

Research Article

Evaluation of the effect of *Musa paradisiaca* L. rhizome on *Saccharomyces* glucose absorption and DPPH free radical scavenging capacity

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Abstract

Introduction: *Musa paradisiaca* is a common edible plant used in traditional medical systems for its medicinal properties. In this study, *in vitro* anti-hyperglycaemic potential and *in vitro* antioxidant activity of *M. paradisiaca* rhizome were investigated. **Methods:** Dried rhizome of *M. paradisiaca* was sequentially extracted to hexane and dichloromethane using cold maceration technique. Stock concentrations of hexane extract (35.35 mg/mL) and dichloromethane extract (22.14 mg/mL) were diluted to prepare different concentrations and were used to evaluate the effects of plant extracts on glucose uptake potential by yeast cells and DPPH free radical scavenging capacity compared to metformin and ascorbic acid standards respectively. **Results:** The extracts improved the glucose uptake through yeast plasma membrane, since a linear relationship in glucose uptake by yeast cells was observed with gradual increase in concentration of the plant extract. In terms of glucose adsorption capacity, dichloromethane extract of plant possessed a significant ($p < 0.05$) activity compared to the hexane extract at higher glucose concentrations, but this activity was significantly less compared to metformin standard ($p < 0.05$). Further, dichloromethane extract demonstrated a higher level of glucose adsorption potential than hexane extract. Same extract had the highest DPPH radical scavenging potential in antioxidant assay. However, this activity was concentration-dependent, and both extracts showed lower radical scavenging potentials compared to the ascorbic acid standard. **Conclusion:** Dichloromethane extract of *M. paradisiaca* rhizome is more biologically active compared to its hexane extract in terms of *in vitro* glucose adsorption potential and antioxidant properties. However, both extracts showed significantly lower activity compared to metformin in glucose uptake assay while ascorbic acid showed the highest activity in free radical scavenging.

Keywords: DPPH, Glucose adsorption, *Musa paradisiaca* rhizome, *Saccharomyces*

Introduction

Diabetes mellitus has become a common non-communicable disease in both developing and developed countries [1]. The metabolic disease is indicated by hyperglycaemia resulting either from deficiency of insulin secretion or defects in insulin action or due to both reasons. The most frequent

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Received: 31 May 2023; Accepted: 21 December 2023

How to cite this article:

Chathurika, P.T.C., Vivitharani, M., Chamikara, L.H.V., Dias, N.T.B. and Siriwardhene, M.A. Evaluation of the effect of *Musa paradisiaca* L. rhizome on *Saccharomyces* glucose absorption and DPPH free radical scavenging capacity, Journal of Health Sciences and Innovative Research, 2023;4(2):11-19

type of diabetes is type 2 diabetes mellitus, a heterogeneous combination of disease syndromes that ultimately contribute to the final common pathway of hyperglycaemia [2]. Two types of therapies are basically used to treat diabetes i.e., insulin replacement therapy and oral antidiabetic agents [3]. Metformin is a popular medicine used as an oral anti-hyperglycaemic agent which has several mechanisms in preventing hyperglycaemia in patients. It improves insulin sensitivity and enhances glucose uptake in peripheral tissues, aiding in glycaemic control. The modulatory action on gut glucose absorption by metformin may influence the absorption of glucose from the gastrointestinal tract [3,4]. In the present study, anti-hyperglycaemic activity of *Musa paradisiaca* L. plant rhizome was evaluated *in vitro* with comparison to the pharmacological activity of metformin. Purified cells of *Saccharomyces cerevisiae* (brewer's yeast) were utilised to simulate the metabolizing, peripheral body cells *in vitro*. *M. paradisiaca* is known as cooking plantain which is the hybrid form of *M. acuminata* × *M. balbisiana* [5]. Different parts of this plant is widely used in traditional medicine due to its antimicrobial, antidiarrhoeal, antidiabetic, and antioxidant activities [6–8]. It was also found that the plant has apoptotic actions against cancers, especially on colorectal cancers [9]. *M. paradisiaca* belongs to the family *Musaceae* and is commonly distributed in the Caribbean islands, South America, and South-East Asia. The pseudo stem of the plant is rolled around one another to form an aerial stem like structure and the rhizomatous is the real stem which is located underground [10]. In Sri Lanka, different plant parts of *M. paradisiaca* have been used since ancient times for the treatment of several diseases and ailments. The rhizome has been used to prepare an antidiabetic medicinal mixture in Ayurvedic medicine, but the reason for incorporating *M. paradisiaca* rhizome in that formulation has not been adequately investigated [11]. Thus, the present study was aimed to reveal

the anti-hyperglycaemic activity of the plant using two distinct *in vitro* methods and also its antioxidant potential using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay.

