

COMPARATIVE EVALUATION OF ALPHA-AMYLASE AND ALPHA-GLUCOSIDASE INHIBITORY POTENTIAL OF AQUEOUS SEED EXTRACT OF *TRIGONELLA FOENUM-GRAECUM* AND *MORINGA OLEIFERA* - AN IN VITRO STUDY

Running title: *In vitro* antidiabetic activity of *Trigonella foenum-graecum* and *Moringa oleifera*

Type of study: Original research

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ABSTRACT:

Background: *Trigonella foenum-graecum* is one of the widely used herbs in food and medicine. *Moringa oleifera* is a source of food, shelter and traditional medicine for many peoples in the developing countries. The seeds of both the plants were investigated for antidiabetic potential.

Methods: The present work was designed to investigate the in vitro anti diabetic potential of the aqueous seed extracts of *trigonella foenum- graceus* and *Moringa oleifera* using the enzymes alpha amylase and alpha glucosidase. Both the extracts were screened for their phytochemicals and antioxidant potential was also analysed. The data were analysed statistically by a one-way analysis of variance (ANOVA) followed by Duncan's multiple range test was used to see the statistical significance among the groups. The results with the $p < 0.05$ level were considered to be statistically significant.

Result: Due to its chemical constituents and active compounds like alkaloids, amino acids, flavonoids, it acts as a good antioxidant. *Trigonella foenum-graecum* seed extract exhibited comparatively higher antidiabetic potential with an IC₅₀ of 300µg/ml than the *Moringa oleifera* extract. Results of the work indicate that the both extracts of plant possessed by inhibition of alpha amylase, alpha glucosidase shows maximum inhibition. Hence concluded that, *Trigonella foenum graecum* leaves might be considered as herbal remedies for diabetes.

Conclusion: *In vitro* antioxidant and antidiabetic potential of seed extracts of *Trigonella foenum-graecum* and *Moringa oleifera* were analysed and compared. This study shows that *Trigonella foenum-graecum* has higher antidiabetic potential than the *Moringa oleifera* extract.

KEY WORDS: Alpha amylase, Alpha glucosidase, Fenugreek, *Moringa oleifera*, Innovative technology, Novel method

INTRODUCTION:

Trigonella foenum-graecum is an herb widely cultivated in India and some parts of China. In India, seeds are used as condiments (1). It is an annual plant, which belongs to the family *leguminosae* (2). Seeds play an important role in the herbal medicine and are recognized for tonic, carminative and aphrodisiac potential (3). *Trigonella foenum-graecum* is a self pollinating crop and it is a native plant used to make maple syrup and the steroid saponin are used widely in industries. *Trigonella foenum-graecum* is rich in calcium, iron, beta carotene, vitamins, lipids (4) and the seeds are rich in carbohydrates and proteins. It is also used as a folk treatment for non insulin dependent diabetes (5). *Trigonella foenum-graecum* is also used to control Hyperglycemia and hyperlipidemia (6). It is also

called fenugreek. It is used to control the glucose, cholesterol, triglyceride and acts as a lowering compounds. It exhibits a long time antidiabetic action (4).

