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A Study on Determination of Antidiabetic Potential of Selected Medicinal Plants from Jharkhand

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ABSTRACT

Diabetes mellitus is a metabolic condition characterized by insufficient or absent insulin production from the pancreas. The development and progression of its long-term complications are closely linked to the extent of hyperglycemia and overall metabolic regulation. Effective management primarily focuses on reducing elevated blood glucose levels and limiting intestinal glucose absorption by inhibiting key carbohydrate-metabolizing enzymes, such as alpha-amylase and alpha-glucosidase. Thus, we aimed to evaluate the aqueous and ethanolic extracts of selected plants from Jharkhand *viz. Dolichos trilobus, Atylosia cajanifolia, Vicia faba,* and *Vigna umbellata* in comparison with standard Acarbose antidiabetic drug. Results revealed that *Vicia faba* had been the most effective ethanolic extract, showing strong inhibition and the lowest IC_{50} value (51.90µg/mL) followed by ethanolic extract of *Atylosia cajanifolia* (68.40µg/mL). While, ethanolic extracts of *Vigna umbellata* (74.20µg/mL) and *Dolichos trilobus* (78.10µg/mL) have shown moderate alpha-amylase inhibition activity. Aqueous (aq.) extract of *Vicia faba* exhibited an effective alpha-amylase inhibition activity with lowest IC_{50} value (61.30µg/mL) followed by aq. extracts of *Atylosia cajanifolia (72.80*µg/mL*), Vigna umbellata* (79.50 µg/mL), and *Dolichos trilobus* (85.40 µg/mL). In conclusion, *Vicia faba* showed the most potent alpha amylase inhibitory activity in both aqueous and ethanolic extractions. Hence, aqueous and/or ethanolic extracts of *Vicia faba* could be explored as natural antidiabetic agent in the development of natural antidiabetic formulations.

Keywords: *Vicia faba*, Alpha-amylase, Acarbose, Antidiabetic, Jharkhand

INTRODUCTION

Multi-purpose medicinal plants continue to attract significant global research and commercial interest due to their rich nutritional and pharmacological properties, making them valuable for the treatment and management of various diseases.[1] These plants produce a wide range of bioactive compounds, including phenols, flavonoids, vitamins, alkaloids, terpenoids, tannins, glycosides, and quinones. Many medicinal plants have been identified, and their therapeutic applications are well documented.[2] Historically, most traditional and modern medicines, including allopathic, ayurvedic, and homeopathic formulations, were derived from plants.[3]

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels caused by impaired insulin secretion and/or action.[4] Type 2 diabetes accounts for 90–95% of cases and is one of the most serious health challenges worldwide, with India ranked among the top three countries with the highest prevalence.[5,6] According to the International Diabetes Federation (IDF), approximately 387 million people were affected by diabetes globally in 2014, and this number is projected to increase to 592 million by 2035.[7] Diabetes is a lifelong endocrine condition that leads to hyperglycemia and severe, often irreversible complications involving both microvascular and macrovascular systems.[8] These include diabetic retinopathy (eyes), diabetic neuropathy (nerves), diabetic nephropathy (kidneys), diabetic foot, atherosclerosis (blood vessels), and cardiovascular diseases (heart). Effective management of hyperglycemia is therefore essential to prevent or minimize these complications.[9]

The development of diabetes involves several pathological processes, with chronic hyperglycemia and insulin resistance playing key roles in the onset of long-term complications.[5] A primary therapeutic strategy for managing diabetes is to reduce elevated blood glucose levels and inhibit intestinal glucose absorption by targeting key carbohydrate-metabolizing enzymes, such as alpha-amylase and alpha-glucosidase.[8]

Herbal medicine remains a crucial component of primary healthcare for about 75%–80% of people in many developing nations due to its cultural acceptance, better compatibility with the human body, and relatively fewer side effects.[2] Moreover, Ayurveda, one of the oldest traditional medical systems in India, emphasizes the use of plants and plant-derived products for healing.[10] Various plant parts, including seeds, roots, leaves, fruits, flowers, or even the entire plant, are utilized for medicinal purposes.[2]

In many developing countries, medicinal plants remain a primary source of healthcare, especially in rural and tribal communities where access to modern medical facilities is limited.[11] Jharkhand, located in eastern India, is one such state, with approximately 26%–28% of its population belonging to Scheduled Tribes.[12] These tribal communities rely heavily on forests for their livelihoods and cultural practices. Forests provide essential resources such as food, fodder, fuel, and building materials, along with medicinal plants that play a central role in traditional healthcare systems.[13] Against this backdrop, the present study was undertaken to evaluate the antidiabetic potential of selected medicinal plants from Jharkhand.

