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In-vitro Study of *Trachyspermum ammi* extract against Diabetes Mellitus

Godavari Amar[*] and Amutha Kuppusamy

Department of Biotechnology, Vels Institute of Science, Technology and Advanced Studies, Tamil Nadu, India

([*]Corresponding author's e-mail: godagene@gmail.com)

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Abstract

Diabetes resulting from the low secretion of insulin and insulin resistance, is a chronic metabolic disorder. Medication from plant materials for treatment of Diabetes Mellitus is advised as good source for novel drug or leading to preparation of novel drug. We investigated the antidiabetic property of fractions and subfractions obtained from the ethyl acetate extract of *Trachyspermum ammi* seeds. TLC analysis of crude extract was done. Then column chromatography was used to isolate the fractions of *T. ammi*. Primary screening of fractions was done by Starch-Agar Gel Diffusion assay for identification of Alpha-amylase inhibitors. Then after chromatographic separation of subfractions, their secondary screening was carried out by Glucose-Uptake assay in Yeast cells. Finally the *in vitro* Anti-diabetic activity of crude and screened subfraction of ethyl acetate extract of *T. ammi* was evaluated in 3T3-L1 cell lines. The results presented the antidiabetic activity of *T. ammi* and their fractions along with subfractions. Further *in vitro* cell line assay also proved the antidiabetic activity of *T. ammi*. It is suggested that the *T. ammi* had antidiabetic activity by inhibiting the α-amylase enzyme, also by facilitating the glucose utilization in adipose tissues and skeletal muscles.

Keywords: Antidiabetic screening, Starch plate assay, Yeast assay, *In vitro* antidiabetic assays, Glucose uptake assay

Introduction

Diabetes Mellitus (DM) is an incurable illness triggered due to defective insulin secretion from pancreas and insulin resistance along with alterations of lipid and protein metabolism which leads to hyperglycemia [1]. Insulin deficiency causes increase in level of glucose in blood leading to damages in body systems, particularly, the blood vessels and nerves. Around 2 - 3 % of the population worldwide has been affected by Diabetes and becomes third 'Killer' of mankind health along with cancer, cardiovascular and cerebrovascular diseases [2]. Prevalence rate of Diabetes is expected to reach up to 4.4 % in 2030 [3]; and highly occurs in India, China and U.S.A [4].

Therapeutic method for treatment of Diabetes is to bring down the post-prandial glucose levels. The α-amylase (α-1, 4-glucan-4-glucanohydrolases; E.C. 3.2.1.1) majorly secreted by pancreas [5] and salivary glands catalyzes the initial breakdown of starch into smaller oligosaccharides through the cleavage of α-D-(1 - 4) glycosidic bonds [6-9]. Delaying glucose absorption through inhibition of carbohydrates-hydrolyzing enzymes, α-glucosidase and α-amylase, present in small intestinal brush border that are responsible for breakdown of oligosaccharides and disaccharides into monosaccharides suitable for absorption helps in reduction of glucose absorption rates and lowers the post-prandial glucose levels since carbohydrates not broken down into glucose molecules [10,11]. Few currently used synthetic suppressors of these enzymes acarbose and miglitol inhibits glycosidases (α-glucosidase and α-amylase) while voglibose inhibits a-glucosidase. In spite of their restrictions are non-specific, produce serious side effects and fail to elevate diabetic complications. Side effects of these inhibitors are gastrointestinal viz., bloating, abdominal discomfort, diarrhea and flatulence [12].

Therapies presently used for Diabetes include Insulin and numerous oral antidiabetic agents such as Sulfonylureas, Biguanides, Thiazolidinedione's, Glucosidase inhibitors and Glinides. They cause a number of serious adverse effects such as hepatotoxicity, abdominal pain, flatulence, diarrhea and hypoglycemic etc. Resistance of drugs to these medications is often conveyed after persistent usage [13].

Thence, investigations to obtain hypoglycemic drugs from therapeutic plants have expanded acceptance in present. 45,000 plants have been recorded in India out of which 7,500 species are of medicinally important [14]. For this reason, search for highly effective and safer hypoglycemic agents is one of the crucial areas of investigation [15]. Traditional Medicine preparations from plant sources are possibly are a good source of peculiar antidiabetic compounds or phytomedicines/supplements [16].

Trachyspermum ammi (Ajwain seeds) comes under the family Apiaceae. *T. ammi* is identified in different regions of the world by different names such as Ajwain, Ajma, Omam, Yaviniki, Bishop's weed, etc., Different herbalists also use the scientific name *Carum copticum* for *Trachyspermum ammi* [17]. Ajwain fruits are bitter, pungent which works as anti-inflammatory, anti-tussive, analgesic, antibacterial, antifungal and antiviral also works as bronchodilatory, anticancer and antioxidant [18,19]. It is also used for relieving flatulence, dyspepsia, spasmodic disorders, flatulence, common cold, acute pharyngitis, sore and congested throat [20]. Nonetheless the antidiabetic activity of *T. ammi* seeds are not yet scientifically established. In the present study we aimed to scientifically establish the glucose lowering potential of *T. ammi* by various assays.

Materials and methods

Sample collection and extraction of phytochemicals

The fresh seeds of *Trachyspermum ammi* were collected from Chennai, Tamilnadu, India. The dried plant seeds were physically cleaned and powdered using a blender and stored in a clean glass ware air tight container. Soxhlet extraction assembly was used for extraction purpose. 50 g dried and powdered seeds were mixed separately with 250 mL of Ethyl Acetate and continuously extraction was done for 5 - 6 h [21]. Then the mixture was filtered using Whatman No.1 filter paper. The crude extract thus obtained was concentrated by using rotary vacuum evaporator under reduced pressure and stored at 4 °C.

Thin layer chromatography of ethyl acetate extract of *T. ammi*

TLC Plates using glass plates were prepared and activated at 110 °C for 30 min for activation [22]. TLC tank glass chamber with airtight lid was developed in saturated glass chambers using the following different solvent systems as mobile phases: Toluene:Methanol:Chloroform (5:1:4), Chloroform:Methanol:Toluene:Acetone (2:1:2:0.5), Chloroform:Ethyl acetate:n-Hexane (70:15:15) and Ethyl acetate:Toluene:Methanol (1:8:1). The 5 µl (10 mg/mL) of extract in ethyl acetate was applied as a small spot on the TLC plates using capillary and solution was dried. Plates were run in the solvent mixtures till the solution reached the line mark [23]. The plates were then air-dried. The plates were placed in the UV light of 312 and 365 nm, many fluorescent spots were observed of varying colors and photographed. TLC plates were then placed in the iodine chamber for the development of brown spots on the TLC plates. R_f value of the bands were calculated by using the formula: R_f= Distance traveled by the solute/Distance traveled by the solvent.

