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Evaluation of Invertase Inhibition Activity and Cytotoxicity of Ethanol and Acetone Extracts of *Swietenia macrophyllia* Leaves, *Syzygium cumini* and *Trigonella foenum-graecum* Seeds

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Abstract

Invertase, the key enzyme responsible for sucrose hydrolysis. Inhibition of invertase can decrease the postprandial blood sugar level in diabetic patients and keep the blood glucose level normal where cytotoxicity to fast-growing cells like those of brine shrimp (*Artemia salina*) nauplii is a great measurement for further important drug development. This study was aimed to investigate potential anti-diabetic and cytotoxic activities of the ethanol and acetone extracts of *Swietenia macrophylla* leaves, *Syzygium cumini* and *Trigonella foenum-graecum* seeds. Invertase inhibition activities of *S. macrophylla* leaves, *S. cumini,* and *T. foenum-graecum* seeds were measured by spectrophotometrically using standard protocols and cytotoxicity were measured by brine shrimp lethality bioassay. Among the plant extracts, all ethanol extracts showed higher invertase inhibition activities than all acetone extracts. *S. cumini* seed ethanol extract showed the highest invertase inhibition activity whereas *S. macrophylla* leaves acetone extract showed the lowest invertase inhibition activity. The maximum toxicity was observed in ethanol extract of *T. foenum-graecum* seed whereas the lowest toxicity was observed in acetone extract of *S. macrophylla* leaves. Both ethanol and acetone extract of *T. foenum-graecum*seeds showed significant cytotoxic activities. This investigation suggested that *S. cumini* and *T. foenum-graecum* seeds possess potential antidiabetic activities and *T. foenum-graecum* seeds have potential cytotoxicity.

Keywords: Plant extracts; Antidiabetic activity; Invertase inhibition activity; Cytotoxicity

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Introduction

Diabetes mellitus is a chronic metabolic disorder in which the blood sugar level remains abnormally high. It occurs due to insufficient insulin secretion (type 1 diabetes) normally happens to younger people (≤30 years), or resistance of receptors against insulin (type 2 diabetes) affecting normally elderly people [1]. Inhibition of carbohydrate digestive enzymes can limit the increase of postprandial blood glucose levels in diabetic patients [2]. There are many synthetic drugs like Acarbose, Metformin, Glibenclamide, Miglitol, and Voglibose that are currently used for controlling postprandial hyperglycemia. Miglitol and Voglibose limits the activity of only α-glucosidase, where Acarbose limits both α-amylase and α-glucosidase, but they have some gastrointestinal side effects and costly [3,4]. In general, plants have lower toxic and minimum/null adverse effects [5]. Production of drugs from plants is less expensive than synthetic drug production which is very important as about 80 % of the diabetic patients are living in low and middle-income countries [6].

Since time immemorial, many plant parts and their extracts have been used to treat different diseases for their properties such as antibacterial, antifungal, antioxidant, antimalarial, antiviral, antidiabetic, cytotoxic, amylase, lipase, and invertase inhibitory activities [7,8]. Plant contains phytochemicals or secondary metabolites such as alkaloids, flavonoids, phenolic acids, tannins, quinines, cardiac glycosides, saponins, sterols, and terpenoids may be responsible for the above-mentioned properties [5]. Therefore, several groups have made their efforts to find α-amylase and α- glucosidase inhibitors from plants, bacteria, marine algae, and fungi [9,10,11,12]. The majority of them have studied the crude extracts (organic or aqueous), and some also have studied pure compounds [13,14]. Invertase is a digestive enzyme that helps to breaks down sucrose into glucose and fructose. The inhibition of this enzyme can decrease the postprandial increase in blood sugar levels. Even though many scientific studies have been done on α-amylase inhibitory and antidiabetic activities of many plant extracts, invertase inhibition activity was not studied so much.

Cytotoxicity is the quality of certain compounds to destroy living cells. It can stop the cell's growth and division, thus decrease the cell's viability [15]. Cytotoxicity to rapidly growing *Artemia salina* (Brine shrimp) nauplii can be evaluated by brine shrimp bio-assay as proposed previously [16,17,18]. The assay is a rapid, reliable, and inexpensive process for primary evaluation of cytotoxicity and considered useful to detect fungal toxins, plant extract toxicity, heavy metals, pesticides, and cytotoxicity testing of dental materials [19,20,21,22]. In chemotherapy different cytotoxic agents are used to kill or damage cells which are reproducing rapidly to destroy the rapidly growing cancer cells. So, evaluation of cytotoxicity to rapidly growing brine shrimp nauplii may

suggest new sources of anticancer drugs. The relationship between the brine shrimp bioassay and growth inhibition of human *in vitro* tumor cell lines were roved by the National Cancer Institute (NCI, USA) is valuable because it exhibits the importance of lethality bioassay as a pre-screening tool for anti-cancer drug research [7].

