International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.11 No.08, pp 32-39, 2018

# GC-MS/MS Analysis and *In Vitro* Anti-Diabetic Activity of leaves of *Trigonella foenum-graecum*

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**Abstract :** The nature has provided abundant plant wealth for all the living creatures, which possess medicinal virtues. Therefore, there is a necessity to explore their uses and to ascertain their therapeutic properties. Hence, the present study aims to open new avenues for the improvement of medicinal uses of *Trigonella foenum-graecum*(Leguminosae) leaves for the selected area for anti-diabetic activity. Dried (crude)ethanolic extracts of leaves of *Trigonella foenum-graecum* was subjected for *in-vitro*anti-diabetic activity. Diabetes mellitus is a heterogenous metabolic disease characterized by altered carbohydrate, lipid and protein metabolism. So many traditional herbs are being used by diabetic patients to control the disease. But very few studies are performed to investigate the efficacy of these herbs clinically. The results obtained indicate that the extracts possessed significant level of activity; the highest concentration of extract was high effective as an anti-diabetic agent. Gas Chromatography-Mass Specrometry analysis to determine the chemical constituents present in ethanol extract of leaves. Totally 32 different compounds from ethanol extract were identified. However, these effects need to be confirmed using *in vivo* models and clinical trials for its effective utilization as therapeutic agents.

**Keywords** : *Trigonellafoenum-geaecum,* GC-MS/MS analysis, *in vitro* antidiabetic activity

## Introduction

Medicinal plants play an appreciable role in the development of modern herbal medicines as many diseases like cancer, liver diseases and arthritis find no complete cure in allopathy. The bioactive compounds of medicinal plants are used as anti diabetic, chemotherapeutic, anti inflammatory, anti arthritic agents where no satisfactory cure is present in modern medicines. Medicinal plants have been used as dietary adjunct and in the treatment of numerous diseases without proper knowledge of their function. Although physiotherapy continues to be used in several countries, few plants have received scientific or medical scrutiny[1].

Plants are frequently used in therapeutics from times immemorial the documentation of therapeutic utility of plants can be seen from vedic period. Now a days therapeutic utility of many plants are identified. Among of them medicinal plants fenugreek are also one of the important plant. *Trigonella foenum-*



**DOI= http://dx.doi.org/10.20902/IJCTR.2018.110803**

*graecum* (Linn.) belonging to the family Papilionaceae commonly known as fenugreek is a aromatic, 30-60 cm tall, annual herb, cultivated throughout the country. A nearly smooth erect annual. Stipulets not toothed. Leaflets to 2-2.5 cm long,oblanceolate-oblong, toothed. Flowers 1-2 axillary, sessile. Calyx-teeth linear. Corolla much exserted. Pod 5-7.5 cm long,with a long persistant beak, often falcate, 10-29 seeded, without transverse reticulations[2].Literature survey revealed that whole plant have a lot of activities for treatment of diseases e.g., fresh leaves are used for the treatment of indigestion, flatulence, and the dried leaves are used as a quality flavor for meat, fish, and vegetable dishes[3]. Moorthy*et al* (2010)[4] reported that GII compound, extracted from aqueous extract of *T. foenum-graecum,* is responsible for hypoglycemic effects in the plant. The antioxidant property of the plant material is due to the presence of many active phytochemicals including vitamins, flavonoids, terpenoids, carotenoids, coumarins, lignin, saponin, and plant sterol etc[5].In India, it was used as a condiment and as medicine for lactic stimulation, treatments of indigestion and baldness. Recently it is also used as anti-diabetic, anti-cancer, immunomodulatory and anti-ulcer in different form such as aqueous extract, methanolic extract, powder and other forms also. Research is going on for determining the different activities of the various parts of plant[6].

## Materials And Methods

### Collection of plant material

The plant *Tigonella foenum-greacum* was collected from in and around Thiruthuaipoondi, Thiuvarur District, Tamilnadu, India. The plant was identified with the help of the flora of presidency of Madras[7].

### Preparation of plant powder

The plant was air dried under shade for 10-15 days. Then the dried material was ground to fine powder using an electric grinder and stored in air tight bottles. The powder material was used for further analysis.

