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In-Vitro Glucose-Uptake Inhibition Activity of *Mikania micrantha* (L.) Willd. Leaf Extract on Yeast Cells

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ABSTRACT
Mikania micrantha (L), commonly known as "mile-a-minute weed," is a perennial climber native to Central and South America. It is widely used in traditional medicine for various therapeutic purposes. In this study, our aim was to evaluate the in-vitro glucose uptake inhibitory effect of Mikania micrantha wild leaf extract on yeast cells. Mikania micrantha leaves were harvested, air dried and ground to a fine powder. The crude extract was obtained by decoction method in a suitable solvent. The extract was then concentrated and used for further experiments. The results showed that Mikania micrantha leaf extract exhibited a dose-dependent inhibition of glucose uptake in yeast cells. With increasing extract concentration, the glucose uptake by yeast cells increased significantly compared to the control group. This inhibition of glucose uptake suggests the presence of bioactive compounds in the extract that could potentially modulate glucose metabolism. The findings add to the growing body of evidence supporting the traditional use of Mikania micrantha in the treatment of glucose-related disorders. Future research should focus on evaluating the extract's efficacy and safety in animal models and possibly in human clinical trials, paving the way for the development of new antidiabetic therapies
Keywords: In-Vitro study; glucose-uptake inhibition; Mikania micrantha; Phytochemicals.

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INTRODUCTION

Diabetes is a group of syndromes characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins and an increased risk of complications from vascular disease. Diabetes is defined as a state in which homeostasis of carbohydrate and lipid metabolism is improperly regulated by insulin [1]. Type 1 diabetes mellitus (DM) and Type2 diabetes are the two major subtypes of DM. T2DM is more prevalent in middle-aged and older adults who have prolonged hyperglycaemia as a result of sedentary lifestyle and eating habits, whereas T1DM is present in children or adolescents. Since the pathogenesis of T1DM and T2DM is very distinct from one another, each type has a different etiology, presentation, and course of therapy [2,3]. In both developing and established nations, 25% of the global population suffers from diabetes mellitus. With an estimated 110 million diabetics, China led the globe, followed by India (70 million) and the United States (30 million) affected by diabetes. According to WHO predictions, diabetes will be the 7th most common cause of mortality by 2030. A significant threat to human health is diabetes with cardiovascular complications, which results in one death every ten seconds [4]. As per international Diabetes Foundation reports from 2014, 8.3% of adults worldwide are expected to have diabetes (387 million), which by the year 2035 is expected to increase to a valuation of 53% (592 million).Type 2 diabetes, which accounts for 90% of all cases of the disease and was once thought to only affect wealthy "Western" nations, has since spread throughout the world and is now a leading cause of disability and mortality, affecting even younger age groups. In India, 77 million people were estimated to have diabetes in 2019, and by 2045, that number is projected to reach over 134 million. About 57% of these people are still unidentified [5]. There is still no drug or reasonable therapy in modern medicine that can effectively treat diabetes. The anti-diabetic medications that are presently available include sulfonylureas, thiazolidinedione, and -glycosidase inhibitors like miglitol and acarbose, a common diabetes medication. However, these medications cause a number of diabetic complications, including diarrhoea, abdominal discomfort, and soft faeces in the colon, in addition to failing to cure the illness [6,7]. Type 2 diabetes