Methods

Collection and authentication of plant material

Fresh rhizomes of *M. paradisiaca* were collected from Gampaha District (7.0850N, 79.9961E) in the Western Province of Sri Lanka. Botanical samples were then taxonomically authenticated by Bandaranayake Memorial Ayurvedic Research Institute, Nawinna, Sri Lanka and a voucher specimen (ACC No-2087) was deposited in the herbarium of the same institute.

Extraction of plant materials

Rhizomes were cut into pieces and air-dried under shade for four days. Then they were oven-dried at 40°C for 48 hours and powdered using a mechanical grinder. Dried powdered rhizome (350.0 g) was extracted with n-hexane and dichloromethane subsequently (Plant material: solvent = 1:4) by cold maceration method [12]. In brief, the powdered rhizome was placed in a stoppered container with n-hexane and was allowed to stand at room temperature for a period of 48 hours with frequent agitation. This mixture was filtered and the solvent present in the filtrate was evaporated using a water bath at 40°C to obtain the crude hexane extract of *M. paradisiaca* (HEMP). Air-dried residue was soaked in dichloromethane for maceration and the same process as previous was followed to obtain the dichloromethane extract of *M. paradisiaca* (DEMP).

Effect of Musa paradisiaca plant extracts on glucose uptake by yeast cells

This assay was carried out with slight modifications to a procedure described previously [13,14]. In brief, commercial baker's yeast was suspended in distilled water and the suspension was maintained at room temperature (28°C±2)

overnight. On the following day, the yeast cell suspension was centrifuged (Kubota, Japan) at 4200 rpm for five minutes. These washed cells were used in the preparation of 20% w/v yeast cell suspension. HEMP and DEMP were mixed with dimethyl sulfoxide (DMSO) separately and samples with concentrations of 35.35 mg/mL and 22.14 mg/mL were obtained respectively. Then 1 mL of each mixture was supplemented with 1 mL of glucose solution in various concentrations (10, 15, and 20 mmol/L) and 100 μ L of yeast cell suspension. These mixtures were then incubated for 60 minutes at 37°C. After the incubation, tubes were centrifuged at 3800 rpm for five minutes and the remaining free glucose content was assessed by glucose oxidase method using an UV-visible spectrophotometer (Evolution 201 UV-Vis, USA) at 520 nm. On the same wavelength, absorbance of the standard positive control, metformin (at glucose concentrations of 10, 15 and 20 mmol/L) was also measured. The percentage increase in glucose uptake by yeast cells was determined using the following equation, compared to DMSO negative control in place of test samples.

$$\% \text{ increase in glucose uptake} = \frac{(\text{Absorption of control} - \text{Absorption of sample})}{\text{Absorption of control}} \times 100\%$$

Determination of glucose adsorption/ binding by *Musa paradisiaca* plant extracts

The glucose adsorption ability of each extract was determined using the method explained by Ou et al., [15]. Exact 1 mL volumes of HEMP (35.35 mg/mL) and DEMP (22.14 mg/mL) prepared in DMSO were added separately to 250 μ L of glucose solutions at different concentrations (5, 10, 15, 20, and 30 mmol/L). Each of these mixtures were thoroughly mixed and incubated in a shaker water bath (Therm Flat - KTH30T) at 37°C for six hours. After incubation, these mixtures were centrifuged at 4800 rpm for 20 minutes, and the glucose content in the supernatant was assessed using the glucose oxidase test (Pointe Scientific Inc., USA). The amount of surface-bound glucose

(glucose adsorption) was determined by absorbance data. A negative control (DMSO mixed with glucose solution) was also tested in the same method.