Column chromatography of ethyl acetate extract of *T. ammi*

Wet packing method was adopted for packing the column. Silica gel (Kieselgel 60, mesh size 60 - 120) was used for column packing. Silica gel slurry was added into a glass column [24]. Gradient elution technique was used which involves the use of a continuously changing eluting medium. The crude ethyl acetate extract (10 g) was dissolved in running solvent n-hexane (10 mL) and loaded onto packed column, followed by graded mixtures of n-hexane: Ethyl acetate, ethyl acetate 100 %, graded mixtures of ethyl acetate: Toluene, toluene 100 %, graded mixtures of toluene: Chloroform, chloroform 100 %, graded mixtures of chloroform: Methanol and finally with 100 % methanol [25]. Eluted fractions were collected at the rate of about 5 mL. Totally 80 fractions were obtained. Each fraction was subjected to TLC on silica gel G. The fractions with same R_f were pooled together and concentrated to obtain major fractions. Totally 9 number of major fractions were obtained.

Primary screening of fractions by starch-agar gel diffusion assay

The 9 fractions obtained were subjected to starch-agar gel diffusion assay to screen the fraction having highest α-amylase inhibition activity. Starch-agar gel diffusion assay was done according to Fossum and Whitaker [26] (1974) with some modifications. 0.5 cm thick 1 % agar containing 0.5 % starch gel was prepared and poured in sterile petri plates. Wells were bored and labeled. The reaction mixtures were prepared by adding 50 µl of α-amylase solution (0.1 mg/mL in phosphate buffer), 50 µl of fraction and 25 µl of 0.02 M phosphate buffer pH 6.9. Blank reaction mixture was prepared without adding fraction; instead 50 µl phosphate buffer was added. Mixtures was prepared at room temperature

and incubated for 10 min to allow enzyme-inhibitor interaction. Standard acarbose solution (1 mg/mL in phosphate buffer) alone served as positive control. 100 µl of each reaction mixture was loaded in the respective wells and allowed to diffuse overnight. After 24 h, the plate was flooded with iodine solution. Clear circular zone of starch hydrolysis was seen in blank well against deep blue or purple background indicating activity of alpha-amylases. Diameters of the zones were measured and used to calculate percentage of inhibition. The fraction with high α-amylase inhibition activity was further sub-fractioned using chromatography techniques and TLC analysis of the subfractions were done to pool them accordingly.

Column separation of fraction in to sub-fractions

1 gm of the fraction-8 was run in column chromatography for sub-fractionation. The solvents used for running the column were hexane (100 %); n-hexane and toluene (2:1, 1:1, 1:2); toluene (100 %); toluene and ethyl acetate (2:1, 1:1, 1:2); ethyl acetate (100 %); ethyl acetate and chloroform (2:1, 1:1, 1:2); chloroform (100 %); chloroform and methanol (2:1, 1:1, 1:2) and methanol (100 %). Then the column sub-fractions were analyzed by TLC. Based on the R_f values 4 major Subfractions were obtained namely subfraction-I, subfraction-II, subfraction-III and subfraction-IV. The subfractions were subjected to glucose uptake activity in yeast cells to further screen the subfraction based on percentage increase in glucose uptake by yeast cells.

Secondary screening of sub-fractions by glucose-uptake assay in yeast cells

Preparation of Yeast cells was done by the method of Cirillo [27], (1962). Baker's yeast dissolved in distilled water was repeatedly centrifuged at 3,000×g, for 5 min until clear supernatant fluids were obtained. 10 % (v/v) of the suspension was prepared in distilled water. Following concentrations of plant extracts (250, 500, 750, 1,000 µg/mL) were added to 1 mL of glucose solution (20 mm) and incubated together for 10 min at 37 °C. Reaction was started by adding 100 µL of yeast suspension followed by vortexing and further incubation at 37 °C for 60 min. After 60 min, the tubes were centrifuged at 2,500×*g*, for 5 min and amount of glucose was estimated in the supernatant. Metronidazole was used as standard drug. The percentage increase in glucose uptake by yeast cells was calculated using the following formula:

$$\text{Increase in glucose uptake (\%)} = \left[\frac{\text{Abs sample} - \text{Abs control}}{\text{Abs sample}}\right] \times 100$$

where, Abs control was the absorbance of the control reaction (containing all reagents except the test sample) and Abs sample was the absorbance of the test sample. All determinations were carried out in triplicate manner and values are expressed as mean ± S.D.

***In Vitro* anti-diabetic activity evaluation in 3T3-L1 cell lines**

3T3-L1 cell culture maintenance

Mouse preadipocytes cell lines 3T3-L1 was procured from cell repository, National Centre for Cell Sciences, Pune. The 3T3-L1 (mouse fibroblast) cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10 % Fetal Calf Serum (FCS), 4 mm Glutamine and 1 % antibiotic/antimycotic solution, in a 5 % CO_2 incubator at 37 °C. The experiments were performed at 90 % confluence.

Cell differentiation and processing

The 3T3-L1 cells were seeded at cell density of 6×10^4 cells/well at a final volume of 1,000:l in a 24 well plate with DMEM containing 10 % FBS and incubated for 48 h until the cells become confluent, then the old media was replaced by % FBS and incubated again for 48 h. Preadipocytes cultured in DMEM containing 10 % FCS, 4 mm Glutamine, 2 % NaHCO3 and antimycotic, in an atmosphere of 5 % CO_2 at 37 °C, which were undifferentiated fibroblasts, were induced to differentiate to mature adipocytes using differentiation Medium (DMEM with 10 % FBS, 1 % antibiotic-antimycotic, 1 µM Dexamethasone, 0.5 mm 3-isobutyl-1-methylxanthine (IBMX) and 1 µg/mL Insulin) for 9 days.