mellitus is a growing health concern in emerging nations as a result of lifestyle modernization. Patients with type 2 diabetes mellitus are frequently put on a restricted diet and told to exercise, mainly for weight control. Pharmacological therapy is recommended if nutrition and exercise are ineffective at bringing blood glucose under control. Regardless of the type of diabetes, patients must maintain blood glucose control by taking medication and/or following a workout and nutrition plan [8]. Certain herbal remedies for diabetes have been shown to relieve symptoms and help avoid the development of secondary complications. Some plants have also been shown to aid in ß-cell regeneration and resistance reduction. Some herbs have also been said to have antioxidant and cholesterol-lowering properties in addition to their ability to keep blood sugar levels regular [9]. As a result, natural derivatives with better efficacy and fewer side effects can be used as an option to insulin. Mikania micrantha (L.) Willd. is a fast-growing tropical plant also known as bitter vine or mile-a-minute. Although it originated in the tropical regions of Central and South America, it is now extensively spread throughout Southeast Asia, the Pacific Islands, South China, etc. It has historically been used as folk medicine in numerous regions of the globe for instance, the juice from the leaves of M. micrantha is used to soothe skin rashes and itches and a poultice produced from the plant's leaves is used to treat venomous bug bites. Its most widely used applications in Jamaica are as folk medicine to encourage the healing of sores and as a dressing for wounds. Additionally, it is employed to treat lung illnesses, rheumatism, fever, jaundice, and stomachaches. Scientific research on modern pharmacological investigations shows that bitter vine has exceptional therapeutic potencies, including antimicrobial, anti-inflammatory, cytotoxic, anticancer, antidiabetic, antioxidant, and wound healing activities. Due to its numerous biological potencies, M. micrantha has attracted the interest of natural product chemists because of its rapid growth and invasion of the local vegetation [9-11]. Phytochemicals like terpenoids (sesquiterpene lactones), alkaloids, flavonoids, steroids, reducing sugars, saponins, tannins, phenolics and flavonoids are abundant in *M. micranth* [6]. Recently attempts have been made to identify M. micrantha rationally on modern line so that benefit can reach to all the persons with low cost. In this context, the present study was therefore, undertaken to evaluate the in-vitro glucose-uptake inhibition activity of Mikania micrantha (L.) Willd. Leaf extract in yeast cells.

MATERIAL AND METHODS

Plant materials

The leaves of Mikania micrantha (L.) Willd. (MM) were collected during April-May from Birkuchi, Guwahati, Assam, and identified with the standard sample preserved in the Botany division of Central Ayurevda Research Institute (CARI), Ministry of AYUSH, Guwahati, Assam.

Preparation of Extract

Aqueous extract of MM (MME) was prepared by adding 300 g of dried, crushed, and powdered leaves of MM in 600mL of distilled water in a round bottom flask and was kept at room temperature for 3 days in the shade. The extract was filtered and the above procedure was repeated twice. The extract filtrate so obtained was cooled and evaporated in the water bath and freeze-dried till it dried. The percent yield of the plant extract was calculated.

Preliminary phytochemical analysis

MME was subjected to qualitative tests for the identification of tannins, glycosides, carbohydrate, protein and amino acids, phenolic compounds, saponins, alkaloids, flavonoids and Steroids & triterpenoids as per the test given below [5].

In-Vitro glucose-uptake inhibition activity

The yeast (Puarmate; Batch no: MC264711) was suspended in distilled water and was repeatedly centrifuged (3000 g, 5 min) until clear supernatant fluids were recovered and 10% (v/v) of the suspension was made. Different concentration of MME (50, 100, 150, 200 and 250 µg) and metronidazole (J.B. Chemicals and Pharmaceuticals Ltd.) (50, 100, 150, 200 and 250 µg) was prepared. 1 ml of glucose solution (5mM) and 1ml of different concentrations MME (50 to 250 g/ml) were combined and incubated at 37°C for 10 minutes. The reaction was initiated by adding 100 µl of yeast suspension, followed by vortexing and a further 60 minutes of incubation at 37°C. The tubes were centrifuged at 2500g for 5 min, and the amount of glucose in the supernatant was calculated. As a typical medication, metronidazole was used as standard. The following formula was used to determine the percentage glucose-uptake by yeast cells [3].

$$\text{Increase in glucose uptake} = \frac{\text{Abs sample} - \text{Abs control}}{\text{Abs sample}} \text{ X } 100$$

Abs sample is the absorbance of the test sample, and Abs control is the absorbance of the control reaction (which contains all of the reagents except for the test sample)

RESULTS AND DISCUSSION

Percent yield of Extract

The percent yield of the *Mikania micrantha* (L.) Willd. (MM) leaves extract was calculated and the yield of the extract was 29.99%. The powdered extract was stored in an airtight container for further use.