In this study, three different plants viz. *Swietenia macrophylla* King (Mahagony), *Syzygium cumini* (L.) Skeels (Jamun), and *Trigonella foenum-graecum* L. (Fenugreek) were selected to evaluate their invertase inhibitory and cytotoxic activities. *S. macrophylla* is grown as an ornamental tree in tropical regions of the world and is the first choice for making high-quality furniture. Fruits of *S. cumini* are eaten in many Asia countries. Seeds of *T. foenum-graecum* are widely used in the preparation of food in South Asian countries. These plants are also of many medicinal importance as they show antioxidant, antibacterial, antifungal, antidiabetic, α-amylase, α-glucosidase, trypsin inhibitory activities so far documented. Most of the antidiabetic studies were performed on diabetic rats or based on α-amylase and α-glucosidase inhibition activities [2,23,24,25]. Some cytotoxic evaluation was also done on some kind of cancer cell line on these plants [26,27,28]. Here, the antidiabetic and cytotoxic effect of ethanol acetone extracts of leaves of *S. macrophylla*, seeds of *S. cumini,* and *T. foenum-graecum* was evaluated through invertase inhibition activity test and brine shrimp lethality bioassay respectively.

Methods

Collection of samples

Fresh leaves of *S. macrophylla* trees from Shahjalal University of Science and Technology (SUST) campus were collected. After collection, the leaves were washed in distilled water for 2 to 3 times and stored at room temperature for 2 weeks to dry. Fruits of *S. cumini* was purchased from the local market of Sylhet, Bangladesh. Fleshy part of the fruits was removed and seeds were washed in distilled water for 2 to 3 times and stored at room temperature for 2 weeks to dry. Seeds of *T. foenum-graecum* was purchased directly from the local market of Sylhet, Bangladesh, and stored at room temperature for 2 weeks to dry. These dry leaves and seeds were ground into a fine powder using an electric homogenizer and stored in plastic vials at 4°C for further utilization.

Chemicals

Invertase was purchased from Sisco Research Laboratories Pvt. Ltd., Maharashtra, India. All other chemicals, reagents, and solvents were of analytical grade and obtained locally.

Preparation of ethanol and acetone extracts

The extraction process was done by the modified shake-flask method [29]. 15gm of each powdered material was weighed and taken into clean, dry, and sterilized in 250 mL conical flasks separately. 150 mL of ethanol/acetone was then added to the conical flask at a 1:10 (gm/mL) ratio. Flask was swirled

a few times to mix the powder and kept in an orbital shaker at a speed of 120 rpm for 48 hours at 40°C. After that the extract was filtered with What-man No. 1 filter paper for at least two times. The filtrate was collected, poured into Petri-dishes, and then kept onto a dryer at 38°C. After complete drying, the dry mass was taken off, weighed onto an aluminum foil paper. The extract was collected in an Eppendorf tube. Finally, the dried extract was suspended in 50 mM sodium acetate buffer, pH 4.8, and the extracts were stored at 4°C for further use.

In vitro Invertase Inhibition Assay

Invertase inhibition activities of the plant extracts were measured according to the method as described previously with some modifications [30]. Briefly- different concentrations of extracts were added separately in test tubes containing 1 mL of 1% sucrose solution (w/v, prepared in 50 mM sodium acetate buffer, pH 4.8). The volume of the solutions was adjusted to 3 mL by 50 mM sodium acetate buffer, pH 4.8. 2 µL (1 unit/µL) invertase enzyme was added carefully to the solution. Then the tubes were transferred into a water bath to incubate the solution at 37°C for 10 minutes. After incubation, 2 mL of 3,5-DNS solution was added to each test tube. Then the test tubes were again placed onto water-bath at 90°C for 7-8 minutes. A 'control' reaction was also prepared by using 50 mM sodium acetate buffer, pH 4.8 instead of plant extract, and a 'blank' was prepared using in 50 mM sodium acetate buffer, pH 4.8 only. The optical density of the test, control, and the blank solutions were measured in a spectrophotometer at a wave length of 540 nm [31]. One unit of invertase activity was defined as the hydrolysis of 1 µM of sucrose per minute under the assay conditions. The invertase inhibition activity (%) was calculated using the formula: [(Enzyme Activity of Control – Enzyme Activity of Test) / Enzyme Activity of Control] × 100 [32].