MATERIALS AND METHODS

Collection and Identification of Plant

Samples of selected lesser-known medicinal plants from Jharkhand state *viz. Dolichos trilobus L., Atylosia cajanifolia, Vicia faba,* and *Vigna umbellata* were collected (Figure 1). Care was

taken to collect a representative part of the plant (leaves) to facilitate accurate identification. The specimens were then pressed and preserved for later identification. The collected plant specimens were identified using standard taxonomic procedures with the help of floras, monographs, and expert consultation at a recognized herbarium.

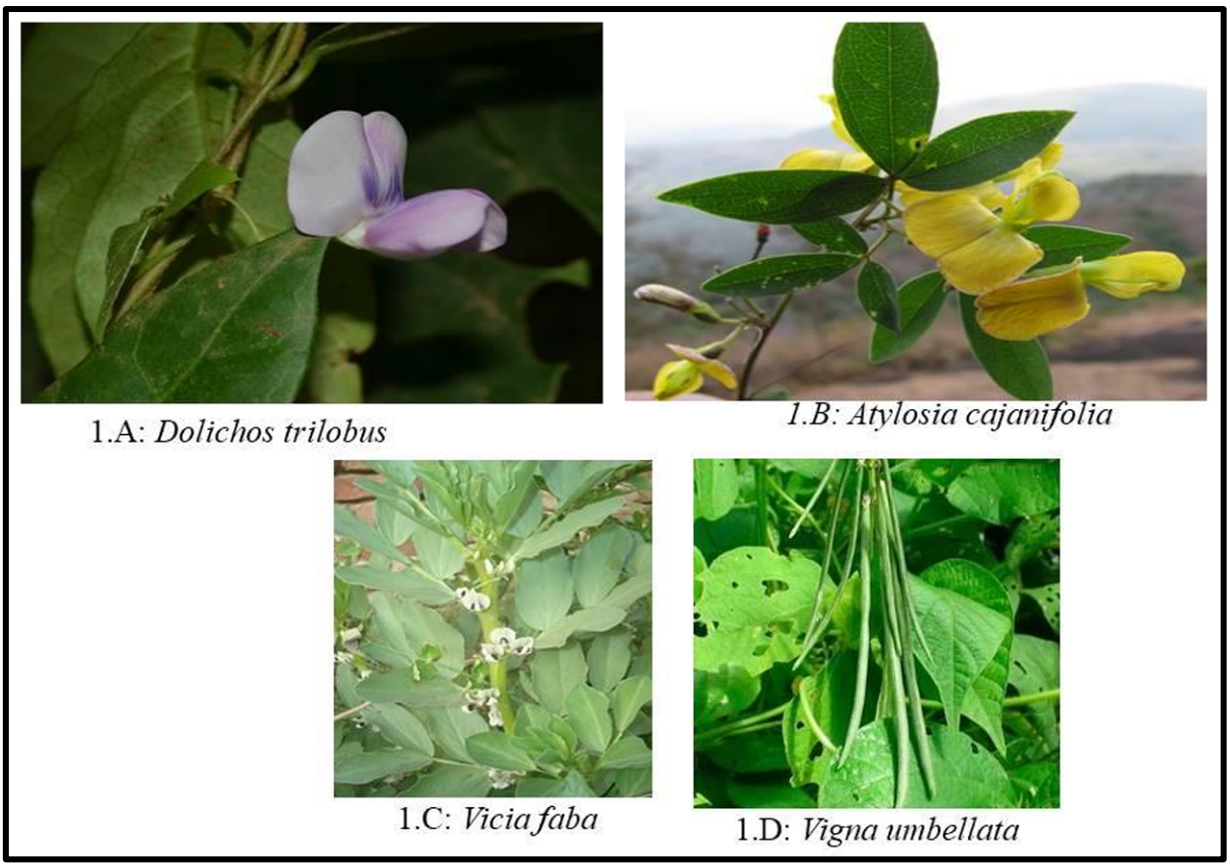

Figure 1. Selected lesser-known medicinal plants from Jharkhand state: *Dolichos trilobus* L. (1A), *Atylosia cajanifolia* (1B), *Vicia faba* (1C), and *Vigna umbellata* (1D)