In the current scenario, phytochemicals have received much attention in the treatment of diabetes. For various reasons, many researchers have focused on isolation of hypoglycemic agents from the medicinal plant. (7) Plant polyphenols and the flavonoids are some of the naturally occurring antidiabetic agents (8). *Trigonella foenum-graecum* contributes to lower postprandial hyperglycemia in diabetes the mechanisms of action involves stimulation of glucose dependent insulin secretion from beta cells of pancreas along with inhibition of activity of enzymes like amylase and glucosidase. (9,10) *Moringa oleifera* is a famous edible plant having therapeutic and the nutritive values. *Moringa oleifera* is also known as *Horseradish tree* and also called as drumstick tree. It is rich in phytochemicals and minerals (9–11). It is a constituent of folk medicine and helps to cure the diabetes. It is commonly called as *Miracle tree*. It also helps to control cancer (12), (13), (14). This medicinal plant is used as a food additive to get relieved from malnutrition. It acts as an antioxidant, anti-inflammatory, antidiabetic, antimicrobial agent and also acts as a natural coagulant. *Moringa oleifera* is also used in the treatment of anemia (15), (16), (17). It has being widely accepted by major population as nutrition and therapeutic agents (18) It can be suggested as a potential herbal source to treat diabetes mellitus and as being widely accepted by major population. Our team has extensive knowledge and research experience that has translate into high quality publications (19), (20), (21), (22), (23), (24), (25), (26), (27), (28), (29), (30), (31), (32), (33), (34) The aim of the study is to compare and evaluate the alpha amylase and alpha glucosidase inhibitory potential of aqueous seed extracts of *Trigonella foenum-gracus* and *Moringa oleifera* through an *in vitro* study.

MATERIALS AND METHODS:

Plant Extract: *Trigonella foenum-graecum* and *Moringa oleifera* seed were collected from a farm in Chennai. Washed, crushed and made into powder. Powder was utilized to prepare an 80% aqueous extract. The extract was prepared by a hot percolation method.

Phytochemical Screening test

Test for phlobatannin

1ml of the extract was treated with 1ml of 1% HCl and boiled for 10 mins. The formation of red color precipitate indicates the presence of phlobatannin.

Test for Carbohydrates

Three to five drops of Molisch reagent was added with 1 mL of the extract and then 1 mL of concentrated sulphuric acid was added carefully through the side of the test tube. The mixture was then allowed to stand for two minutes and diluted with 5 mL of distilled water. The development of red or dull violet rings at the junction of the liquids showed the presence of carbohydrates.

Test for Flavonoids

Few drops of 1% liquid ammonia were taken in a test tube and along with it 1ml of the extract was added resulting in the formation of yellow color thereby indicating the presence of flavonoids.

Test for Alkaloids

2ml of sample was mixed with 2ml of HCl. Then 6 drops of HCN was added and further 2 drops of picric acid was added that resulted in a creamish pale yellow ppt indicating the presence of alkaloids.

Test for Terpenoids

2 ml of sample along with 2ml of chloroform and 3ml of con. H_2SO_4 was added. Red color ppt obtained indicates the presence of terpenoids.

Test for proteins

One milliliter of ninhydrin was dissolved in 1 mL of acetone and then a small amount of extract was added with ninhydrin. The formation of purple colour revealed the presence of protein.

Detection of saponins

Foam test: A fraction of the extract was vigorously shaken with water and observed for persistent foam.

Test for steroids

One milliliter of chloroform was mixed with 1 mL of extract and then ten drops of acetic anhydride and five drops of concentrated sulphuric acid were added and mixed. The formation of dark red colour or dark pink colour indicates the presence of steroids.

Antioxidant activity:

DPPH free radical scavenging activity

Scavenging of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radicals was assessed by the method of (35). DPPH solution (1.0 ml) was added to 1.0 ml of extract at different concentrations (0.1 to 0.5 mg/ml). The mixture was kept at room temperature for 50 minutes and the activity was measured at 517 nm. Ascorbic acid at the same concentrations was used as standard. The capability to scavenge the DPPH radical was calculated and expressed in percentage (%) using following formula:

$$\text{DPPH radical scavenged (\%)} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

Control OD

Alpha amylase inhibitory activity of aqueous seed extract of *Trigonella foenum-graecum* and *Moringa oleifera* :