***In Vitro* MTT Assay**

MTT assay was carried out based on the method described by Mosmann [28] (1983). The MTT reagent was prepared by dissolving 5 mg of 3-(4,5-dimethylthiazol-2- yl)-2,5-diphenyl tetrazolium

bromide (MTT) in 1mL of phosphate buffer saline (PBS). Preadipocyte were seeded at density, 10,000 cells per well in 96-well were allowed to attach for 24 h prior to the cell viability assay. To study the toxicity of extract on 3T3-L1 cell viability, the monolayer cell culture was trypsinized and the cell count was made 1.0×10^5 cells/mL in DMEM with 10 % FBS. In each well, 0.1 mL of the diluted cell suspension was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µl of crude and Sub-fraction of Ethyl acetate extract at different concentrations (10, 50, 100, 250 and 500 µg/mL) were added. The plates were then incubated at 37 °C for 3 days in 5 % CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the drug solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well. Plates were gently shaken and incubated for 3 h at 37 °C in 5 % CO_2 atmosphere. Supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured at a wavelength of 570 nm using a microplate reader. The percentage growth inhibition was calculated using the following formula, Cell viability (%) was calculated by using the formula:

$[(A_C - A_T)/A_C]\times100$

where, A_C - O.D of Control at 570 nm, A_T - O.D of Test samples at 570 nm.

In Vitro glucose uptake activity

3T3-L1 cells monolayer was maintained at sub confluent conditions in growth media containing DMEM with 4.5 g/l glucose, 100 IU/mL penicillin, 100 µg/mL streptomycin, and 10 % fetal bovine serum. Cells were cultured on 6 well plates and incubated for 48 h at 37 °C in a CO_2 incubator. When semi confluent monolayer was formed, the culture was renewed with serum free DMEM containing 0.2 % BSA and incubated for 18 h at 37 °C in the CO_2 incubator. Insulin was used as a positive control and wells containing medium and cells served as the negative control. After 18 h, the media was discarded and cells were washed with PBS buffer once. The cells were treated with Insulin, Crude and Sub-Fraction of varying concentration (10, 50 and 100 µg/mL), then glucose (1M) was added and incubated for half an hour. The supernatant was collected for glucose estimation and glucose uptake was terminated by washing the cells thrice with 1 mL ice-cold DPBS buffer. Cells were subsequently lysed by freezing and thawing thrice. Cell lysate was collected for glucose estimation [29]. Glucose uptake was calculated as the difference between the initial and final glucose content in the incubated medium by GOD-POD method.

Glucose concentration in mg/dl = $(A_{sample}/A_{standard})\times100$

whereas, A_{sample} - Absorbance of test sample,

$A_{standard}$ - Absorbance of standard drug.

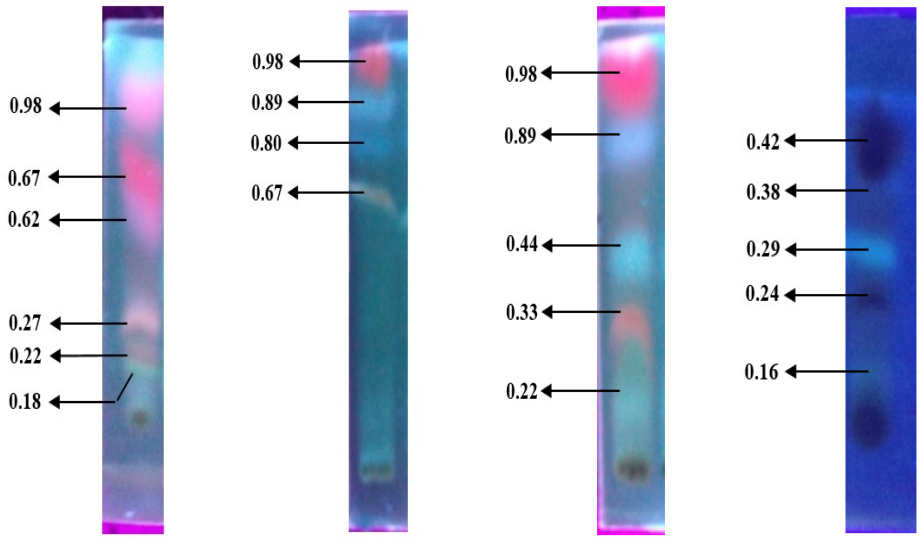
Results

TLC analysis of ethyl acetate extract of *T. ammi*

The ethyl acetate extract yielded a dark greenish-brown substance which was subjected to TLC. Prepared TLC Plates were developed with solvent systems which separated components into a wide range of R_f values as shown in **Table 1** and **Figure 1**. The bioactive compounds present in ethyl acetate crude extract of *T. ammi* were identified using TLC. The most suitable TLC solvent mixture was Toluene:Methanol:Chloroform (5:1:4) with the largest discriminating power under ultra violet light at 365 nm (**Figure 1(a)**). Different solvent systems and the R_f values obtained in each solvent systems is shown in **Table 1**. The components in TLC plates were visualized by placing under visible and U.V. light (312 and 365 nm). The best resolution of compounds was identified in UV Light of 365 nm as shown in **Figures 1(a) - 1(d)** shows the under UV light of 312 nm.

Table 1 R_f Values of Crude Ethyl Acetate Extract of *T. ammi* in Different Running Solvent Mixtures.

S. No	Solvent Mixtures	R_f value
1	Toluene:Methanol:Chloroform (5:1:4)	0.18, 0.22, 0.27, 0.62, 0.67, 0.98
2	Chloroform:Methanol:Toluene:Acetone (2:1:2:0.5)	0.67, 0.80, 0.89, 0.98
3	Chloroform:Ethyl acetate:Hexane (70:15:15)	0.22, 0.33, 0.44, 0.89, 0.98
4	Ethyl Acetate:Toluene:Methanol (1:8:1)	0.16, 0.24, 0.29, 0.38, 0.42



1(a) **1(b)** **1(c)** **1(d)**

Figures 1(a) - 1(d) Compound Separation by Thin Layer Chromatography in Different Running Solvent Mixtures. (a) TLC Plate in Toluene:Methanol:Chloroform (5:1:4) at UV-365 nm, (b) TLC Plate in Chloroform:Methanol:Toluene:Acetone (2:1:2:0.5) at UV-365 nm, (c) TLC Plate in Chloroform:Ethylacetate:Hexane (70:15:15) at UV-365 nm, and (d) TLC Plate in Ethyl Acetate:Toluene:Methanol (1:8:1) at UV-312 nm.

Column chromatography for separations of fractions

80 eluted column fractions were collected at regular volume of 5 mL each and run in TLC to separate the similar compounds with same R_f values. They were pooled together giving 9 number of major fractions.