Preliminary phytochemical analysis

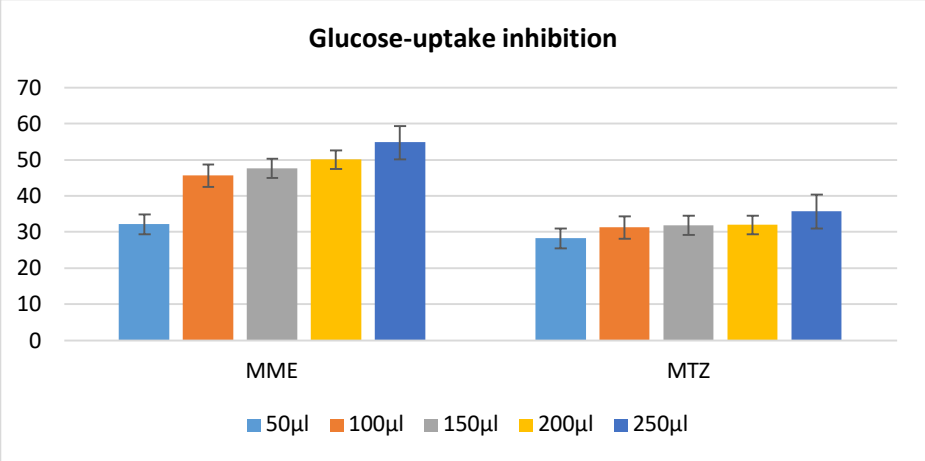
Dried leaves powder of MM were extracted with distilled water following the standard procedure and used for phytochemical screening. The preliminary phytochemical screening of the MME showed the presence of tannins, glycosides, carbohydrate, protein and amino acids, phenolic compounds, saponins, alkaloids, flavonoids and Steroids & triterpenoids (Table 1).

Table 1. Data showing the preliminary phytochemical screening of MME

Phytochemical	Test	Observation	Interference
Alkaloids	Hagers test	Yellow ppt formed	+
	Mayers test	White or creamy ppt not formed	+
	Dragendroffs test	Orange ppt is formed	+
	Wagners test	Reddish brown ppt is formed	+
Carbohydrates	Fehlings test	Brick red ppt formed	+
Amino acid	Ninhydrin test	Characteristic purple/violet colour formed	+
Cardiac Glycosides	Baljet's test	Orange colour is formed	+
Tannins	Ferric chloride test	Green colour is formed	+
Flavanoids	Lead acetate test	Yellow ppt	+
Steriods and terpenoids	Sulphur powder test	Sink at the bottom	+
Coumarin		Yellow colour is formed	+
Saponins	Frothing test	Froth is formed	+
Phenols	Ferric Chloride test	Dark green colour is formed	+

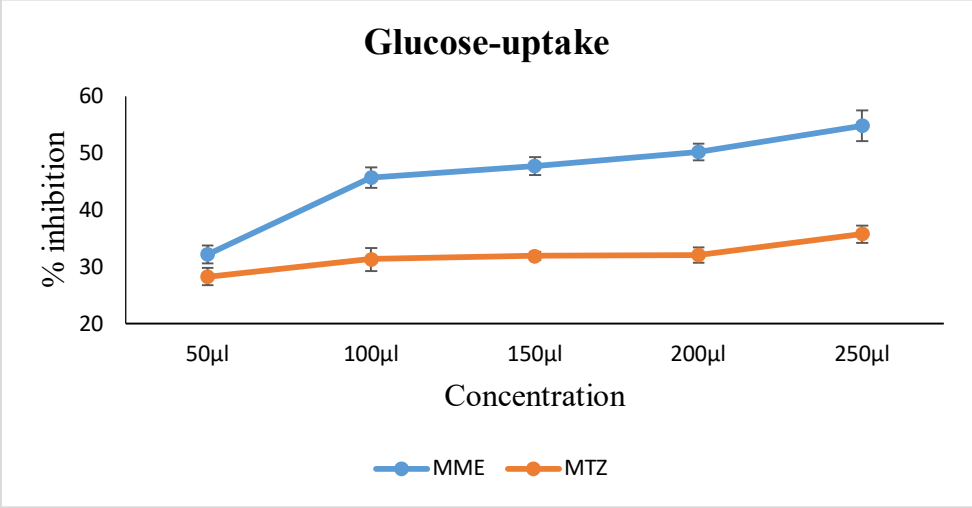
In-Vitro glucose-uptake inhibition activity