IC50 (50% inhibition concentration) values for each extract were calculated using the regression analysis model method from the following equation: Y = MX+C, where Y = 50 (medium inhibition), X = Concentration of the extracts, M and C both are constant.

Measurement of cytotoxicity using brine shrimp lethality bioassay

Brine shrimp lethality bioassay, described by Meyer et al. with some modifications was used to determine the cytotoxicity of *S. macrophylla* leaves, *S. cumini* seeds, and *T. foenum-graecum* seeds [15,32]. The fresh and viable eggs of brine shrimp (*Artemia salina*) were collected from the local market and stored at room temperature. The eggs of brine shrimp were hatched in a vessel with constant oxygen supply at 25°C. The nauplii, which were hatched for about 48 hours, were at the first stage of development after leaving the egg. The sample extract solution was prepared by dissolving the required amount of extracts in a specific volume of pure dimethyl sulfoxide (DMSO) and seawater. The nauplii were taken in separate test tubes con-

taining 5 mL DMSO and seawater mix. Then required volume of samples was added into the test tubes to get a final concentration of 5 µg/mL, 10 µg/mL, 20 µg/mL, 40 µg/mL and 80 µg/ml. After incubating for 24 hours at 37°C the test tubes were observed and the number of surviving nauplii in each test tube was counted. The percentage of mortality of the brine shrimp nauplii was calculated for each concentration of extract by using the following formula: Mortality (%) = (Nt/N0) ×100; Where, Nt = Number of dead nauplii after 24 hours of incubation, N0 = Number of total nauplii transferred. Then using the percentage of mortality rate, the LC50 (median lethal concentration) of the extract was calculated.

LC50 (50% lethality concentration) values for each extract were calculated using the regression analysis model method from the following equation: Y = MX+C, where Y = 50 (medium inhibition), X = Concentration of the extracts, M and C both are constant.

Statistical Analysis

For each concentration, experiments were done three times. Data were collected and saved in a Microsoft Office Excel file. Finally, the results were expressed as mean ± standard deviation (SD).

Results

Inhibition assay of invertase activity

In this investigation, ethanol extract of *S. cumini* seeds showed the highest invertase inhibition activity (65.20%) at the concentration of 1.25 µg/mL while the lowest invertase inhibition activity (20.16%) at the concentration of 0.25 µg/mL with an IC50 value of 1.10 ± 0.48 µg/ml. The ethanol extract of *T. foenum-graecum* seeds performed the maximum invertase inhibition activity (60.62%) at the concentration of 1.25 µg/mL and the least inhibitory potential (25.26%) at 0.25 µg/mL concentration of extracts with an IC50 value of 1.20 ± 0.12 µg/ml. Moderate invertase inhibition activity was observed in *S. macrophylla* leaves ethanol extracts with the highest invertase inhibiting activity of 43.47% and the lowest inhibiting activity of 32.92% at the same highest and lowest concentration of the extracts respectively with an IC50 value of 2.04 ± 0.17 µg/ml. So, in between all the ethanol extracts, *S. cumini* seeds showed the highest invertase inhibition activity and *T. foenum-graecum* seeds showed the least invertase inhibition activity (Figure 1 and Table 1).

Among the acetone extracts of three plants, the highest invertase activity (48.24%) was found in *T. foenum-graecum* seeds at the concentration of 1.25 µg/mL with an IC50 value of 1.40 ± 0.22 µg/mL whereas the lowest invertase activity (13.32%) was observed in *S. cumini* seeds at the concentration of 0.25 µg/mL with an IC50 value of 5.80 ± 0.94 µg/mL (Figure 2 and Table 1). The other acetone extracts showed invertase inhibitory potentials in between these two values (13.32% and 48.24%) at different concentrations (Figure 2).

In between all the extracts, ethanol extract of *S. cumini* seeds performed the maximum invertase inhibition activity (65.20%) with the least IC50 value (1.10 ± 0.48 µg/mL) while the lowest invertase activity (13.32%) with the highest IC50 value (5.80 ± 0.94 µg/mL) was found in acetone extract of the same plant materials (Figure 1 and Figure 2). Invertase inhibition activity of the organic solvents extract of the other plants at different concentrations were found in between 13.32%-65.20% (Figure 1 and Figure 2).