Primary screening of fractions by starch-agar gel diffusion assay for α-amylase inhibition

The fractions were subjected to starch agar-gel diffusion assay to screen the fraction having high α-amylase inhibiting activity. All the fractions exhibited different percentage of α-amylase inhibitory activity as given in **Table 2** and **Figure 2**. The activity observed in blank was highest which was considered as 100 % amylase activity. Acarbose inhibited 100 % amylase activity acting as standard.

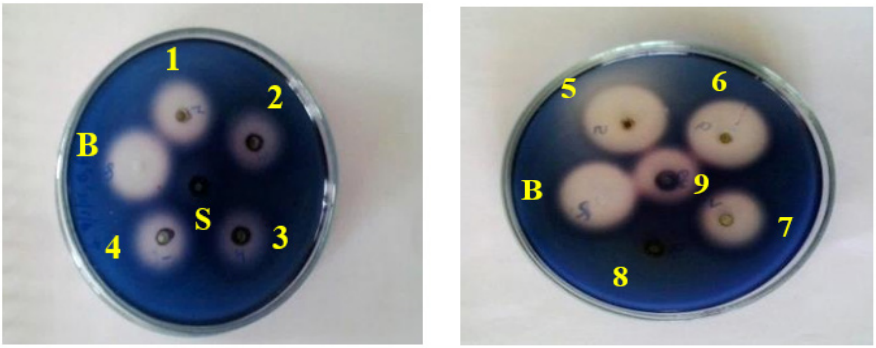


Figure 2 Starch-Agar gel diffusion assay for α-Amylase inhibition by fractions of *T. ammi,* B: Blank (Alpha-amylase), S: Standard (Acarbose), 1: Fraction 1, 2: Fraction 2, 3: Fraction 3, 4: Fraction 4, 5: Fraction 5, 6: Fraction 6, 7: Fraction 7, 8: Fraction 8, 9: Fraction 9.

Table 2 Percent Inhibition of α-amylase Activity by the 9 Different Fractions of Ethyl Acetate Extract of *T. ammi.*

S. No	Fractions	Zone of inhibition in cm
B	Blank (Alpha-amylase)	1.54 ± 0.04
S	Standard(Acarbose)	0
1	Fraction 1	1 ± 0.14
2	Fraction 2	0.77 ± 0.02
3	Fraction 3	0.53 ± 0.01
4	Fraction 4	0.86 ± 0.02
5	Fraction 5	NA
6	Fraction 6	NA
7	Fraction 7	1.25 ± 0.03
8	Fraction 8	0
9	Fraction 9	0.48 ± 0.01

Values are means ± S.D. of triplicates. NA: No inhibitory activity exhibited by fractions.

The fraction with high activity was found to be fraction-8 screened by starch-agar gel diffusion assay. This fraction was further subjected to column chromatography to separate in to subfractions. The TLC of fraction performed the solvent system was further used for obtaining subfractions. These subfractions were run in TLC and based on the same R_f values they were pooled together leading to main 4 subfractions namely subfraction-I, subfraction-II, subfraction-III and subfraction-IV. All the 4 subfractions were subjected to further determining of hypoglycemic activity by *in vitro* glucose uptake assay by yeast cells.

Secondary screening of sub-fractions by glucose uptake assay by yeast cells

Amount of transportation of glucose across the cell membrane was studied in an *in vitro* system comprising of yeast cells suspended in 20 mm glucose concentration. The rate of glucose transport across cell membrane in yeast cells was directly proportional to the increase in the concentration of subfractions of ethyl acetate extract of *T. ammi*. Subfraction-I showed maximum activity than other subfractions. The high uptake of the glucose was seen in 20 mm glucose concentration. The results were depicted in **Figure 3**. The amount of glucose remaining in the medium after a specific time interval serves as an indicator of glucose uptake by the yeast cells. The Subfraction-I exhibited significantly higher glucose uptake activity than the other subfractions. Therefore, the active subfraction-I along with crude ethyl acetate extract of *T. ammi* was further subjected to *in vitro* testing in 3T3-L1 Cell line.

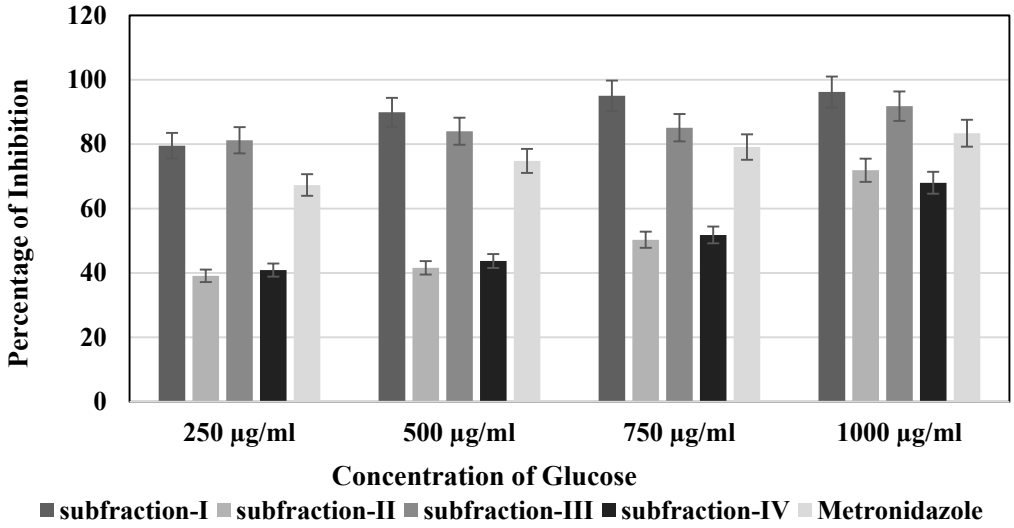


Figure 3 Effect of Subfractions of Ethyl Acetate Extract of *T. ammi* on the uptake of Glucose (20 mm) by Yeast Cells.

***In vitro* toxicity testing by MTT assay**

MTT assay measures the viability and proliferation of cells. This assay works with the principle of reduction of yellow color (MTT) to purple formazan by the enzyme reductase which is present in living cells. The cytotoxicity of crude extracts and subfraction-I on 3T3-L1 cell line at various concentrations was evaluated in the present study (**Table 3**). The control (untreated cells) showed 100 % cell viability. The cells treated with crude extracts and subfraction-I showed no toxicity between 10 - 500 µg/mL concentration (**Figure 4**).