Alpha amylase inhibitory activity of extract was carried out according to the standard method of Ademiluyi et al 2013, In a test tube a reaction mixture containing 500 mu/l phosphate buffer (100mM ; pH=6.8). 100 mu alpha amylase (2 mu/l) and varying concentration of extract (0.1 - 0.5 mg/ml) was incubated at 37 degree Celsius for 20 minutes. Then the 200 mu/l of 1% soluble starch (100 MM phosphate buffer 6.8) was added as a substrate and incubated further at 37 degree Celsius for 30 minutes, 1000 mu/l of the 3,5 Dinitrosalicylic acid [DNS], DNS colour reagent was then added and boiled for 10 minutes. The absorbance of the resulting mixture was measured at 540 nm using a multi plate reader. Acarbose at various concentrations (0.1-0.5 mg/ml) was used as a standard.

$$\text{Inhibitory activity [\%]} = (1 - \text{AS}/\text{AC}) \times 100$$

AS= absorbance in the presence of test substance ; AC=absorbance of control.

Alpha glucosidase inhibitory activity of aqueous seed extract of *Trigonella foenum-graecum* and *Moringa oleifera*:

Alpha glucosidase inhibitory activity of extract was carried out according to the method of Ademiluyi et al (2013) Reaction mixture containing 500 mu/l phosphate buffer(100mM pH 6.8), 100mu/l glucosidase (10 ml) and varying concentration of extract (0.1 to 0.5 mg /ml) was pre incubated at 37 degree Celsius for 15 minutes. Then 200 mu/l p-NPG(5mM) was added as a substrate and incubated further at 37degree Celsius for 30 minutes. The reaction was stopped by adding 50 mu/l sodium carbonate (0.1M). The absorbance of the released p- nitrophenol was measured at 405 nm using multiple readers. Acarbose at various concentrations (0.1-0.5mg/ml)was used as a standard.

Inhibitory activity [%]=(1-AS/AC)100

AS=absorbance of test substance; AC= absorbance of control.

Statistical Analysis

The data were subjected to statistical analysis using two-way analysis of variance (ANOVA) and Tukey's multiple range test to assess the significance of individual variations between the groups. In Tukey's test, significance was considered at the level of $p < 0.05$.

RESULTS :

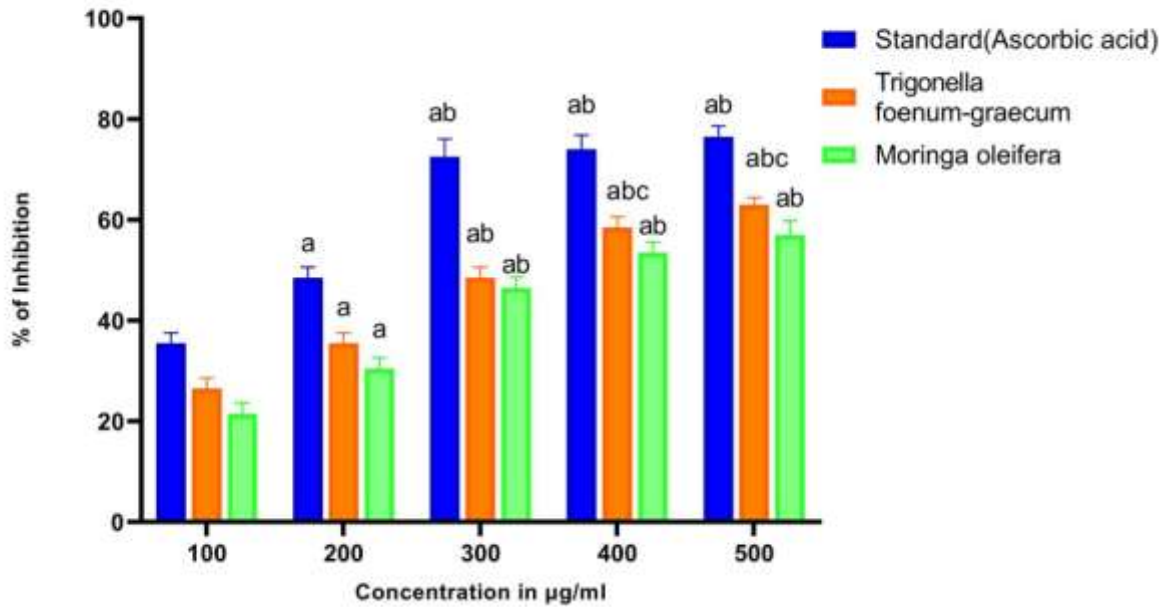
Table 1:Phytochemical analysis of aqueous seed extract of *Fenugreek* and *Moringa oleifera*