Among these three plant extracts, ethanol extract of *S. cumini,* acetone extract of *S. macrophylla,* and both ethanol and acetone extract of *T. foenum-graecum* were showed to have significant anti-diabetic activities. The study also showed that all ethanol extracts possessed comparatively higher antidiabetic activities than all acetone extracts. So, ethanol is a better solvent than acetone considering antidiabetic activities.

Table 1. IC50 values of the organic solvent extracts of *S. macrophylla* leaves, *S. cumini* and *T. foenum-graecum* seeds

Organic Solvent	Plant parts	(IC50 (µg/mL
	S. macrophylla leaves	2.04 ± 0.17
Ethanol	*S. cumini* seeds	1.10 ± 0.48
	T. foenum-graecum seeds	1.20 ± 0.12
	S. macrophylla leaves	1.79 ± 0.33
Acetone	*S. cumini* seeds	5.80 ± 0.94
	T. foenum-graecum seeds	1.4 ± 0.22

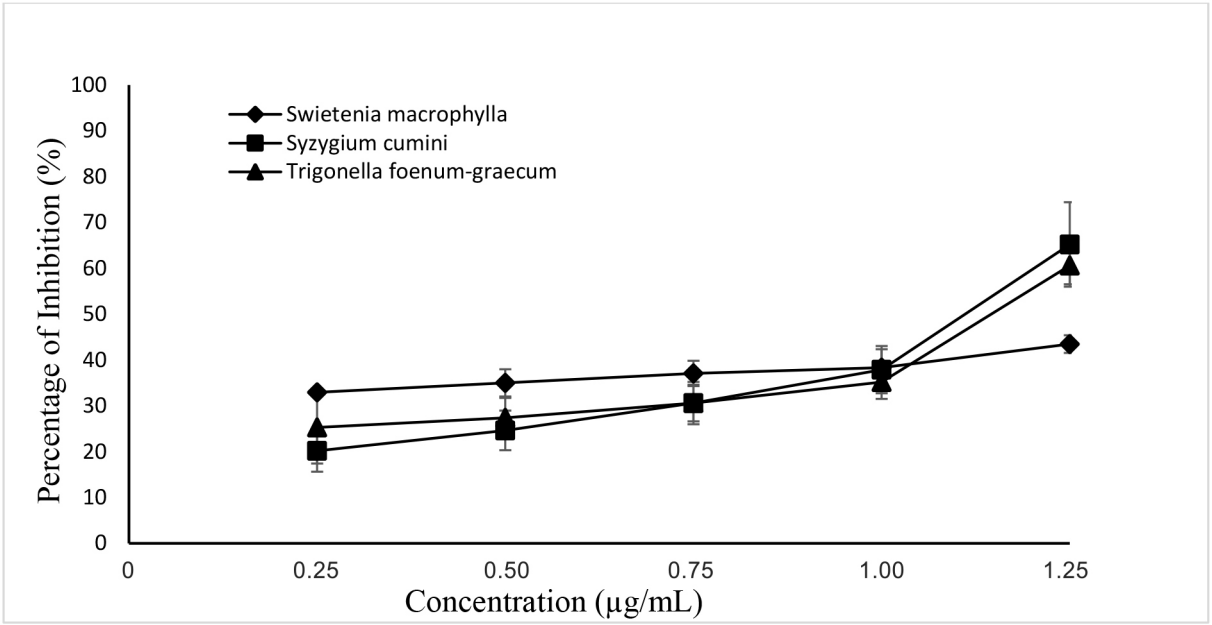

Figure 1. Invertase inhibition activity of *S. macrophylla* leaves, *S. cumini* and *T. foenum-graecum* seeds ethanol extracts