Determination of DPPH radical scavenging ability of *Musa paradisiaca* plant extracts

DPPH free radical scavenging capacities of *M. paradisiaca* rhizome extracts were evaluated according to an established method with minute modifications [14]. A 0.2 mL aliquot of HEMP (15, 20, 25, 30 mmol/L) and DEMP (5, 10, 15, 20 mmol/L) dissolved in DMSO were mixed separately with 3.8 mL of 0.1 mmol/L ethanolic DPPH solution. The mixture was then mixed vigorously for one minute using an electric vortex and was then incubated in darkness at room temperature for 30 minutes. After incubation, absorbance was taken at 517 nm. DMSO was used as the control and ascorbic acid as the standard.

Statistical analysis

Results of each of the test assay was given as mean \pm standard deviation (SD). All the tests were triplicated (n=3) unless specified in each case. Statistical analysis was done using SPSS 22 software. Suitable statistical tests were employed and are indicated where necessary.

Results

Effect on glucose uptake capacity of yeast cells

The percentage yield of *M. paradisiaca* rhizome was 0.19% (w/w) in hexane and 0.09% (w/w) in dichloromethane compared to the crude dry powder of the plant. With a gradual increase in the concentration of plant extract from 3.54×10^{-2} ng/mL to 3.54×10^6 ng/mL, a linear relationship in glucose absorption was observed *in vitro* with *Saccharomyces* cells as shown in Table 1 and 2. The dichloromethane extract had a higher effect on glucose absorption by yeast cells than hexane extract in all three tested glucose concentrations. Moreover, the absorption capacity of both HEMP and DEMP were higher in high glucose

Table 1: The percentage increase in glucose uptake by *Saccharomyces* cells when treated with hexane extract of *Musa paradisiaca* and metformin compared to the control

Concentration (ng/mL)	HEMP			Metformin		
	10 mM glucose	15 mM glucose	20 mM glucose	10 mM glucose	15 mM glucose	20 mM glucose
0.04	2.93 ± 0.22	3.72 ± 1.21	0.57 ± 0.20	35.34 ± 0.22	62.98 ± 1.08	70.69 ± 0.20
0.35	4.94 ± 0.44	6.01 ± 0.13	1.36 ± 0.30	37.04 ± 0.44	64.41 ± 0.13	71.62 ± 0.10
3.54	5.71 ± 0.22	7.63 ± 0.27	4.53 ± 0.10	37.65 ± 0.00	64.89 ± 0.27	71.91 ± 0.10
35.35	6.79 ± 0.44	8.87 ± 0.40	7.54 ± 0.51	37.96 ± 0.44	66.89 ± 0.40	71.91 ± 0.10
353.5	7.56 ± 0.22	8.68 ± 0.13	13.22 ± 0.20	38.73 ± 0.22	68.03 ± 0.13	72.13 ± 0.20
3 535.0	11.11 ± 0.44	12.79 ± 0.27	24.63 ± 0.36	43.83 ± 0.87	68.13 ± 0.00	72.27 ± 0.20
35 350.0	12.04 ± 0.44	13.74 ± 0.54	50.25 ± 0.56	46.60 ± 0.87	68.61 ± 0.40	72.49 ± 0.10
353 500.0	18.67 ± 1.09	14.60 ± 0.40	62.07 ± 0.20	52.31 ± 1.96	69.85 ± 0.27	72.84 ± 0.00
3 535 000.0	22.69 ± 0.22	16.32 ± 0.13	69.21 ± 0.26	63.43 ± 0.65	71.95 ± 0.27	73.92 ± 0.30

HEMP: Hexane extract of *Musa paradisiaca*; values given as mean ± SD

concentrations compared to lower glucose concentrations. Both extracts showed significantly lower activity compared to metformin ($p < 0.05$). However, in higher glucose concentrations, more similar activities were observed between higher concentrations of the plant extract and metformin.