Table 3 MTT assay for crude and fraction-8 of ethyl acetate extract of *T. ammi* treated in 3T3-L1 cell line.

Concentration of samples in µg/mL	Percentage of viability (%)	
	Crude	Fractions
10	92.66 ± 2.52	93.33 ± 6.03
50	80 ± 2.64	91.33 ± 1.53
100	68.33 ± 3.05	78.33 ± 7.64
250	45.33 ± 3.51	62.33 ± 3.05
500	20.66 ± 1.15	50.67 ± 1.15

Results are expressed as Mean ± S.D; n = 3

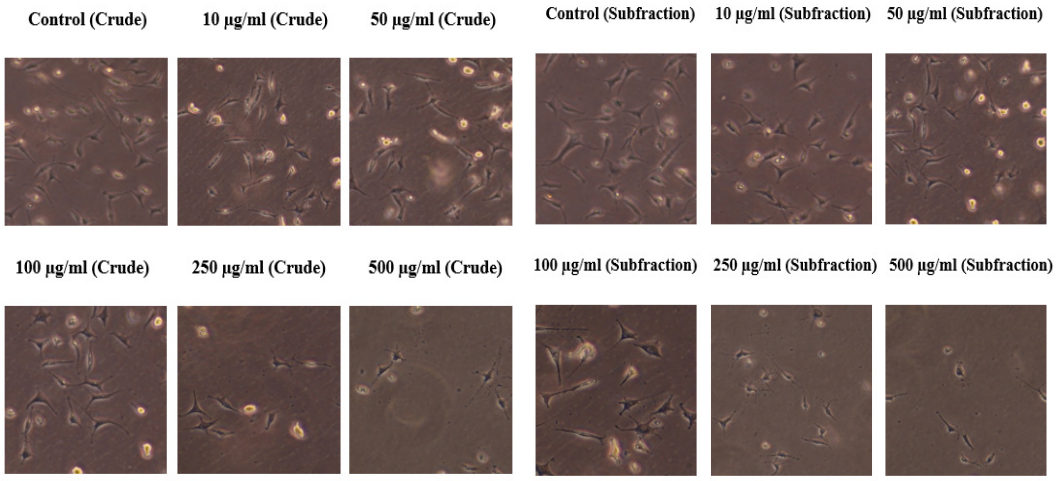


Figure 4 Cytotoxic activity of crude and Sub-Fraction-1 of ethyl acetate extract of *T. ammi* in 3T3-L1 cell line.

Glucose uptake activity (insulin mimetic activity) in 3T3-L1 cell line

In 3T3-L1 cell lines the glucose uptake assays, a significant level of insulin mimetic activities were observed in the case of crude extract and subfraction-I of *T. ammi*. All observed values of glucose uptake activity are compared using the control (untreated cells). These values are normalized with MTT cell viability assay values (10, 50, 100, 250 and 500 µg/mL) for the crude and subfraction-I. The insulin mimicking activity was found to be the best at 100 µg/mL concentration of subfraction-I (**Figure 5**).

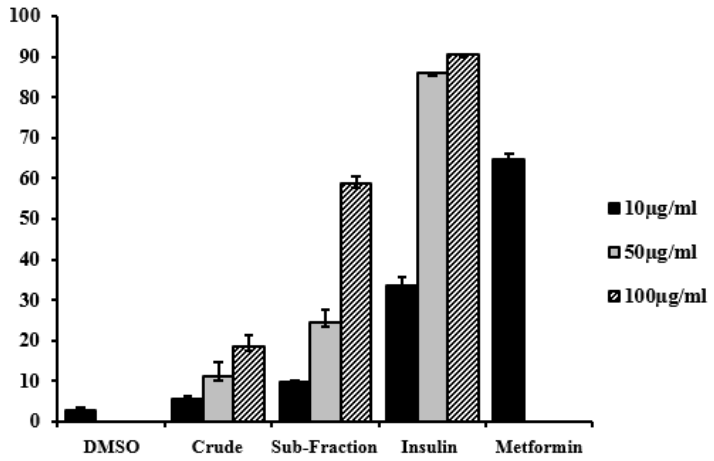


Figure 5 Glucose uptake activity in 3T3-L1 cells. Cells were incubated with crude, sub-fraction and Insulin along with 1M glucose.

Discussion

Traditional medication from plant materials for treatment of Diabetes Mellitus is advised as good source for novel drug or leading to preparation of novel drug. Prescribed by the traditional healers, different plant extract preparations conceivably been accustomed by users for Diabetes Mellitus. Ethno botanical information reports almost 800 plants possess anti-diabetic potential [30,31]. Glucose Control in blood is important to help lower the diabetic complications. Acarbose and miglitol are presently used synthetic inhibitory drugs which have negative effects such as diarrhea, vomiting, abdominal pain, nausea, abnormal weight gain, low blood glucose or allergic reactions [32,33]. Plant natural products are useful in discovering lead candidates with potential antidiabetic activity which plays an important role in

prospective drug development [34]. Acarbose and miglitol, competitive inhibitors of α-amylase and α-glucosidase reduces the digestion of starch and polysaccharides, disaccharides, thereby resulting in lower absorption of glucose through the inhibition of carbohydrates hydrolyzing enzymes in the digestive tract delaying carbohydrate digestion time [35,36]. In the present study, acarbose was used as a standard which showed inhibitory activity on α-amylase during starch assay.

The inhibitory activities of the extracts may be as a result of phytochemicals like alkaloids, steroids and fatty acids which are majorly present in them. Previous studies on medicinal herbs recommend that a number of capable inhibitors belong to alkaloids and steroids class that has features to inhibit α-amylase and α-glucosidase [37]. Phytomolecules such as flavonoids, phenols, alkaloids, glycosides, saponins, glycolipids, dietary fibres, polysaccharides, peptidoglycans, carbohydrates, amino acids and others obtained from various plant sources have been reported with hypoglycemic and antihyperglycemic agents [38,39]. Plant based α-amylase and α-glucosidase inhibitors were proved to contain potential therapeutic strategy to control hyperglycemia [40,41]. In this study, fraction-8 of *T. ammi* exhibited high inhibitory activity suggesting it to be a good source of α-amylase inhibitors.