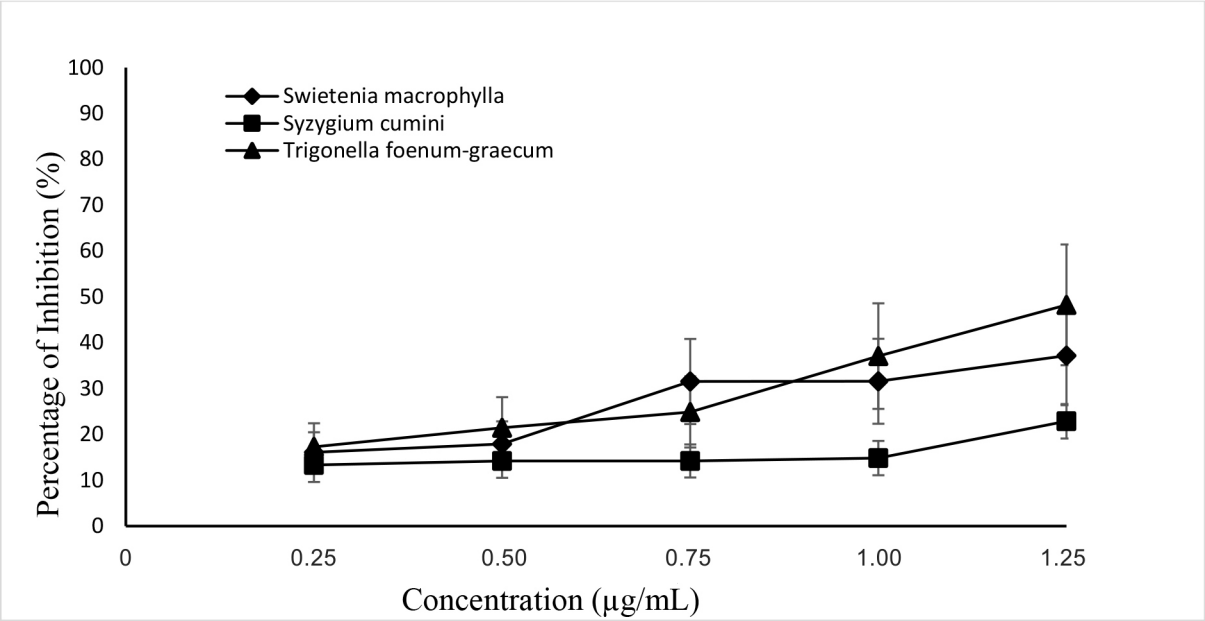

Figure 2. Invertase inhibition activity of *S. macrophylla* leaves, *S. cumini* and *T. foenum-graecum* seeds acetone extracts

Cytotoxicity bio-assay using brine shrimp nauplii

Among the ethanol extracts, *T. foenum-graecum* seeds performed the highest cytotoxicity (100%) at the concentration of 80 µg/mL while the lowest cytotoxicity (3.33%) was observed in *S. macrophylla* leaves at the concentration of 5 µg/mL (Figure 3 and Table 2). Other ethanol extracts of the plant at different concentrations resided in between these two values (Figure 3 and Table 2). At all concentration of ethanol extracts, *T. foenum-graecum* seeds showed the highest cytotoxicity with a LC50 value of 30.42 ± 4.43 µg/mL, whereas *S. cumini* seeds ethanol extract gave almost the mildest cytotoxicity with a LC50 value of 61.50 ± 7.17 µg/mL and *S. macrophylla* leaves exhibited the least cytotoxicity with a LC50 value of 66.43 ± 9.96 µg/mL (Figure 3 and Table 2).

Table 2. LC50 values of the organic solvent extracts of *S. macrophylla* leaves, *S. cumini* and *T. foenum-graecum* seeds.

Organic Solvent	Plant parts	LC50 (µg/mL)
Ethanol	*S. macrophylla* leaves	66.43 ± 9.96
	S. cumini seeds	61.50 ± 7.17
	T. foenum-graecum seeds	30.42 ± 4.43
Acetone	*S. macrophylla* leaves	79.13 ± 8.99
	S. cumini seeds	75.26 ± 7.61
	T. foenum-graecum seeds	35.30 ± 5.11

Among the acetone extracts, the highest cytotoxicity was found in *T. foenum-graecum* seeds extracts with a LC50 value of 35.30 ± 5.11 µg/mL whereas *S. macrophylla* and *S.*

cumini seeds extracts showed almost the similar cytotoxicity with LC50 values of 79.13 ± 8.99 µg/mL and 75.26 ± 7.61 µg/mL respectively, at all the concentrations (Figure 4 and Table 2). The maximum apparent cytotoxicity (80%) was observed in acetone extract of *T. foenum-graecum* seeds at a concentration of 80 µg/mL while *S. macrophylla* and *S. cumini* seeds extracts showed the least cytotoxicity (6.66%) at a concentration of 5 µg/mL (Figure 4).