Sample preparation

Leaves were collected by hand using clean scissors and gloves to minimize contamination. After collection, leaves were placed in sterile, labeled polythene bags lined with moist tissue paper to maintain freshness during transport to the laboratory. Leaves were first rinsed under running tap water in the laboratory to remove dust, soil particles, and insect residues. This was followed by a rinse with distilled water to eliminate any remaining contaminants. In some cases, a mild sterilization step using 0.1% sodium hypochlorite solution was employed. Cleaned leaves were spread on blotting papers and air-dried under shade to remove surface moisture before further processing. After complete drying, leaves were ground into fine powder using a sterile mechanical grinder The powdered samples were sieved through a mesh (60–80 µm) to obtain uniform particle size. The powdered leaves were stored in airtight amber glass bottles. Bottles were kept in desiccators with silica gel at room temperature until further experimental analysis.

Extraction

The leaves of *Dolichos trilobus L., Atylosia cajanifolia, Vicia faba,* and *Vigna umbellata* were subjected to both aqueous and ethanolic extraction techniques to obtain crude extracts. The

aqueous extracts of the selected plant leaves had been prepared using the maceration method. A standard ratio of 1:10 (w/v) i.e., 100g of powdered leaf material macerated in 1000 ml of distilled water had been maintained for uniformity across species. The powdered material was placed in sterile glass conical flasks, and freshly boiled and cooled distilled water was added. The flasks were sealed with parafilm to prevent contamination and kept at room temperature (25 ± 2°C) for a period of 72 hours. During this period, the macerated mixture was stirred intermittently with a sterile glass rod to facilitate maximum solubilization of bioactive constituents. After completion of the maceration period, the mixture was filtered through a double layer of muslin cloth followed by filtration through Whatman No. 1 filter paper to remove coarse plant debris. The filtrates obtained were further clarified by centrifugation at 5000 rpm for 10 minutes, ensuring removal of suspended particles. The aqueous extracts were collected in pre-labeled sterile amber-colored bottles and stored in refrigerator at -20°C.

For ethanol-soluble phytochemicals, extraction had been performed using the Soxhlet extraction apparatus, a widely accepted method for exhaustive extraction of plant metabolites. Approximately 50g of powdered leaves of each species was accurately weighed and placed in a cellulose thimble inside the Soxhlet extractor. Analytical grade ethanol (95%) was used as solvent, and a solvent-to-sample ratio of 200 ml per 50g was maintained. The system was heated on a heating mantle, allowing ethanol vapors to rise, condense, and repeatedly wash over the powdered leaf material. Each extraction cycle took approximately 30 minutes, and the process was continued for 8–10 hours, until the siphon tube of the Soxhlet ran clear, indicating exhaustive extraction. The ethanolic extracts were collected and concentrated immediately after extraction, and stored in pre-labeled sterile amber-colored bottles in refrigerator at -20°C.

Assay of Alpha-amylase Inhibition Activity

The alpha-amylase inhibition activity of extracts of aq. and ethanolic extracts of *Dolichos trilobus L., Atylosia cajanifolia, Vicia faba*, and *Vigna umbellata* was assayed as per standardized method described by Suthindhiran et al.[14] Briefly, 500µl of extracts at different concentration (50, 100, 200, 400, 800 µg/ml) and 500µl of 0.02M Sodium phosphate buffer (pH 6.9) containing alpha-amylase (0.5 mg/ml) were incubated at 25°C for 10 minutes. After pre-incubation 500µl of 1% starch solution in 0.02M phosphate buffer was added to the reaction, and the reaction mixture was incubated at 37°C for 30 minutes. Then reaction was stopped by adding 1ml of DNS reagent. The test tubes were then incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted by adding 10ml distilled water and absorbance was measured at 540nm. The antidiabetic drug Acarbose was used as standard in alpha-amylase inhibition assay. The inhibition (%) was calculated using the following formula:

$$\% \ Inhibition = [Abs_{control} - Abs_{sample} / Abs_{control}] \times 100$$

RESULTS

The results on *in-vitro* antidiabetic activity of aqueous extracts of selected plants *viz. Dolichos trilobus, Atylosia cajanifolia, Vicia faba,* and *Vigna umbellata* at 100µg/mL in comparison with standard Acarbose were represented in Table 1 and plotted in Figure 2. Results depicted that *Atylosia cajanifolia* had shown 65.2 ± 1.8% inhibition with an IC_{50} value of 72.80 ± 2.50µg/mL, reflecting stronger activity than *Dolichos trilobus*. *Vicia faba* had recorded the highest inhibitory activity among the tested species with 74.1 ± 2.5% inhibition and the lowest IC_{50} value of 61.3 ± 2.1µg/mL signifying the most potent alpha-amylase inhibition. *Dolichos*

trilobus had exhibited 58.5 ± 2.1% inhibition, with an IC_{50} value of 85.4 ± 3.2µg/mL indicating moderate inhibitory potential. *Vigna umbellata* had demonstrated 61.8 ± 2.0% inhibition with an IC_{50} of 79.5 ± 2.8 µg/mL, suggesting moderate effectiveness, comparable to *Dolichos trilobus*.