### Preparation of ethanol extract

Ethanolic extract was prepared according to the methodology of Indian pharmacopoeia. The coarse powder material was subjected to Soxhlet extraction separately and successively with 210ml ethanol and 90ml distilled water. These extracts were concentrated to dryness in flash evaporator under reduced pressure controlled at a temperature ($40^0$C-$50^0$C). The paste form of the exracts was put in an air tight container stored in refrigerator[8].

### Preliminary Phytochemical screening

Qualitative phytochemical analysis was cartied out for all the extracts as per the standard methods [9].

### GC-MS/MS Analysis

### Analysis of Samples

The given sample was extracted with ethanol and analyzed through Gas Chromatography – Mass Spectrometry/ Mass Spectrometry for identification of different compounds.

### Gas Chromatograpy-Mass Spectrometry Analysis

For quantitation (area%), the GC analysis were carried out by using JEOL JMS-700 by the electron impact method where an electronic accelerating voltage of 75eV and an ion accelerating voltage of 8-10kV.The reservoir inlet systems were used. The capillary columns were: nonpolar column DB-5MS (J&W Scientific; 30 m x 0.25 mm, film thickness 0.25µm) and polar column TC-Wax (60 m x 0.25 mm,film thickness 0.25µm).The dynamic range for the peak intensities was 3 digits, and the accuracy of the mass number was 0.5.The oven temperature was programmed from 40˚-240˚C at a rate of 4˚C/min and held at 240˚C for 5 min. The injector and detector temperatures were 240˚C and 280˚C.The flow rates of the carrier gas (He) were 1.8mL/min.GLC data reported are given as area percentage. He at 49.9 Kpa was used as carrier gas and the FID detector was maintained at 250˚C.The oil constituents were identified on the basis of their retention data and by using GC/MS analytical conditions similar to that of GC/FID. The mass spectra were recorded on a mass

spectrometer coupled to a JEOL JMS-700 gas chromatograph (EI mode 70 eV, source temperature 230˚C, scanned mass ranged 35 - 350 amu).The characteristic fragmentation patterns have been analyzed and compared to those of Wiley 275.L database[10].

**Identification of compounds**

The identification of the compounds was based on comparison with the library spectra (NIST-I, NIST-2,Wiley 275 and Adams libraries) of their relative retention indices with literature values. The relative percentage amount of each component was calculated by comparing its average peak to the total areas. The name, molecular weight, molecular formula and structure of the component of the test materials were determined and the data are presented.

## *In vitro* antidiabetic activity

**Method for In vitro Anti-diabetic Activity**

**Inhibition of Alpha-amylase Enzyme**

Starch solution (0.1% w/v) was prepared by stirring 0.1g of potato starch in 100 ml of 16 mM of sodium acetate buffer. The enzyme solution was prepared by mixing 27.5 mg of alpha-amylase in 100 ml of distilled water. The colorimetric reagent was prepared by mixing sodium potassium tartarate solution and 3, 5 Di-nitro salicylic acid solution at 96 mM concentration. Both control and plant extracts were separately added with starch solution and left to react with alpha- amylase solution under alkaline conditions at 25°C. There action was measured after 3 minutes. The generation of maltose was quantified by the reduction of 3, 5 Di-nitro salicylic acid to 3-amino-5- nitro salicylic acid. This reaction is detectable at 540 nm[11]**.**

**Inhibition of Alpha-glucosidase Enzyme**

The inhibitory activity of alpha-glucosidase enzyme was determined by incubating 1 ml solution of starch substrate (2 % w/v maltose or sucrose) with 0.2 M Tris buffer pH 8.0 and plant extracts separately for 5 minutes at 37°C. There action was initiated by adding 1 ml of alpha-glucosidase enzyme (1U/ml) to it followed by incubation for 40 minutes at 35°C. Then the reaction was terminated by the addition of 2 ml of 6N HCl. Then the intensity of the colour was measured at 540nm[12].