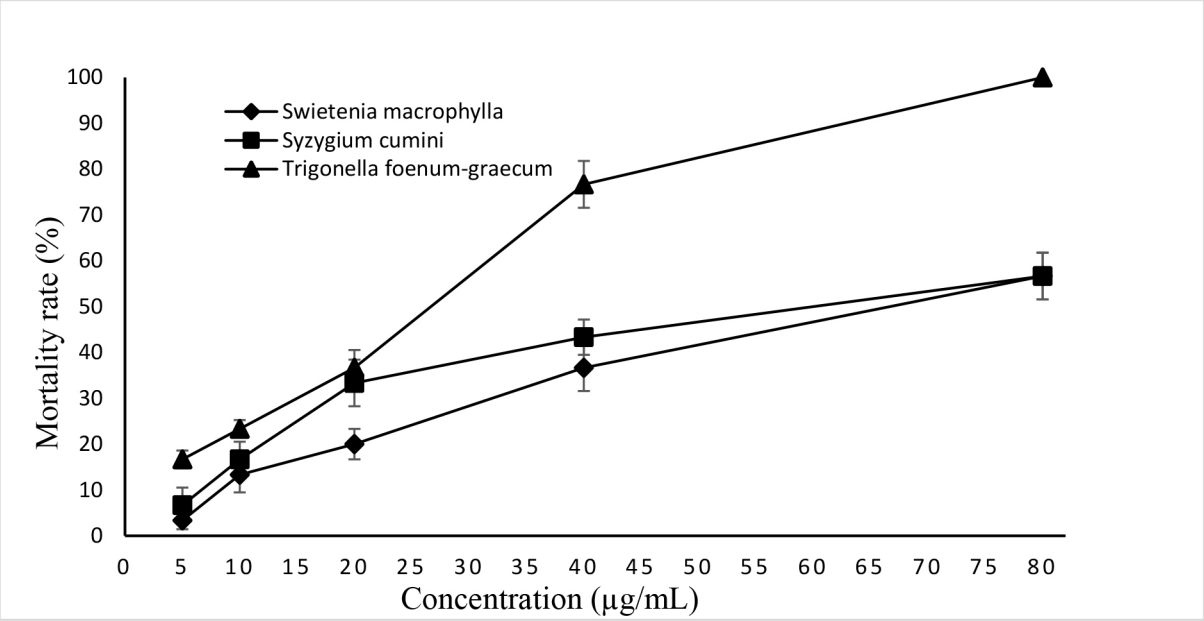

Figure 3. The mortality rate of *S. macrophylla* leaves, *S. cumini* and *T. foenum-graecum* seeds ethanol extracts