PHYTOCHEMICALS	FENUGREEK	MORINGA OLEIFERA
Amino Acids	++	-
Flavonoids	++	++

Alkaloids	++	+
Terpenoids	+	+
Steroids	++	++
Carbohydrates	-	-

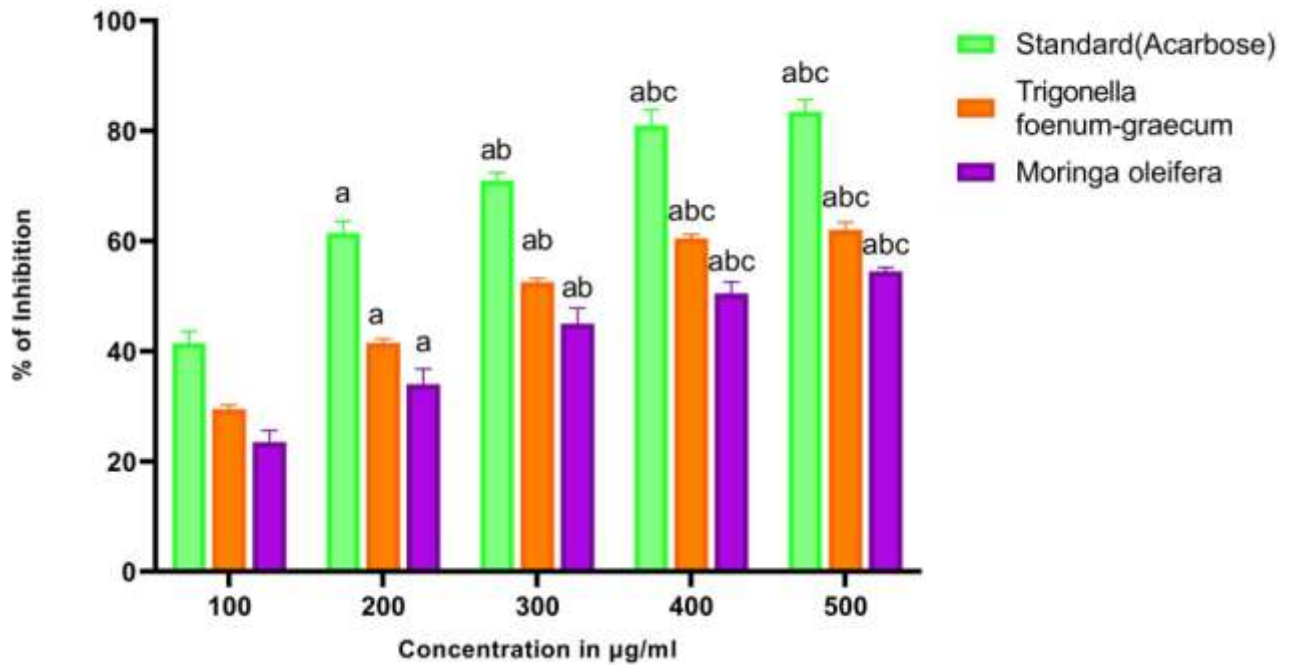
Trigonella foenum-graecum shows the presence of Amino Acids, Flavonoids, Alkaloids which have higher antidiabetic potential than Terpenoids and carbohydrates. In *Moringa oleifera* , the presence of flavonoids, steroids indicates the higher antidiabetic potential than Alkaloids and carbohydrates.