Glucose adsorption by the plant extracts

The effect of HEMP and DEMP on *in vitro* glucose adsorption is shown in Figure 1. Results of the

present study indicated a significant ($p < 0.05$) glucose adsorption capacity in DEMP when compared to HEMP. Adsorption of glucose by DEMP was proportional to the glucose concentration (which were $> 80\%$ for all glucose concentrations) as the minimum adsorption was recorded at 5 mmol/L glucose concentration and the maximum was at 30 mmol/L. However, HEMP showed variable glucose adsorption with varying glucose concentrations. The standard drug

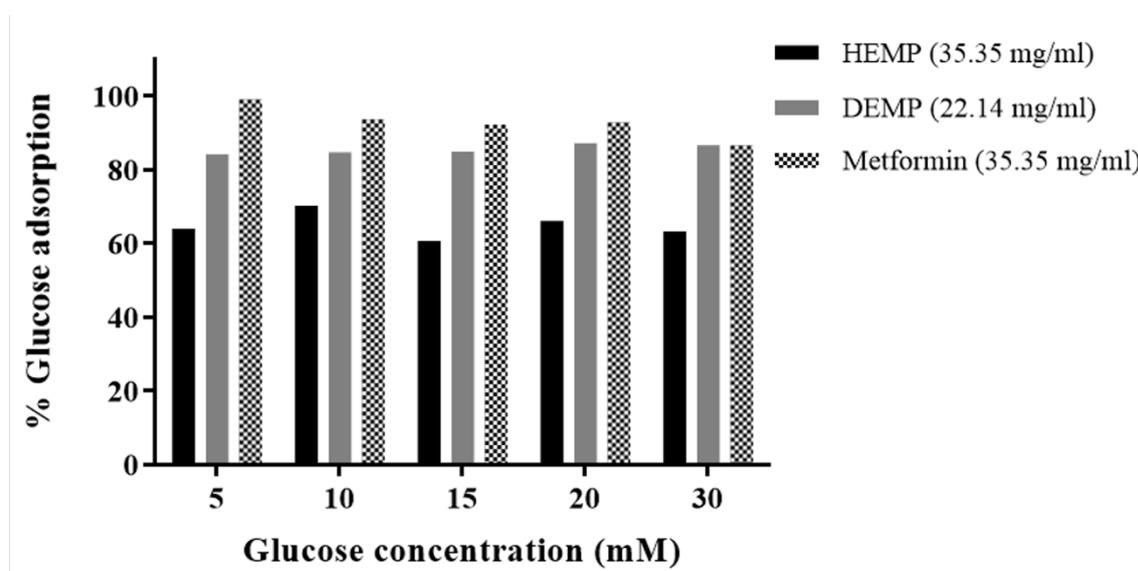


Figure 1: *In vitro* glucose adsorption capacity of hexane- and dichloromethane- extracts of *Musa paradisiaca*, and metformin

metformin (35.35 mg/ml) showed a significant ($p < 0.05$) glucose binding which was actually inversely proportional to the glucose concentration, which is also shown in Figure 1.

DPPH radical scavenging activity

As shown in Figure 2, free radical scavenging activities of HEMP and DEMP were significantly lower ($p < 0.05$) when compared with ascorbic acid standard. At 30 mg/mL glucose concentration, HEMP showed the maximum free radical scavenging activity. DPPH radical scavenging potentials of DEMP was higher than those of HEMP in 15 mg/mL and 20 mg/mL concentrations. In all the tests, ascorbic acid standard showed the highest DPPH scavenging with $>40\%$ activity.

Discussion

The peripheral glucose absorption process is essential for lowering of post-prandial blood glucose levels in blood. In type 2 diabetes mellitus, this mechanism may get impaired [16]. Metformin is a widely used medicine in this context, which improves insulin sensitivity in peripheral tissues and modulates gut glucose absorption which will lead to delayed absorption of glucose and then a

slower rise in blood glucose levels after meals [17]. As an attempt to reveal the unexplored medicinal properties in common edible plants with nutraceutical values, this study was executed to investigate the antidiabetic potential of *Musa paradisiaca* L. plant, the “cooking plantain”. There are a number of reasons for choosing these type of herbal materials to treat diseases [18]. Numerous studies have scientifically proven that some edible plant materials can be used as antidiabetic agents to reduce elevated blood glucose levels, though their activity is not comparable with synthetic single-molecule medicines [19–21]. Many of these studies have employed different *in vivo* methods to determine anti-hyperglycaemic effects of a wide range of test substances, where alpha-amylase inhibition and alpha-glucosidase inhibition assays are apparently the popular *in vitro* models [22,23]. Besides those common methods, assay of glucose uptake by *Saccharomyces* yeast cells is another underutilised method that can be used in this approach, which simulates animal peripheral body cells by yeast cells. The intestinal glucose adsorption/ binding ability of plant fibres and constituents has also been studied in some investigations, though there