**Determination of Glucose uptake in Yeast cells**

The commercial baker's yeast was washed by repeated centrifugation (3,000×g; 5 minutes) in double distilled water until the supernatant fluids were clear and a 10% (v/v) suspension was prepared in distilled water. Various concentrations of plant extracts (25 - 1000 µg/mL) were added to 1mL of glucose solution (5, 10 and 25 mM) and incubated together for 10 minutes at 37 °C. Reaction was started by adding 100 µL of yeast suspension, vortex and further incubated at 37 °C for 60 minutes. The tubes were then centrifuged (2,500 × g, 5 minutes) and amount of glucose was estimated in the supernatant. Metronidazole was taken as standard drug[13]. The percent increase in glucose uptake by yeast cells was calculated using the following formula:

**Increase in glucose uptake (%) = Absorbance (sample) – Absorbance (control) X 100**

Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample) and Abs sample is the absorbance of the test sample. All the experiments were triplicated.

## Results and Discussion

**Qualitative phytochemical screening**

In the present study the *Trigonella foenum- greacum* leaves was analyzed. The biologically active compounds are screened by dissolving the crude powder in ethanol. Phytochemical studies of ethanolic extract of *Trigonella foenum- greacum* were investigated. The finding of the phytochemicals test result was depicted in the Table 1.Various chemical tests were performed using the standard procedures to identify the

phytoconstituents. These screening tests revealed the presence of various plants constituents present in the plant. Among the various phytoconstituents, phenols, flavonoids and alkaloid were majorly present compounds

**Table 1: Phytochemical analysis of ethanolicexract of *Trigonella foenum-greacum* leaves**

| S.No. | Phytochemical constituents | Name of the Test | Ethanolic extract |
|---|---|---|---|
| **1.** | Alkaloids | Mayer'test<br>Dragondraff test<br>Wagner test | +<br>+<br>+ |
| **2.** | Carbohydrates | Molish test<br>Fehling test<br>Benedicts test | +<br>+<br>+ |
| **3.** | Tannins | Lead acetate | + |
| **4.** | Pseudo tannins | Ferric chloride | + |
| **5.** | Cholorogenic acid | Ammonia | _ |
| **6.** | Steroidal glycosides | Salkowaski | + |
| **7.** | Anthocyanin | $H_2So_4$ | _ |
| **8.** | Steroidal glycosides | Liebermann's burchad test | _ |
| **9.** | Saponins glycosides | $H_2So_4$ | + |
| **10.** | Flavonoids | Ammonia | _ |
| **11.** | Flavones | Shinoda's test | + |
| **12.** | Phenols | Ferric chloride | + |
| **13.** | Coumarin | Sodium chloride | _ |
| **14.** | Phytochemical Constituents | Bontrage's test | _ |

**GC-MS/MS Analysis**

GC-MS is one of the best techniques to identify the constituents of volatile matter, long chain, branched chain hydrocarbons, alchocols, acids and esters etc. The GC-MS analysis of *Trigonella foenum- greacum* leaves revealed the presence of 32 compounds (phytochemical constituents) that could contribute the medicinal quality of the plant (Table 2 and Figure 1). The results revealed that 1, 2,5- Oxadiazole(3.57), Ethoxyacetylene (4.05), Trans-4- Oxo-2-pentenoice acid(4.92), 3-amino-4,5-dimethyl-2(5H)(5.46), 7-Hydroxy-6-methyl-oct-3-Enoic acid(6.27), undecylenic acid(6.86),L-Arginine, methyl ester(7.90),2- Carboxymethyl-3-methyl-cyclopentanecarboxylic acid(9.80), 2H-Pyran-2-one, tetrahydro-4-(2-methyl-3-methylene-1-Buten- 4-yl)-(9.91), 3-0-methyl –d-glucose(11.8), 3-Hexadecyne(13.41), 7-Octadecyne,2-methyl-(13.71), 1-pentadecyne(13.94), 9-eicosyne(17.02), 9,12,15-Octadecatrienoic acid,2,3-dihydroxypropyl ester,(Z,Z,Z)-(17.77), Oleic acid(19.65), 9,12-Octadecadienoic acid(Z,Z)-(20.95), Retinol(21.25),(Dihydroxypropyl-9,12,15-Octadecatrienoic acid,2,3-ester,(Z,Z,Z)(22.18),Cis-5,8,11,14,17-Eicosapentaenoicacid(22.28), Oxiranedodecanoic acid, 3-octyl-,cis-(22.56), Choletan-3-ol, 2-methylene-, (3β,5α)-(23.70), E)-13-Docosenoic acid(25.47), Ursodeoxycholic acid (27.38), 9-Hexacosene(28.26), Gitoxigenin (28.99), i-Propyl 5,8,11,14,17-eicosapentaenoate(30.93), Octadecane, 3-ethyl-5-(2-ethylbutyl)-(31.30), Vitamin E(31.72), Campesterol(33.59), Diosgenin (34.83), β-Sitosterol(35.73).