Table 1. Alpha-amylase inhibition assay of aqueous plant extracts

Aqueous plant extracts (100µg/mL)	Inhibition (%)	IC_{50} (µg/mL)
Dolichos trilobus	58.50 ± 2.10	85.40 ± 3.20
Atylosia cajanifolia	65.20 ± 1.80	72.80 ± 2.50
Vicia faba	74.10 ± 2.50	61.30 ± 2.10
Vigna umbellata	61.80 ± 2.00	79.50 ± 2.80
Acarbose (Positive Control)	91.50 ± 1.50	35.10 ± 1.20

Values were expressed as mean ± standard deviation (SD)

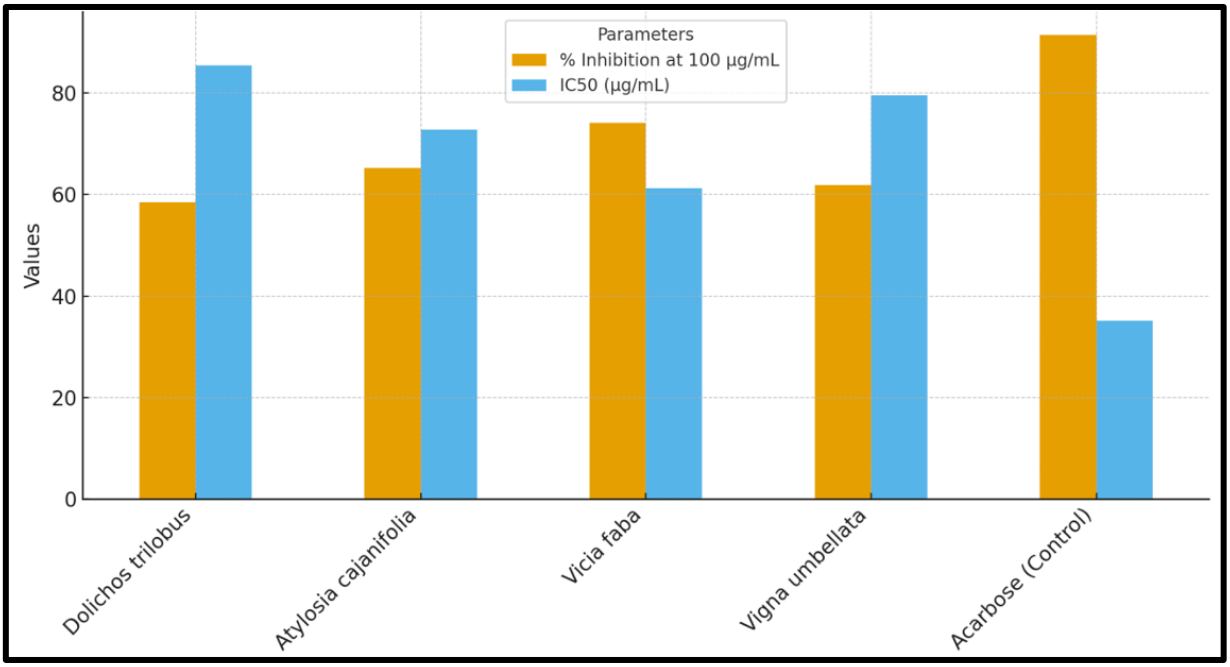

Figure 2. Alpha-amylase inhibition assay of aqueous plant extracts

Overall, all four aqueous extracts have exhibited significant alpha-amylase inhibitory activity, though none had matched the potency of Acarbose. Among the species, *Vicia faba* had been the most effective followed by *Atylosia cajanifolia, Vigna umbellata*, and *Dolichos trilobus*. These results suggested that the aqueous extracts particularly *Vicia faba*, could serve as promising natural sources for the development of anti-diabetic agents.

The results on *in-vitro* antidiabetic activity of ethanolic extracts of selected plants *viz. Dolichos trilobus, Atylosia cajanifolia, Vicia faba,* and *Vigna umbellata* at 100µg/mL in comparison with standard Acarbose were represented in Table 2 and plotted in Figure 3.