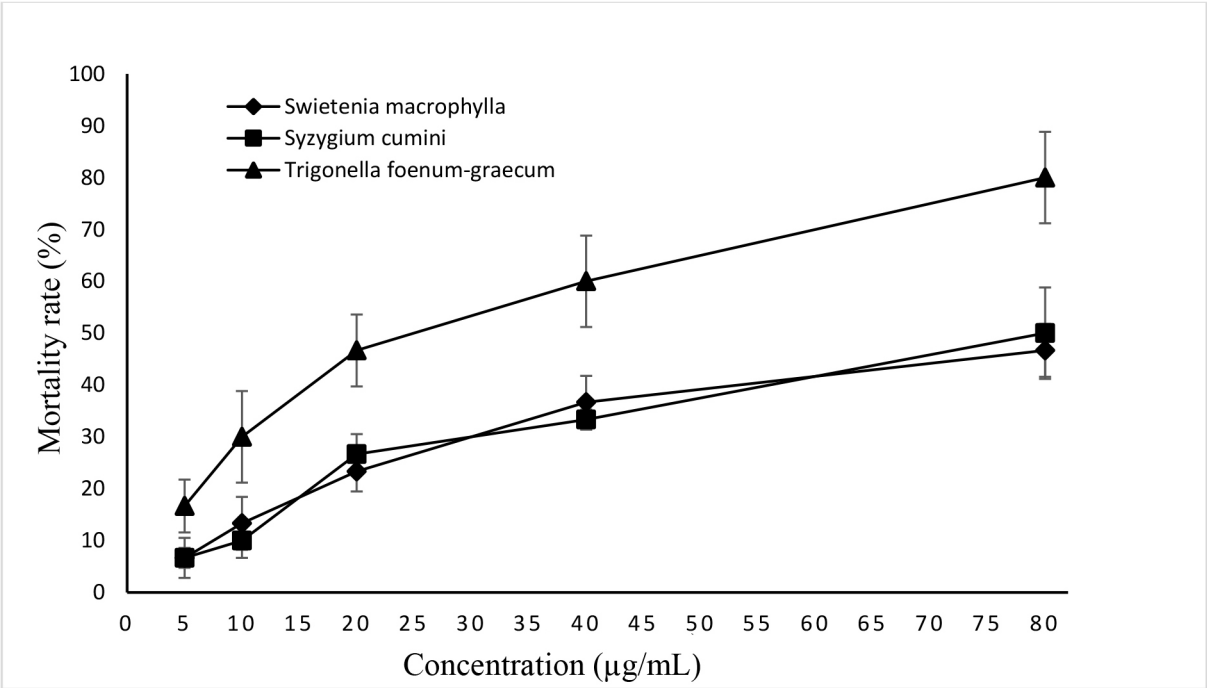

Figure 4. The mortality rate of *S. macrophylla* leaves, *S. cumini* and *T. foenum-graecum* seeds acetone extracts.

Discussion

Diabetes is a chronic metabolic disorder relating to carbohydrate metabolism which can be effectively controlled by using the glycosidic inhibitors that inhibit enzymes such as α-amylase, invertase, trehalase, maltase, and isomaltase [33]. Plants having rare deleterious effects, have many therapeutics produced by it, so they have been used to treat many diseases since time immemorial. Traditionally many plant extracts are being used to reduce blood glucose level because of their potential antidiabetic activities. More than 400 plants around the world have been recorded as helpful to control diabetics [34,35]. Many plants and herbs were reported as having antidiabetic behavior when taken by mouth [36]. In this study, antidiabetic activities of *S. macrophylla* leaves, *S. cumini* seeds, and *T. foenum-graecum* seeds were evaluated and proved through invertase inhibitory activity of their ethanol and acetone extracts, where different extracts of *S. macrophylla* leaves, *S. cumini* seeds, and *T. foenum-graecum* seeds showed potential invertase inhibitory activities. Solvent is also important as shown in this study that ethanol extract has higher invertase inhibitory activities. In earlier studies, *S. macrophylla* showed significant antidiabetic activity when evaluated in diabetic rats [22]. *In vivo* studies of both *T. foenum-graecum* and *S. cumini* were also proved to have significant antidiabetic activities [23,25].

Among the ethanol and acetone extracts, the highest and lowest cytotoxicity were observed in ethanol extracts. *T. foenum-graecum* seeds ethanol extract performed the highest cytotoxicity whereas *S. macrophylla* leaves ethanol extract showed the least cytotoxicity (Figure 3).

Brine shrimp lethality bioassay is a rapid, reliable, and inexpensive method as proved previously [15]. Cytotoxicity of different plant extracts can be evaluated easily using this method. The criteria for brine shrimp toxicity of plant extract were established as- a) LC50 value above 1000 µg/mL are considered non-toxic, b) LC50 value having the range between 500 and 1000 µg/mL are considered as weak toxic and c) value below 500 µg/mL are toxic [37]. In this study, LC50 values for all extracts were found below 500 µg/mL. So, all *S. macrophylla* leaves, *S. cumini* seeds, and *T. foenum-graecum* seeds have strong cytotoxicity, where *T. foenum-graecum* showed comparatively higher cytotoxicity.

Conclusion

The above study suggested that *S. cumini* and *T. foenum-graecum* possessed potential invertase inhibitory activities and *T. foenum-graecum* has a strong cytotoxic effect. So, it can be concluded that *S. cumini* and *T. foenum-graecum* would be used as a good alternate against synthetic drugs to manage diabetes mellitus through invertase inhibition for which further study is needed to specify the component responsible for these activities. *T. foenum-graecum* could be a potential source of natural products that could contribute to developing an anticancer agent as

it kills rapidly dividing cells of brine shrimp nauplii. More research should be carried out to investigate the efficacy of the above-mentioned plant parts for controlling diabetes and developing drugs.

Specific author contributions

MJA conceived the study. MJA and AD designed the study protocol. AD conducted the research work and drafted the manuscript. MJA, MFM, MRK and MS contributed to revise the manuscript. All authors approved the final manuscript.

Conflicts of Interest

The authors disclose no conflicts of interest.

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