**Table 2: GC-MS/MS analysis of photochemical compounds in ethanolic extract of *Trigonella foenum-graecum sa***

| No. | RT | Name of the compound | Molecular Formulae | Molecular Weight | Peak Area (%) |
|---|---|---|---|---|---|
| **1** | 3.57 | 1,2,5-Oxadiazole | $C_2H_2N_2O$ | 70 | 3.92 |
| **2** | 4.05 | Ethoxyacetylene | $C_4H_6O$ | 70 | 3.72 |
| **3** | 4.92 | trans-4-Oxo-2-pentenoic acid | $C_5H_6O_3$ | 114 | 1.95 |
| **4** | 5.46 | 3-Amino-4,5-dimethyl-2(5H) | $C_6H_9NO_2$ | 127 | 0.98 |
| **5** | 6.27 | 7-Hydroxy-6-methyl-oct-3-enoic acid | $C_9H_{16}{}^{O}{}_3$ | 172 | 0.46 |

| | | | | | |
|---|---|---|---|---|---|
| **6** | 6.86 | Undecylenic acid | $C_{11}H_{20}O_2$ | 184 | 0.45 |
| **7** | 7.90 | L-Arginine, methyl ester | $C_7H_{16}N_4O_2$ | 188 | 0.94 |
| **8** | 9.80 | 2-Carboxymethyl-3-methyl-cyclopentanecarboxylic acid | $C_9H_{14}O_4$ | 186 | 0.95 |
| **9** | 9.91 | 2H-Pyran-2-one, tetrahydro-4-(2-methyl-3-methylene-1-buten-4-yl)- | $C_{11}H_{16}O_2$ | 180 | 0.96 |
| **10** | 11.8 | 3-0- methyl-d-glucose | $C_7H_{14}O_6$ | 194 | 4.01 |
| **11** | 13.41 | 3-Hexadecyne | $C_{16}H_{30}$ | 222 | 4.43 |
| **12** | 13.71 | 7-Octadecyne, 2-methyl- | $C_{19}H_{36}$ | 264 | 1.97 |
| **13** | 13.94 | 1-Pentadecyne | $C_{15}H_{28}$ | 208 | 1.53 |
| **14** | 17.02 | 9-Eicosyne | $C_{20}H_{38}$ | 278 | 6.52 |
| **15** | 17.77 | 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)- | $C_{21}H_{36}O_4$ | 352 | 0.31 |
| **16** | 19.65 | Oleic Acid | $C_{18}H_{34}O_2$ | 282 | 0.34 |
| **17** | 20.95 | 9,12-Octadecadienoic acid (Z,Z)- | $C_{18}H_{32}O_2$ | 280 | 0.61 |
| **18** | 21.25 | Retinol | $C_{20}H_{30}O$ | 286 | 0.48 |
| **19** | 22.18 | dihydroxypropyl -9,12,15-Octadecatrienoic acid, 2,3- ester, (Z,Z,Z)- | $C_{21}H_{36}O_4$ | 352 | 0.56 |
| **20** | 22.28 | cis-5,8,11,14,17-Eicosapentaenoic acid | $C_{20}H_{30}O_2$ | 302 | 1.28 |
| **21** | 22.56 | Oxiranedodecanoic acid, 3-octyl-, cis- | $C_{22}H_{42}O_3$ | 354 | 0.78 |
| **22** | 23.70 | Cholestan-3-ol, 2-methylene-, (3β,5α)- | $C_{28}H_{48}O$ | 400 | 0.87 |
| **23** | 25.47 | E)-13-Docosenoic acid | $C_{22}H_{42}O_2$ | 338 | 1.41 |
| **24** | 26.38 | Ursodeoxycholic acid | $C_{24}H_{40}O_4$ | 392 | 0.87 |
| **25** | 28.26 | 9-Hexacosene | $C_{26}H_{52}$ | 364 | 8.94 |
| **26** | 28.99 | Gitoxigenin | $C_{23}H_{34}O5$ | 390 | 7.37 |
| **27** | 30.93 | i-Propyl 5,8,11,14,17-eicosapentaenoate | $C_{23}H_{36}O_2$ | 344 | 0.34 |
| **28** | 31.30 | Octadecane, 3-ethyl-5-(2-ethylbutyl)- | $C_{26}H_{54}$ | 366 | 1.88 |
| **29** | 31.72 | Vitamin E | $C_{29}H_{50}O_2$ | 430 | 0.38 |
| **30** | 33.59 | Campesterol | $C_{28}H_{48}O$ | 400 | 2.41 |
| **31** | 34.83 | Diosgenin | $C_{27}H_{42}O_3$ | 414 | 12.97 |
| **32** | 35.73 | β-Sitosterol | $C_{29}H_{50}0$ | 414 | 21.82 |