Acarbose-like drugs, which inhibit α-glucosidase present in the brush border of epithelial lining, were demonstrated in decreasing post-prandial hyperglycemia [42] and improved impaired glucose metabolism in NIDDM patients [43]. These drugs are useful for patients taking sulfonylurea medication or metformin, to keep their blood glucose level within a safe range. Hence, delayed absorption of carbohydrates with a plant based α-amylase and α-glucosidase inhibitors offers a prospective therapeutic approach for the management of type 2 Diabetes Mellitus and borderline patients [44]. With this regard the ethyl acetate extract of *Trachyspermum ammi* seems to be a promising alternative for the treatment of Diabetes Mellitus by reducing postprandial hyperglycemia.

TLC technique was used for the separation of compounds present in the crude EA extract and to select the solvent system capable of showing better resolution. TLC fingerprints of plant extracts shows complex mixtures of non-polar to polar compounds. TLC helps in monitoring composition of the extracts to ensure that no components are lost during processing. Toluene:Methanol:Chloroform solvent system was a suitable solvent mixture for fractionation of ethyl acetate extract of *T. ammi*, since this solvent system was giving good resolution and showing 5 - 6 spots on exposure to iodine vapor by forming the intense brown color at the spotted area. So the same solvent systems have been selected for the further column chromatographic study [45].

With the help of column chromatography, ethyl acetate crude extract of *T. ammi* yielded 9 major fractions which were screened by α-amylase inhibition assay by starch-agar gel diffusion method. The highest activity was observed in blank containing only α-amylase solution which was considered as 100 % amylase activity having 1.54 ± 0.04 as zone of measurement. Acarbose was used as a positive control which inhibits α-amylase enzyme and does not produce zone of measurement, considered as 100 % inhibitory activity on amylase enzymes. The percentage of inhibition shown by fraction 8 was highest (100 %) since no zone of measurement was obtained. Fraction 8 inhibited α-amylase enzymes which was comparable to standard acarbose. The present study reports were in agreement with Bale and Gawade, [46] (2014) in which aqueous stem bark extracts of 34 plants species were screened for their α-amylase inhibitory activity by using Starch-agar gel diffusion assay out of which bark extracts of 5 plant species showed 100 % inhibition equivalent to acarbose. Similarly, Chandrashekar *et al.* [47] (2012) investigated antidiabetic activity of aqueous extracts of stem bark of *Bauhinia purpurea*, which showed significant α-amylase inhibitory activity as compared to acarbose standard drug.

For a second time the fraction 8 was run in column chromatography yielding 4 subfractions. These subfractions were subjected to glucose uptake assay by yeast cells. It was studied that the glucose uptake rate increased with increasing concentration of the extract and decreased with increasing extracellular glucose. The subfraction-I exhibited up to 96 % increase in glucose uptake by yeast cells. Transport of glucose across yeast cell membrane occurs by facilitated diffusion down the concentration gradient. Hence glucose transport occurs only if the intracellular glucose is effectively utilized [48]. The reports obtained clearly suggests that the subfraction-I is capable of effectively enhancing glucose uptake which in turn suggests that it is capable of enhancing effective glucose utilization thereby controlling blood glucose level.

Scharff, [49] (1961) explained the mechanism of glucose transport across the yeast cell membrane has been receiving attention as *in vitro* screening method for hypoglycemic effect of various compounds/medicinal plants. It has been reported that glucose transport in yeast cells (*Saccharomyces cerevisiae*) is extremely complex and it is generally agreed that glucose is transported in yeast cells was by facilitated diffusion process. Facilitated carriers are specific carriers that transport solutes down the concentration gradient. This means that effective transport is only attained if there was removal of

intracellular glucose [50]. Heera *et al.* (2014) testified the *in vitro* antidiabetic activity of methanolic and ethyl acetate extracts of *Alpinia galangal*. The plant extracts were studied for their effect on increased glucose uptake percentage rate into yeast cells which was found to be linear in different glucose concentrations used. Maximum increase was seen in 20 mm glucose concentration exhibited by methanolic extract of *Alpinia galangal* [51]. Hence, 20 mm glucose concentration was used here for the glucose uptake assay by yeast cells.

From the present investigation, Crude and subfraction-I of ethyl acetate extract of *T. ammi* was screened using MTT for its cytotoxicity against 3T3-L1 cell line at different concentrations which was found to be dose dependent. Skeletal muscle is a major tissue involved in insulin induced stimulation of glucose uptake. Functional transport of glucose molecules increases insulin uptake in skeletal muscles in the plasma membrane. Contractile activity also stimulates transport of glucose in skeletal muscles. Defects in insulin mediated glucose uptake in skeletal muscle are common pathological condition of NIDDM. Medicinal plants enhances glucose uptake by glucose transport molecules (GLUT4) translocation and were proven by *in vitro* glucose model. 3T3-L1 cell lines are finest studied cellular model for glucose uptake study [52]. Hence, in this study 3T3-L1 cell lines are used to determine the glucose uptake activity of ethyl acetate extract of *T. ammi*. Insulin and Metformin were standard antidiabetic drugs.

Several plant bioactive compounds (alkaloids, polyphenols) have been reported for antidiabetic activity in 3T3-L1 cell lines. Berberine alkaloid from *Cortidis Rhizoma* increased glucose-stimulated insulin secretion in Min6 cells via an enhanced insulin/insulin-like growth factor-1 signaling cascade [53]. Akuammicine, an indole alkaloid, isolated from chloroform extract of seeds of *Picralima nitida* (Apocynaceae) stimulated glucose uptake activity in differentiated 3T3-L1 adipocytes cell line [54]. Kumar *et al.* (2013) treated the 3T3-L1 cells with mangiferin and mahanimbine which increased the glucose utilization in a dose-dependent manner [55]. Based on these reports, it can be concluded that the alkaloid content of *T. ammi* may be responsible for the antidiabetic activity in 3T3-L1 cell lines.

The results in 3T3-L1 cell line assay on glucose uptake demonstrated that crude and subfraction-I of ethyl acetate extract of *T. ammi* could increase the glucose consumption. Subfraction-I of ethyl acetate extract of *T. ammi* showed better glucose uptake effect in 3T3-L1 cell line than the crude extract. Insulin and metformin had highest glucose uptake effect in 3T3-L1 cell lines. It is suggested that the *T. ammi* had antidiabetic activity by facilitating the glucose utilization in adipose tissues and skeletal muscles.