Table 2: The percentage increase in glucose uptake by *Saccharomyces* cells when treated with dichloromethane extract of *Musa paradisiaca* and metformin compared to the control

Concentration (ng/mL)	DEMP			Metformin		
	10 mM glucose	15 mM glucose	20 mM glucose	10 mM glucose	15 mM glucose	20 mM glucose
0.02	17.75 ± 0.22	54.66 ± 0.13	67.22 ± 0.10	44.29 ± 0.22	64.35 ± 0.13	65.71 ± 0.00
0.22	22.69 ± 0.22	55.23 ± 0.13	67.29 ± 0.00	46.76 ± 0.65	65.49 ± 0.13	67.43 ± 0.20
2.21	24.54 ± 0.22	55.80 ± 0.00	67.93 ± 0.10	46.91 ± 0.00	66.16 ± 0.00	68.65 ± 0.10
22.14	25.00 ± 0.44	55.32 ± 0.27	68.36 ± 0.10	47.38 ± 0.22	67.02 ± 0.13	69.51 ± 0.10
221.4	25.31 ± 0.00	56.08 ± 0.27	68.29 ± 0.00	54.78 ± 0.22	67.40 ± 0.13	70.52 ± 0.10
2 214.0	27.16 ± 0.44	56.75 ± 0.13	69.33 ± 0.15	56.17 ± 0.00	68.25 ± 0.22	70.09 ± 0.10
22 140.0	31.33 ± 0.22	57.32 ± 0.13	69.66 ± 0.10	56.64 ± 0.22	69.01 ± 0.22	70.73 ± 0.00
221 400.0	32.72 ± 0.44	58.46 ± 0.13	70.01 ± 0.00	59.41 ± 0.65	69.49 ± 0.13	70.80 ± 0.10
2 214 000.0	50.31 ± 0.44	61.98 ± 0.27	73.96 ± 0.10	61.57 ± 0.22	69.77 ± 0.22	71.02 ± 0.20

DEMP: Dichloromethane extract of *Musa paradisiaca*; values given as mean ± SD

is no specific allopathic medicine discovered so far with similar action on glucose absorption [3]. According to a study carried out in 2020, antihyperglycaemic effects of hydroethanolic extracts of *M. paradisiaca* leaf and fruit peel were demonstrated in rats whose diabetes was induced by nicotinamide-streptozotocin reagent [24]. The treatment with leaf and fruit peel extracts significantly improved the oral glucose tolerance in diabetic rats and significantly increased the serum C-peptide levels ($p < 0.05$) as well. Same investigators have further correlated the antihyperglycaemic activity of plant extract to various phytochemicals identified in a GC-MS analysis such as octadecatrienoic acid, hexadecanoic acid, β -sitosterol, and stigmasterol. In another study, anti-hyperglycaemic and anti-dyslipidaemic activities of *M. paradisiaca* flowers and bracts were also evidenced in diabetic rats with significant ($p < 0.05$) improvements in rat biochemical markers such as fasting blood glucose, hexokinase, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) in comparison to a control animal group [25]. However, the present study aspires to *in vitro* anti-hyperglycaemic activity of *M. paradisiaca* rhizome with reference to the standard medicine metformin. Both hexane and dichloromethane extracts of the plant exhibited significant ($p < 0.05$) activities in glucose

uptake assay compared to the negative control. However, the activity was found to be significantly less compared to metformin. The dichloromethane extract demonstrated a higher glucose absorption than hexane extract in all the tested glucose concentrations. This may be linked to the variable activity of phyto-substances that would be extracted into each of the organic solvents [26]. Dichloromethane is a more polar solvent compared to hexane [27], thus suggesting that more polar phyto-compounds included in dichloromethane extract considerably affected the glucose absorption through *Saccharomyces* cell membrane. It is possible that increased glucose uptake by yeast in the presence of HEMP and DEMP is attributable to stimulation of facilitated diffusion across yeast cell membrane [14].