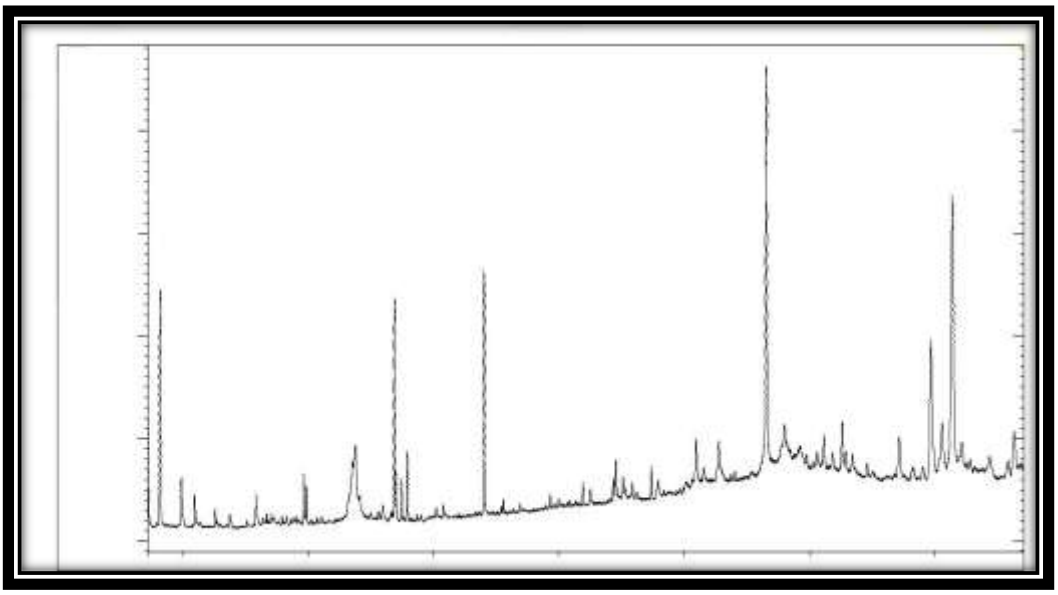

**Figure 1: GC-MS/MS analysis of photochemical compounds in ethanolic extract of *Trigonella foenum-graecum***

Karpagasundari and Kulothungan[14]screened the bioactive components of Physalisminima leaves have been evaluated using GCMS. GC/MS analysis of extract of Physalisminima leaves revealed the existence of Heneicosanoic acid (25.22), Bicyclo [4.1.0] Hepta-2, 4-dien (27.41) Octadecanoic acid (CAS), Stearic acid (31.19) and Octadeca-9, 12-dienoic acid (32.02).

The GC-MS analysis of Caesalpiniaitalica leaves revealed the presence of seventeen compounds. The identified compounds possess many biological properties. For instance, 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-Linolenic acid possesses anti-inflammatory, insectifuge, hypocholesterolemic, cancer preventive, nematicide, hepatoprotective, antihistaminic, antieczemic, antiacne, 5-alpha reductase inhibitor, antiandrogenic, antiarthritic and anticoronary properties. n-Hexadecanoic acid - palmitic acid can be an antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant activities and hemolytic 5-alpha is a reductase inhibitors. PhytolDiterpene is an antimicrobial, anticancer, antiinflammatory and diuretic agent[15]. 9, 12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-, n-Hexadecanoic acid, 1,2-Benzenedicarboxylic acid and di-isooctyl ester were present in *Caesalpinia sappan* ethanol extract [16]. Similar types of compounds were identified among the twenty compounds of this present study.