The MME inhibit the in-vitro glucose absorption in yeast cell in a dose-dependent manner with the comparison of standard drug. 250micro liter dose of MME showed highly significant effects and inhibit glucose transportation (Fig. 1 & 2). In the comparison study, the MME showed better effect and significant inhibition comparison to standard drug metronidazole (Fig. 3).



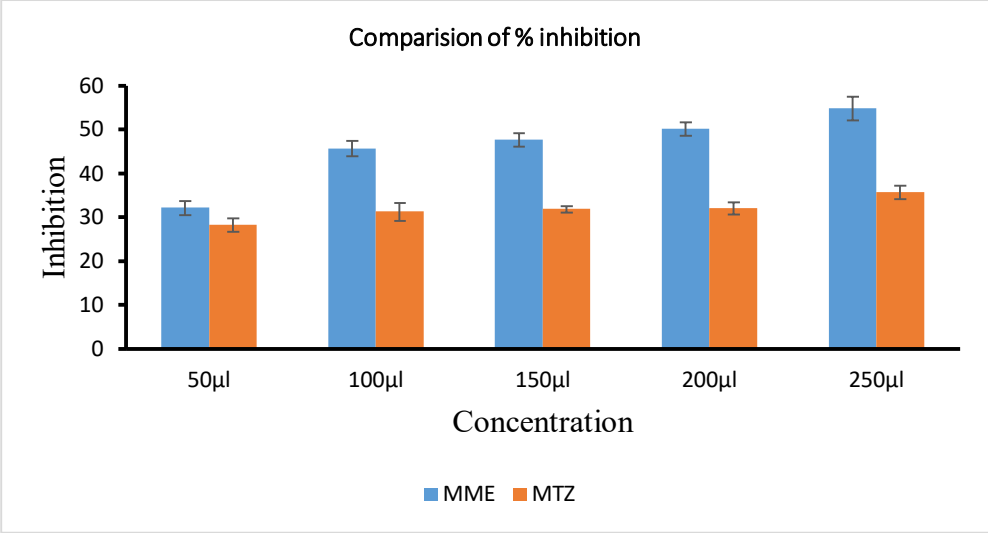
Values are mean ± SD of 3 experiments in each group

Figure 1. Effect of graded concentration of MME and MTZ on glucose uptake inhibition by yeast cells



Values are mean ± SEM of 3 experiments in each group

Figure 2. Glucose uptake inhibition by graded concentration of MME and MTZ



Values are mean ± SEM of 3 experiments in each group

Figure 3. Comparison of glucose uptake inhibition by MME & MTZ

Diabetes mellitus (DM) is a complex metabolic disorder involving various organs leading to development of both macro and micro-vascular complications, including neuropathy, nephropathy, retinopathy, delayed wound healing and foot ulcer. It is a chronic metabolic disease affecting a large number of people of all ages, races and socio-economic classes throughout the world [12]. Although several drugs targeted for carbohydrate hydrolysing enzymes (psuedosaccharides), release of insulin from pancreatic β-cells (sulphonyl ureas), glucose utilization (biguanides), insulin sensitizers, PPAR agonists (glitazones) are in clinical practices, the growing diabetes market observes a number of changes. Some of these drugs are linked to liver toxicity (troglitazone), including a number of deaths from hepatic failure and raising the symptoms and risk factors of heart disease leading to heart failure (rosiglitazone). Therefore, as the long term of risk and effect on the complications of diabetes related with these drugs are not clear. On the other hand, traditional medicinal plants with various active principles and properties have been used since ancient times by physicians and laymen to treat a great variety of human diseases such as diabetes, coronary heart disease and cancer. The beneficial multiple activities like manipulating carbohydrate mechanism by various mechanism, preventing and restoring integrity and function of β-cells, insulin-releasing activity, improving glucose uptake and utilization and the antioxidant properties present in medicinal plants offer exciting opportunity to develop them in to novel therapeutics [13]. In our present study, we selected *Mikania micrantha* (L.) Willd. (Leaves), on the basis of their traditional uses and some reported pharmacological activities. The preliminary phytochemical screening of the MME showed the