The influence of solvent polarity can be further postulated from the results of glucose adsorption assay, as the hexane extract in this assay has again shown significantly ($p < 0.05$) lesser activity compared to the dichloromethane extract. This might be a result of impaired extraction of fibrous matter from plant rhizome to hexane due to its non-polar nature. Fibrous matters in plants are primarily larger polysaccharides (such as cellulose and hemicellulose) which have the ability to bind glucose in meal and also to increase the viscosity

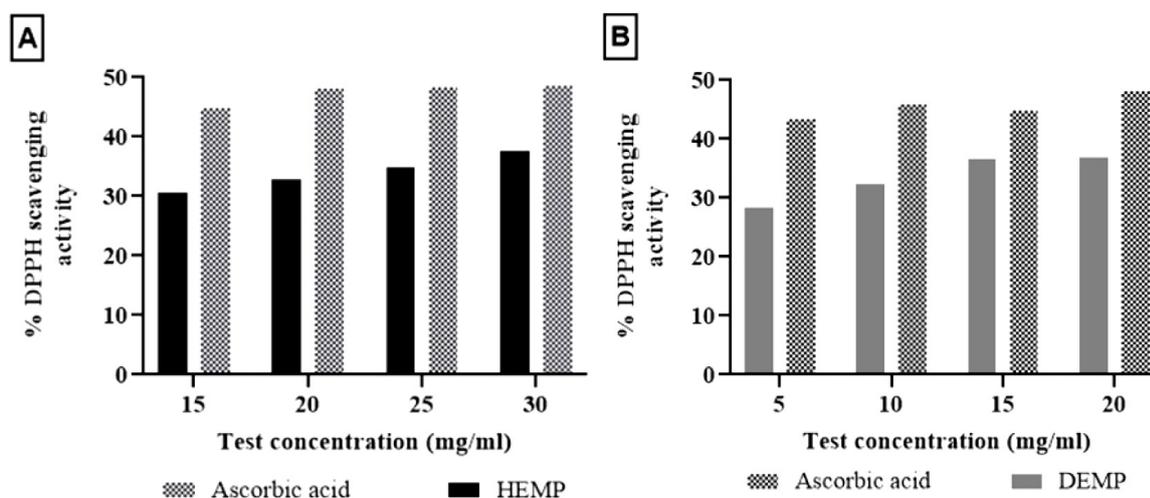


Figure 2 : *In vitro* antioxidant activity of A) hexane- and B) dichloromethane- extracts of *Musa paradisiaca* in DPPH scavenging assay

of intestinal juice [15]. They are generally insoluble in non-polar solvents like hexane since these components have strong polar groups in their structure [28]. It is suggested to use a more suitable standard drug in future studies on glucose adsorption, since metformin is known to reduce glucose absorption in intestine by inhibiting sodium-glucose co-transporters, which is a different pathway for the reduction in glucose absorption exhibited by the test plant extracts. Moreover, glucose adsorption ability of metformin observed in this study requires further investigations to establish a molecular mechanism.

Several studies have been done on the evaluation of *in vitro* antioxidant activity of different parts of *M. paradisiaca*, but not on the rhizome of the plant. In an intensive investigation conducted by Mahmood and co-workers on *M. paradisiaca* flowers [29], extractions with different solvents i.e., petroleum ether, ethanol, and water have been examined and the existence of phytochemicals such as alkaloids, saponins, glycosides, and tannins were confirmed. The ability of some phytochemicals (in HEMP and DEMP) with acidic functional groups can donate H⁺ ions to scavenge DPPH radicals *in vitro* and is thought to be responsible for the antioxidant properties discovered in the present study.

Another study has previously determined the anti-glucosidase and α -amylase inhibitory activities of Sri Lankan *M. paradisiaca* variety [30] emphasising anti-diabetic potential of the plant. However, the present study offers two other distinct mechanisms to establish the anti-hyperglycaemic activity of the plant interested. Furthermore, present investigators suggest that more elaborated *in vivo* studies could reveal the exact mechanisms of *M. paradisiaca* plant on different animal tissues.

Conclusion

The study discovered significant *in vitro* anti-

hyperglycaemic effects and DPPH free radical scavenging activity in dichloromethane and hexane extracts of *Musa paradisiaca* rhizome. Dichloromethane extract was the most active test sample which had considerably higher activities compared to the hexane extract. However, both HEMP and DEMP extracts showed significantly lower activities compared to metformin ($p < 0.05$) in glucose uptake assay, while the ascorbic acid standard showed the highest DPPH free radical scavenging activity in the antioxidant assay.

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