyphenolic compounds like flavonoids, phenolic acids, and tannins are considered the primary contributors to the antioxidant properties of medicinal plants. The antioxidant effects of polyphenolic compounds stem from their redox properties, allowing them to function as reducing agents, singlet oxygen quenchers, and hydrogen donors [31].

Antioxidants have been shown to reduce the risk of diabetes development, improve glucose balance, and mitigate related complications [32-34]. Oxidative stress has been associated with the pathogenesis and progression of various degenerative diseases, including naturally occurring and chemically induced diabetes mellitus. Furthermore, diabetes mellitus disrupts antioxidant defense mechanisms in the body, leading to increased production of free radicals [36]. The methanolic extract of *Doronicum hookeri* root displayed substantial antioxidant activity in all conducted experiments, indicating its strong antioxidant properties. The presence of a significant amount of polyphenolic compounds in the methanolic extract of *Doronicum hookeri* may account for its antioxidant activity. These results are consistent with

previous research where similar outcomes were reported for *Doronicum hookeri* root extracts [37]. Deguchi's study confirmed that the in vitro anti-diabetic activity of *Doronicum hookeri* root is more prominent in the methanolic extract and efficiently inhibits glucose utilization compared to other solvents, following specific standard protocols [38]. Therefore, the current investigation provides pharmacological evidence supporting the anti-diabetic potential of plant leaves with both anti-diabetic and antioxidant properties.

6. Conclusion

Diabetes is a disorder of metabolism in which high blood sugar (hyperglycemia) abnormally resulting from insufficient levels of the hormone insulin. Treatment of diabetes by natural resources seems to be a promising approach and can be favored for inhibition of alpha-amylase. Further study is required on the isolation and characterization of the principal bioactive compounds of the medicinal plant extracts and that can be safely used in clinical research for long-term administration of the natural plant extracts for type 2 diabetes. The screening of phytochemicals shows the presence of phenols, flavonoids, antioxidant and antidiabetic compounds in *Doronicum hookeri*. The *Doronicum hookeri* extracts showed the highest amylase and lipase inhibitory activity. So results show that *Doronicum hookeri* has better results against diabetes. However, *Doronicum hookeri* can be studied further for *in-vivo* anti-diabetic potentials of plants.

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