presence of alkaloids, saponins, flavonoids, phenolic compounds, amino acids, carbohydrates, cardiac glycosides, steroids, coumarin, and tannins. Saponins are reported to have cholesterol lowing properties by preventing its re-absorption [14]. It is also reported to have antioxidants, immune-modulator, antitumor and anti-mutagenic activities [15]. Various bioactivities of phenolic compounds are responsible for their chemo-preventive properties (e.g., antioxidant, anti-carcinogenic, or anti-mutagenic and anti-inflammatory effects) and also contribute to their inducing apoptosis by arresting cell cycle, regulating carcinogen metabolism and ontogenesis expression, inhibiting DNA binding and cell adhesion, migration, proliferation or differentiation, and blocking signaling pathways [16]. Alkaloids are well known for their antimicrobial activity [5,17]. Tannins have been reported to possess antioxidant, wound healing and antimicrobial activities [18]. It has been discovered that certain phytochemicals aid in controlling blood sugar levels. For instance, substances like quercetin (found in apples, onions, and berries), berberine (found in various plants), and resveratrol (found in grapes and red wine) have shown the capacity to increase glucose uptake by cells, improve insulin sensitivity, and inhibit enzymes involved in carbohydrate metabolism. When taken by people with diabetes, these acts can help them better control their blood sugar levels [19].Some phytochemicals may have effects that are insulinotropic, or ones that boost the pancreas' capacity to produce insulin. As an illustration, it has been shown that components in cinnamon and fenugreek seeds can boost insulin release and support glucose management [20,21]. Diabetes mellitus is a non-contagious illness that is frequently hereditary in origin but can also develop as a result of lifestyle choices. There is no approved, efficient therapy or drug to treat diabetes in current medicine [22]. There are a number of factors that could explain MME increased glucose absorption. First off, it might promote the movement of glucose transporters (GLUTs) to the plasma membrane, which would make it easier for glucose to enter cells. The manner of action of some anti-diabetic medications, including metformin, is comparable to this effect [23]. MME may also stimulate important enzymes involved in the metabolism of glucose, including pyruvate kinase, hexokinase, and glucokinase. These enzymes can be activated to improve the intracellular glycolytic pathway, increasing glucose uptake and energy production [24]. Furthermore, MME might have antioxidant characteristics that can lessen the effects of oxidative stress-related insulin resistance. The antioxidant activity of MME may contribute to its anti-diabetic properties because oxidative stress is known to play a role in the etiology of diabetes [25].

CONCLUSION

Extract of Mikania micrantha (L.) Wild. Showed significant glucose-uptake inhibition in yeast cells. It might promote the movement of glucose transporters (GLUTs) to the plasma membrane, which would make it easier for glucose to enter cells. The preliminary phytochemical screening of the MME showed the presence of saponins, flavonoids, phenolic compounds, amino acids, carbohydrates, cardiac glycosides, steroids, coumarin, and tannins. Phytochemicals aid in controlling blood sugar levels and have shown the capacity to increase glucose uptake by cells, improve insulin sensitivity, and inhibit enzymes involved in carbohydrate metabolism. Therefore, it can be concluded that MME has good potential to be used as an anti-diabetic agent and may be used as complementary alternative medicine. However, further work is required in the field of molecular research to authenticate their use.

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