Conclusions

The present work of α-amylase inhibition activity of fraction-8 from ethyl acetate extract of *T. ammi* confirmed its antidiabetic nature. Glucose uptake assay in yeast model showed the hypoglycemic nature of subfractions of ethyl acetate extract of *T. ammi*. Further the *in vitro* antidiabetic activity by cell line assay revealed their hypoglycemic nature of crude and subfraction-I of ethyl acetate extract of *T. ammi.* Hence the hypoglycemic activity of ethyl acetate extract of fruit of *T. ammi* has been proven to be significant in few *in vitro* experimental models. Since *in vitro* activity does not always correlate to the corresponding *in vivo* activity, proof of concept have to be demonstrated in animal studies to extend its scientific use.

References

[1] K Mangesh, G Vidyasagar and K Ashwini. Diabetes and complications at a glance for pharmacy researchers. *Int. J. Pharmaceut. Sci. Rev. Res*. 2014; 25, 281-9.

[2] A Chauhan, PK Sharma, P Srivastava, N Kumar, R Dudhe and R Dudhe. Plants having potential antidiabetic activity: A review. *Scholars Res. Libr*. 2010; 2, 369-87.

[3] M Badran and I Laher. Type II diabetes mellitus in arabic-speaking countries. *Int. J. Endocrinol*. 2012; **2012**, 902873.

[4] ME Tucker. IDF Atlas: About 415 million adults worldwide have diabetes. *In*: Proceedings of the International Diabetes Federation 2015 World Congress, Columbia, Canada. 2015.

[5] DC Whitcomb and ME Lowe. Human pancreatic digestive enzymes. *Dig. Dis. Sci*. 2007; **52**, 1-17.

[6] J Iulek, OL Franco, M Silva, CT Slivinski, C Bloch, DJ Rigden and MFGD Sá. Purification, biochemical characterisation and partial primary structure of a new α-amylase inhibitor from Secale cereale (rye). *Int. J. Biochem. Cell. Biol*. 2000; **32**, 1195-204.

[7] L Kandra. α-Amylases of medical and industrial importance. *J. Mol. Struct*. 2003; **666-667**, 487-98.

[8] GC Brayer, Y Luo and SG Withers. The structure of human pancreatic a-amylase at 1.8 A resolution and comparisons with related enzymes. *Protein Sci*. 1995; **4**, 1730-42.

[9] S Tangphatsornruang, M Naconsie, C Thammarongtham and J Narangajavana. Isolation and characterization of an α-amylase gene in cassava (*Manihot esculenta*). *Plant. Physiol. Biochem.* 2005; **43**, 821-7.
[10] KJ Naquvi, J Ahamad, SR Mir, M Ali and M Shuaib. Review on role of natural alpha-glucosidase inhibitors for management of diabetes mellitus. *Int. J. Biomed. Res.* 2011; **2**, 374-80.
[11] K Hullatti and M Telagari. *In vitro* α-amylase and α-glucosidase inhibitory activity of *Adiantum caudatum* Linn. and *Celosia argentea* Linn. extracts and fractions. *Indian J. Pharmacol*. 2015; **47**, 425-9.
[12] AYY Cheng and IG Fantus. Oral antihyperglycemic therapy for type 2 diabetes mellitus. *Can. Med. Assoc. J*. 2005; **172**, 213-26.
[13] T Fujisawa, H Ikegami, K Inoue, Y Kawabata and T Ogihara. Effect of two α-glucosidase inhibitors, voglibose and acarbose, on postprandial hyperglycemia correlates with subjective abdominal symptoms. *Metabolism* 2005; **54**, 387-90.
[14] AY Oubré, TJ Carlson, SR King and GM Reaven. From plant to patient: an ethanomedical approach to the identification of new drugs for the treatment of NIDDM. *Diabetologia* 1997; **40**, 614-7.
[15] A Saxena and NK Vikram. Role of selected Indian plants in management of Type 2 Diabetes: A Review. *J. Alternative Compl. Med*. 2004; **10**, 369-78.
[16] RJ Marles and NR Farnsworth. Antidiabetic plants and their active constituents. *Phytomedicine* 1995; **2**, 137-89.
[17] MH Boskabady, S Alitaneh and A Alavinezhad. *Carum copticum* Linn: A herbal medicine with various pharmacological effects. *BioMed Res. Int*. 2014; **2014**, 569087.
[18] MR Moein, K Zomorodian, K Pakshir, F Yavari, M Motamedi and MM Zarshenas. *Trachyspermum ammi* (L.) sprague: Chemical composition of essential oil and antimicrobial activities of respective fractions. *J. Evid. Base. Integr. Med*. 2014; **20**, 50-6.
[19] M Lateef, Z Iqbal, U Rauf and A Jabbar. Anthelmintic activity of *Carum copticum* seeds against gastrointestinal nematodes of sheep. *J. Anim. Plant Sci.* 2006; **16**, 34-7.
[20] B Ranjan, S Manmohan, SR Singh and RB Singh. Medicinal uses of *Trachyspermum ammi*: A Review. *Pharmacologyonline* 2012; **5**, 247-58.
[21] S Javed, AA Shahid, MS Haider, A Umeera, R Ahmad and M Sobia. Nutritional, phytochemical potential and pharmacological evaluation of *Nigella sativa* (Kalonji) and *Trachyspermum ammi* (Ajwain). *J. Med. Plants Res*. 2012; **6**, 768-75.
[22] E Stahl. *Thin layer chromatography*. A Laboratory hand book. 2nd ed. Springer Berlin, Heidelberg, Germany, 1969.
[23] DM Steinberg, LJ Sokoll, KC Bowels, JH Nichols, R Roberts, SK Schultheis CM O,Donnell. Clinical evaluation of toxi-prep™: A semiautomated solid-phase extraction system for screening of drugs in urine. *Clin. Chem*. 1997; **43**, 2099-105.
[24] MM Parvez, MA Rahman, MK Molla and A Akter. Compound isolation and purification by chromatographic method of stem bark of *Anisoptera scaphula* (Roxb.). *Int. J. Pharmaceut. Sci. Rev. Res.* 2012; **1**, 1-6.
[25] RS Alm, RJP Williams and A Tiselius. Gradient elution analyses I. A General Treatment. *Acta Chem. Scand*. 1952; **6**, 826-36.
[26] K Fossum and JR Whitaker. Simple method for detecting amylase inhibitors in biological materials. *J. Nutr*. 1974; **104**, 930-36.
[27] VP Cirillo. Mechanism of glucose transport across the yeast cell membrane. *J. Bacteriol*. 1962; **84**, 485-91.
[28] T Mosmann. Rapid colorimeter assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Meth*. 1983; **65**, 55-63.
[29] S Sarasvathy. Antioxidant and insulin-like properties of extracts from wheat grains fermented by selected indigenous *ganoderma* spp / *Sarasvathy Subramaniam*. Master Thesis. University of Malaya, Kuala Lumpur, Malaysia.
[30] JK Grover, S Yadav and V Vats. Medicinal plants of India with anti-diabetic potential. *J. Ethnopharmacol*. 2002; **81**, 81-100.
[31] M Jung, M Park, HC Lee, K Yoon-Ho, ES Kang and SK Kim. Antidiabetic agents from medicinal plants. *Curr. Med. Chem*. 2006; **13**, 1203-18.
[32] K Vijayalakshmi, C Immanuel Selvaraj, S Sundhu and P Arumugam. *In vitro* investigation of antidiabetic potential of selected traditional medicinal plants. *Int. J. Phcog. Phytochemical Res*. 2014; **6**, 856-61.