Antioxidant potential of aqueous seed extract of *Trigonella foenum-graecum* and *Moringa oleifera*



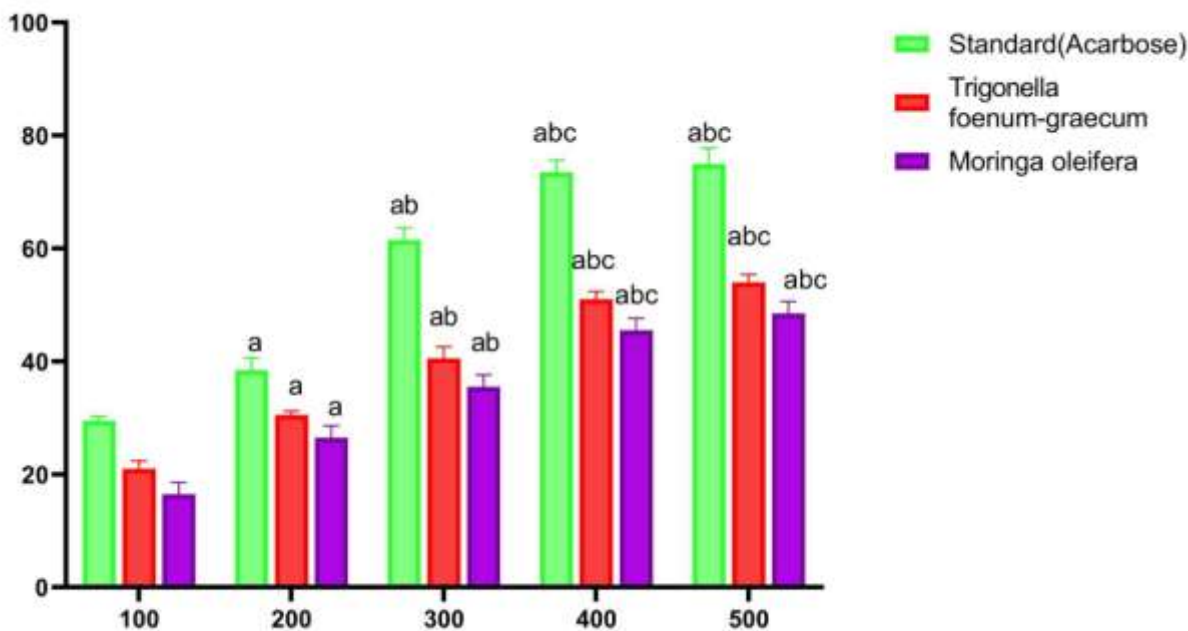
Graph 1 represents Antioxidant potential of aqueous seed extracts of *Trigonella foenum-graecum* and *Moringa oleifera* compared with standard Ascorbic acid. X axis represents the concentration in µg/ml and Y axis represents the inhibitory potential of the extracts. Blue bar represents standard Ascorbic acid, orange bar represents *Trigonella foenum-graecum* and green bar represents *Moringa oleifera*. Each bar represents Mean \pm SEM of 3 independent observations. Significance at $p < 0.05$.

α amylase inhibitory potential *Trigonella foenum-graecum* and *Moringa oleifera*



Graph 2 represents Alpha Amylase Inhibitory potential of aqueous seed extracts of *Trigonella foenum-graecum* and *Moringa oleifera*. X axis represents the concentration in $\mu\text{g/ml}$ and Y axis represents the Alpha amylase inhibitory potential of the extracts. Green bar represents standard Acarbose, orange bar represents *Trigonella foenum-graecum* and purple bar represents *Moringa oleifera*. Each bar represents Mean \pm SEM of 3 independent observations. Significance at $p < 0.05$.