Results inferred that *Dolichos trilobus* had exhibited 62.3 ± 2.4% inhibition with an IC_{50} value of 78.1 ± 3.5µg/mL, suggesting moderate inhibitory activity. *Atylosia cajanifolia* had shown a higher inhibition of 69.8 ± 2.0%, with an IC_{50} value of 68.4 ± 2.3 µg/mL, which indicated better potency compared to *Dolichos trilobus*. *Vicia faba* had demonstrated the most significant inhibitory activity among the ethanolic extracts, with 81.5 ± 2.8% inhibition and the lowest IC_{50} value of 51.9 ± 2.0µg/mL, highlighting it as the most effective candidate. *Vigna umbellata* had recorded 65.9 ± 2.3% inhibition with an IC_{50} value of 74.2 ± 3.0µg/mL which reflected moderate inhibitory effects, better than *Dolichos trilobus* but less potent than *Atylosia cajanifolia* and *Vicia faba*. In comparison, Acarbose (positive control) had shown the highest inhibition of 91.5 ± 1.5% and the lowest IC_{50} value of 35.1 ± 1.2 µg/mL, confirming its superior efficacy as a standard drug.

Table 2. Alpha-amylase inhibition assay of ethanolic plant extracts

Ethanolic plant extracts (100µg/mL)	Inhibition (%)	IC_{50} (µg/mL)
Dolichos trilobus	62.30 ± 2.40	78.10 ± 3.50
Atylosia cajanifolia	69.80 ± 2.00	68.40 ± 2.30
Vicia faba	81.50 ± 2.80	51.90 ± 2.00
Vigna umbellata	65.90 ± 2.30	74.20 ± 3.00
Acarbose (Positive Control)	91.50 ± 1.50	35.10 ± 1.20

Values were expressed as mean ± standard deviation (SD)

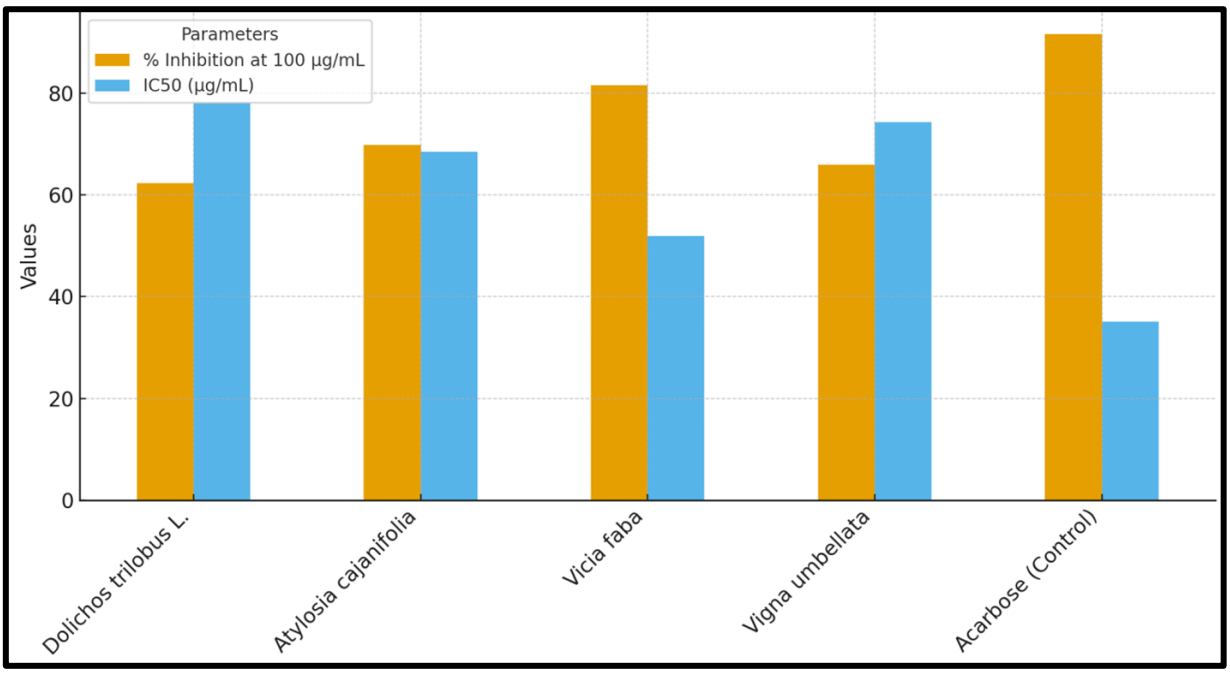

Figure 3. Alpha-amylase inhibition assay of ethanol plant extracts

Overall, the ethanolic extracts had displayed significant alpha-amylase inhibitory activities, though their potencies had been lower than that of Acarbose. Among the tested