[33] N Anbu, V Musthafa and Velpandian. Anti-diabetic activity of polyherbal formulation aavaraiyathichurnam in alloxan induced diabetic rats. *Int. J. Toxicol. Pharmacol. Res*. 2012; **4**, 77-80.
[34] IV Asharani, M Gowtham, M Paridhavi and D Thirumalai. A comprehensive review on folklore antidiabetic plants. *Int. J. Pharmaceut. Sci. Rev. Res*. 2016; **39**, 213-5.
[35] FAVD Laar. Alpha-glucosidase inhibitors in the early treatment of type 2 diabetes. Vas. Heal. Risk. Managt. 2008; **4**, 1189-95.
[36] N Chiniwala and S Jabbour. Management of diabetes mellitus in the elderly. *Curr. Opin. Endocrinol. Diabetes. Obes.* 2011; **18**, 148-52.
[37] SB Gaikwad, GK Mohan and MS Rani. Phytochemicals for diabetes management. *Pharmaceut. Crop*. 2014; **5**, 11-28.
[38] G Brahmachari. *Bio-flavonoids with promising antidiabetic potentials: A critical survey*. *In*: VK Tiwari and BB Mishra (Eds.). Opportunity, Challenge and Scope of Natural Products in Medicinal Chemistry. 1st ed. Research Signpost, Ahmedabad, India, 2011, p. 187-212.
[39] S Kumar, R Malhotra and D Kumar. Antidiabetic and free radicals scavenging potential of *Euphorbia hirta* Flower Extract. *Indian J. Pharmaceut. Sci*. 2010; **72**, 533-7.
[40] M Mosaddegh, F Naghibi, H Moazzenia, A Pirania and S Esmaeili. Ethnobotanical survey of herbal remedies traditionally used in Kohghiluyeh va Boyer Ahmad province of Iran. *J. Ethnopharmacol*. 2012; **141**, 80-95.
[41] A Gholamhoseinian, H Fallah, F Sharifi-far and M Mirtajeddini. The inhibitory effect of some Iranian plants extracts on the alpha glucosidase. *Iranian J. Basic Med. Sci*. 2008; **11**, 1-9.
[42] AAF Sima and S Chakrabarti. Long-term suppression of postprandial hyperglycaemia with acarbose retards the development of neuropathies in the BB/W-rat. *Diabetologia* 1992; **35**, 325-30.
[43] JM Carrascosa, JC Molero, Y Fermin, C Martinez, A Andres and J Satrustegui. Effects of chronic treatment with acarbose on glucose and lipid metabolism in obese diabetic wistar rats. *Diabetes Obes. Metabol*. 2001; **3**, 240-8.
[44] P McCue, YI Kwon and K Shetty. Anti-diabetic and anti-hypertensive potential of sprouted and solid-state bioprocessed soybean. *Asia Pac. J. Clin. Nutr*. 2005; **14**, 145-52.
[45] H Wagner and S Bladt. *Plant drug analysis.* A thin layer chromatography atlas. 2nd ed. Springer Berlin, Heidelberg, Germany, 1996.
[46] SR Bale and AE Gawade. Screening of aqueous bark extracts of some plant species for α-amylase inhibitory activity. *Bionano Frontier* 2014; **7**, 75-7.
[47] G Daksha, Chandrashekar, R Lobo, Y Nayak and G Nilesh. *In-vitro* Antidiabetic activity of stem bark of *Bauhinia purpurea* Linn. *Der Pharmacia Lettre* 2012; **4**, 614-9.
[48] F Ahmed, S Sairam, A Urooj. Effect of various ayurvedic formulations and medicinal plants on carbohydrate hydrolyzing enzymes and glucose uptake by yeast cells-an *in vitro* study. *J. Pharm. Res*. 2009; **2**, 563-8.
[49] TG Scharff. Evidence for hexose transport in acetone-dried yeast. *Arch. Biochem. Biophys*. 1961; **95**, 329-35.
[50] MA Bhutkar, SD Bhinge, DS Randive and GH Wadkar. Hypoglycemic effects of berberis aristata and *Tamarindus indica* extracts *in vitro*. *Bull. Facul. Pharm. Cairo Univ*. 2017; **55**, 91-4.
[51] P Heera, L Inbathamizh and J Ramachandran. An *invitro* study on antidiabetic activity of different solvent extract from *Alpinia galanga. Int. J. Ethnomedicine Pharmacol. Res*. 2014; **2**, 1-10.
[52] DS Reddy, SR Avanapu and PH Ananth. *In vitro* antioxidant and glucose uptake effect of *Trichodesma indicum* in L-6 cell lines. *Int. J. Pharma Bio Sci*. 2012; **3**, 810-9.
[53] BS Ko, SB Choi, SK Park, JS Jang, YE Kim and S Park. Insulin sensitizing and insulinotropic action of Berberine from *Cortidis Rhizoma*. *Biol. Pharmaceut. Bull*. 2005; **28**, 1431-7.
[54] H Shittu, A Gray, B Furman and L Young. Glucose uptake stimulatory effect of akuammicine from *Picralima nitida* (Apocynaceae). *Phytochemistry Lett*. 2010; **3**, 53-5.
[55] BD Kumar, K Krishnakumar, SK Jaganathan and M Mandal. Effect of Mangiferin and Mahanimbine on glucose utilization in 3T3-L1 cells. *Phcog. Mag*. 2013; **9**, 72-5.