Similarly the work was done in GC-MS analysis of bioactive components of Hugoniamystax L. (Linaceae). Thirteen compounds were identified. 1,2-Benzenedicarboxylic acid, diisooctyl ester (48.75%) was found to be major component followed by n- Hexadecanoic acid (13.52%), Phytol (9.25%), Squalene (6.41%), Vitamin E (4.09%), Dianhydromannitol (3.56%), 9,12 – Octadecadienoic acid (Z,Z) – (3.20%) and 3,7,11,15 – tetramethyl -2- hexadecen -1-ol (2.85%) .The presence of various bioactive compounds justifies the use of the leaf for various ailments by traditional practitioners. So it is recommended as a plant of phytopharmaceutical importance[17].

***In vitro* Anti diabetic activity**

Plants are important source of medicinal uses and potentially bioactive constituents for the development of new chemotherapeutic agents. The results of *in vitro*antidiabetic activity was tabulated in Table 3, 4 & 5 which are evaluated against two enzymes (alpha-amylase and alpha-glucosidase) and standard drug Metformin *Trigonella foenum-geacum* leaves extract showed significant activity against alpha-amylase and alpha-glucosidase enzymes in different extracts.

The antidiabetic property by glucose uptake by yeast cells. Glucose transport across the yeast cell membrane is gaining significant importance. The above conducted *in vitro* anti diabetic studies depict an appreciable increase in the glucose uptake by the yeast cells in the combination with the plant extracts. The maximum of glucose uptake by yeast cells observed at 10mM glucose concentration 1000µg of flower extracts. Similarly the work was done in *Peltophorum pterocarpum* flowers showed potent antidiaabetic activity compared to that standard drug[18].

**Table 3 :*In vitro* antidiabetic activity alpha-amylase method**

| S.No. | Concentration (µg/ml) | Standard drug | % Inhibition |
|---|---|---|---|
| **1.** | 25 | 0.01±0.01 | 22 |
| **2.** | 50 | 0.85±0.05 | 31 |
| **3.** | 75 | 0.10±0.00 | 42 |
| **4.** | 100 | 0.17±0.30 | 66 |
| **5.** | 200 | 0.18±0.03 | 69 |
| **6.** | 500 | 0.26±0.07 | 81 |
| **7.** | 1000 | 0.29±0.34 | 84 |
| **8.** | Standard drug Metformin (100µg/ml) | 0.37±0.02 | 91.5 |

**Table 4 :*In vitro* antidiabetic activity of alpha-glucosidase method**

| S.No. | Concentration (µg/ml) | Standard drug | % Inhibition |
|---|---|---|---|
| **1.** | 25 | 0.01±0.01 | 12 |
| **2.** | 50 | 0.16±0.05 | 21 |
| **3.** | 75 | 0.19±0.06 | 32 |
| **4.** | 100 | 0.22±0.07 | 38 |
| **5.** | 200 | 0.24±0.09 | 49 |
| **6.** | 500 | 0.26±0.10 | 52 |
| **7.** | 1000 | 0.29±0.12 | 69 |
| **8.** | Standard drug Metformin(100µg/ml) | 0.36±0.73 | 76 |

**Table 5 : Glucose uptake in yeast cell in 10Mm glucose concentration**

| S.No. | Concentration (µg/ml) | Standard drug | % Inhibition |
|---|---|---|---|
| **1.** | 25 | 0.06±0.13 | 26.6 |
| **2.** | 50 | 0.10±0.21 | 50.4 |
| **3.** | 75 | 0.13±0.27 | 55.1 |
| **4.** | 100 | 0.18±0.36 | 68 |
| **5.** | 200 | 0.22±0.45 | 68.9 |
| **6.** | 500 | 0.26±0.05 | 72.8 |
| **7.** | 1000 | 0.53±0.68 | 73.4 |
| **8.** | Standard drug Acarbose (100µg/ml) | 0.34±0.26 | 76.4 |

## Acknowledgement

The authors are thankful to Dr. V, Dhivaharan, Correspondent, Sengamala Thayaar Educational Trust Women's College, Mannargudi, for providing facilities and to carry out my work.

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