legumes, *Vicia faba* had been the most effective ethanolic extract, showing strong inhibition and the lowest IC_{50} value followed by *Atylosia cajanifolia.* While, *Vigna umbellata* and *Dolichos trilobus* had shown moderate activity. These results suggested that ethanol had been an effective solvent in extracting phytochemicals with anti-diabetic potential particularly in *Vicia faba*.

DISCUSSION

Several herbs have been used historically in Ayurvedic medicine to treat a wide range of illnesses. The greatest bioresource for pharmaceutical intermediates, modern and traditional medicine, nutraceuticals, food supplements, folk remedies, and chemical entities for synthetic drugs is found in medicinal plants.[15] One of the therapeutic approaches for decreasing of blood glucose rise after a meal is to retard the absorption of glucose by inhibition of carbohydrate hydrolyzing enzymes such as alpha-amylase and alpha-glucosidase. The use of synthetic drugs *viz.* acarbose, miglitol and voglibose are the regular practices in control of postprandial hyperglycemia despite its gastrointestinal adverse effects. Hence, the research for new group of agents from natural resources especially from traditional medicines became an alternative approach for the treatment of postprandial hyperglycemia.[4] Hence in the current study we aimed to evaluate the selected aqueous and ethanolic extracts of plants from Jharkhand state *viz. Dolichos trilobus, Atylosia cajanifolia, Vicia faba,* and *Vigna umbellata* in comparison with standard Acarbose antidiabetic drug.

The results of the *in-vitro* anti-diabetic assay in the current study demonstrated that all tested aqueous and ethanolic plant extracts *viz. Dolichos trilobus, Atylosia cajanifolia, Vicia faba,* and *Vigna umbellata* exhibited significant alpha-amylase inhibitory activity, a key mechanism in managing post-prandial blood glucose levels. Both aqueous and ethanolic extracts showed a dose-dependent inhibitory effect. However, the ethanolic extracts consistently showed a stronger inhibitory effect with lower IC_{50} values compared to their aqueous counterparts. This suggested that the active phytochemicals responsible for alpha-amylase inhibition were more soluble in ethanol than in water. Among all the tested plants, *Vicia faba* showed the most potent inhibitory activity in both aqueous and ethanolic extractions. Its ethanolic extract recorded the lowest IC_{50} value of 52µg/mL, indicating its superior anti-diabetic potential.

Concurrently, previous studies suggest the anti-diabetic and free radical scavenging property of *Vicia faba* points out hypoglycemic and antioxidant effect.[16] The presence of vicine or divicine in *Vicia faba* seeds,[17] may be one cause of antidiabetic activity. Furthermore, *Vicia faba* with antioxidants help in rejuvenation of β-cells of pancreas by scavenging free radicals and defends cytotoxic streptozotocin effect which helps in treating diabetes mellitus.[16] A rapid decline in blood glucose and lipid levels was observed along with increase in insulin hormone level, high density lipoproteins, ferritin, haemoglobin, superoxide dismutase, catalase, glutathione peroxidase and glutathione-S-transferase.[18]

It was understood from the literature that plant-based alternative medications and functional foods that modulate physiological effects in preventing and treating diabetes and obesity are becoming more popular.[5] The plant kingdom could be a vast resource for finding natural, effective oral hypoglycemic medicines with little or no adverse effects. Over 1200 plant species have been identified as being utilized effectively for hypoglycemic action over the

world.[19] As a result, natural alpha-glucosidase and alpha-amylase inhibitors derived from the plant kingdom offer promising leads for hyperglycemia management.[20]

CONCLUSION

In conclusion, our study findings clearly demonstrated that among all the tested plants, *Vicia faba* showed the most potent alpha-amylase inhibitory activity in both aqueous and ethanolic extractions. Hence, aqueous and ethanolic extracts of *Vicia faba* could be explored as natural antidiabetic agent in the development of natural antidiabetic formulations. Furthermore, this preliminary pilot study provided a scientific basis for the traditional use of *Vicia faba* in managing diabetic symptoms and highlighted *Vicia faba* as a promising candidate for further research and the development of natural anti-diabetic agents.

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