α glucosidase inhibitory potential of *Trigonella foenum-graecum* and *Moringa oleifera*



Graph 3 represents Alpha Glucosidase Inhibitory potential of aqueous seed extract of *Trigonella foenum-graecum* and *Moringa oleifera*. X axis represents the concentration in µg/ml and Y axis represents the alpha glucosidase inhibitory potential of the extracts. Green bar represents standard Acarbose, red bar represents *Trigonella foenum-graecum* and purple bar represents *Moringa oleifera*. Each bar represents Mean \pm SEM of 3 independent observations. Significance at $p < 0.05$.

DISCUSSION:

Phytochemicals are secondary metabolites only present in plants. Plants possess various biologically active compounds that protect and help the human body. (36) Phytochemical analysis has revealed (Table 1) a strong presence of alkaloids, flavonoids and terpenoids in *trigonella foenum-graecum* and *Moringa oleifera* seed extracts (3). The presence of phytochemicals like alkaloids, glycoside, tannins, carbohydrates, phenols indicates that the extract is potential for further in vitro analysis like antioxidant activity and antidiabetic activity.

Antioxidant activity of aqueous seed extracts of *trigonella foenum-graecum* and *Moringa oleifera* was determined by performing DPPH free radical scavenging assay. Free radicals are molecules possessing an unpaired electron (37). Herbal extracts which are rich in phenolic compounds have great importance in free radical scavenging. The effect of antioxidants on DPPH free radical scavenging was

considered to be due to their hydrogen donating ability. The extracts were analysed for their antioxidant potential. Both the extract exhibited a significant antioxidant potential, when compared *trigonella foenum-graecum* exhibited a strong antioxidant potential with IC_{50} of 320 $\mu\text{g/ml}$ then *Moringa oleifera* (IC_{50} of 360 $\mu\text{g/ml}$) (Graph 1) as compared with the standard vitamin C.

In vitro Antidiabetic activity was analysed for the extract and compared with the standard drug metformin Both Alpha amylase and Alpha glucosidase inhibitory potential was estimated to analyse antidiabetic potential.(38).The ethanolic seed extract of *trigonella foenum-graecum* exhibited significantly more inhibition for Alpha amylase and Alpha glucosidase(Graph 2and 3) with IC_{50} of 280 and 390 $\mu\text{g/ml}$ respectively.Previous studies on antidiabetic potential of *trigonella foenum-graecum* has also exhibited a significant inhibitory potential, where aqueous seed extract of *trigonella foenum-graecum* a dose of 25mg/kg body weight given to experimental animals for 60days,significantly decreased blood glucose and liver enzymes level.However,no histological liver protective role was reported.(3)

The extracts are tested for its role as a starch blocker(39).Since,it prevents or slows the absorption of starch mainly by blocking the hydrolysis of glycosidic linkage in starch(40).There is a positive relationship between phytoconstituents contents and its ability to inhibit alpha glucosidase and alpha amylase.From the results,it was evident that both the aqueous seed extract of *trigonella foenum* and *Moringa oleifera* possess antioxidant and antidiabetic potential and increased in a dose dependent manner as compared to standard.(41)The extracts are capable of inhibiting the absorption of glucose.(42)Further research is needed to evaluate the *in vivo* antidiabetic potential of the extracts. Further validation of the drug's ability to decrease glucose concentration in serum can help in the drug formulation.

CONCLUSION

Even though there are many drugs available for diabetes, a complete cure remains a dream. Current *In vitro* findings on the antioxidant and antidiabetic potential of seed extracts of *Trigonella foenum-graecum* and *Moringa oleifera* showed that both the plant extracts have potential antidiabetic activity due the presence of the bioactive compounds.

Source of funding:

The present study was supported by the following agencies.

- Saveetha Dental College
- Saveetha Institute of Medical and Technical Sciences (SIMATS)

- Saveetha University
- Jyothi gas agency

Acknowledgment

The authors would like to thank Saveetha Dental College and Hospitals, Saveetha Institute of medical and technical sciences, Saveetha University for providing research laboratory facilities to carry out the study.

Conflict of interest

All the authors declare that there was no conflict of interest in the present study.

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