

COMPARATIVE EVALUATION OF ANTI-DIABETIC POTENTIAL OF AQUEOUS SEED EXTRACTS OF *MOMORDICA CHARANTIA*, SEED KERNEL EXTRACT OF *MANGIFERA INDICA* AND ITS HERBAL FORMULATION - AN *IN VITRO* STUDY

Running title: Anti-diabetic potential of aqueous seed extracts of *Momordica charantia*, seed kernel extract of *Mangifera indica*, an in vitro analysis.

ABSTRACT:

Introduction: A condition in which the body's ability gets impaired to process blood glucose level is called diabetes, otherwise known as blood sugar. It ranked sixteenth among leading causes of death around the globe. There are estimated 72.96 million cases diagnosed with diabetes in the adult population of India. *Momordica charantia* possess anti-diabetic, anti hyperglycemic and anti inflammatory properties. *Mangifera indica* is traditionally used to treat diarrhoea, cancer, diabetes and tooth aches.

Aim: Aim of this study is to analyse comparative evaluation of anti-diabetic potential of aqueous seed extracts of *Momordica charantia*, seed kernel extract of *Mangifera indica* through *in vitro* analysis.

Methods: *Momordica charantia* and *Mangifera indica* were collected from local farms, and the extracts were analysed for its phytochemicals. Antioxidant and Anti-diabetic potential of the seed extract were evaluated along with standards. 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.

Results: *Momordica charantia* and *Mangifera indica* and its herbal formulation showed significant antioxidant and anti-diabetic potential.

Conclusion: The results showed both the seed and seed kernel extracts of *Momordica charantia* and *Mangifera indica* possessed anti-diabetic activity but the herbal formulation possessed much more significant anti-diabetic activity than the individual extract.

Key words: *Momordica charantia*, *Mangifera indica*, anti-diabetic, *in vitro*, therapeutic efficacy, Innovative technology, Novel method

INTRODUCTION:

A condition in which the body's ability gets impaired to process blood glucose level is called diabetes, otherwise known as blood sugar. Diabetes Mellitus is a major and rapidly increasing health problem in most of the countries around the world, and is an important cause of prolonged ill health and early death. It ranked sixteenth in the cause of global mortality in 1990, for about 5,71,000 deaths (1). Recent studies of geographical and ethnical influences have shown and concluded that people of Indian origin are highly prone to diabetes (2). The number of adults affected from diabetes in India is predicted to increase three times from 19.4 million in 1995 to 57.2 million in 2025 (3). The global diabetic prevalence in 2019 is estimated to be 9.3%. Different types of diabetes can occur, and managing the condition depends on the type. Not all forms of diabetes occur because of an inactive lifestyle. In fact, some are present from childhood. There are 3 types of diabetes classified into type 1, type 2 and gestational diabetes. Type 1 diabetes, also known as juvenile diabetes, occurs when the body fails to produce insulin. People with type 1 diabetes are insulin dependent, in which people must be injected with artificial insulin to stay alive. Type-2 diabetes is affected by the way the body uses insulin. While the

body still produces insulin, unlike in Type-1, the cells present in the body do not respond effectively as they once did. This is the most common type of diabetes, according to the National Institute of Diabetes and Digestive and Kidney Diseases, and it is very much related to obesity. Gestational diabetes occurs in women during pregnancy when the body becomes less sensitive towards insulin. Gestational diabetes does not occur in all women but usually continues in women even after giving birth.

Antioxidant activity is the property of a limitation of proteins, lipids, DNA or other oxidation of molecules. Primary antioxidants show effect directly in scavenging free radicals, and secondary antioxidants indirectly cause prevention of free radicals through Fenton's reaction (4) Free radicals play a very significant role in tissue development in living organisms (5)(6). *Momordica charantia* and *Mangifera indica* possess various antioxidant properties.

Medicinal plants are used as primary health care, as an alternative option to modern synthetic drugs in developing countries (7). During the past few years *Momordica charantia* has grabbed attention from researchers. *Momordica charantia* also called as "bitter melon" or African cucumber is a plant of *Cucurbitaceae* family widely cultivated in tropical and sub-tropical regions and is commonly used in Mediterranean traditional medicine for its anti-diabetic properties and antihyperglycemic and anti-inflammatory activities. The fruits, stems, leaves and roots of bitter melon are all used as traditional medicine to treat hyperlipidemia, digestive disorders, microbial infections and menstrual problems (8). The plant possesses traditional usage and modern scientific evidence of benefits of *Momordica charantia*, it is one of the most promising plants for the usage in treating diabetes (9) (10).

Mangifera indica, commonly known as mango, belongs to the *Anacardiaceae* family. *Mangifera* genus contains 69 species. The bark of the mango tree is traditionally used to treat diarrhea, cancer, diabetes, toothache, skin infections and used as diuretic, antiseptic, hepatoprotective agent (11). The mango plant shows both invitro and invivo antioxidant properties (12). Parts of the plant also show hypoglycemic activity (13). Plant seeds have also shown antibacterial activity (14). Though there is several work done on the various parts of the plants, much work has not been documented on the seed and seed kernel extracts of the plants. The aim of the study is to analyze and compare the anti-diabetic potential of aqueous seed extract of *Momordica charantia*, seed kernel extract of *Mangifera indica* and its herbal formulation as an in vitro study.

Even though there are many drugs, the main disadvantage of current drugs is that they have to be given throughout the life and they produce side effects. By exploring the natural herbs to replace the synthetic drugs, prevention can be done naturally without any side effects if significant research is done. Our team has extensive knowledge and research experience that has translate into high quality publications(15),(16),(17),(18),(19),(20),(21),(22),(23),(24),(25),(26),(27),(28),(29),(30),(31), (32),(33),(34)The present study was undertaken to analyze the anti-diabetic effect of *Momordica charantia* and *Mangifera indica*. Alternate hypothesis is that there is an anti-diabetic activity of *Momordica charantia* and seed kernel extract of *Mangifera indica*.

MATERIALS AND METHODS:

Plant Extract:

Momordica charantia and seed kernel of *Mangifera indica* were collected from a farm in Chennai, washed, crushed and made into powder. Crushed seed powder was utilized to prepare an 80% aqueous extract. Equal volume of the extract was mixed and a formulation was prepared. 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 a red or dull violet ring 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. H₂SO₄ 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(13).

Antioxidant activity:

DPPH free radical scavenging activity:

Scavenging of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was assessed by the method of Hatano et al^[1989]. DPPH solution (1.0 ml) was added to 1.0 ml of extract at different concentrations (0.1 to 0.5mg/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 scavenging potential was calculated and expressed in percentage (%) using following formula:

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

Control OD

Alpha amylase inhibitory activity of aqueous seed extract of *Momordica charantia* , seed kernel extract of *Mangifera indica*

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° 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.

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

AS = Absorbance in the presence of test substance

AC = Absorbance of control

Alpha glucosidase inhibitory activity of aqueous seed extract of *Momordica charantia*, seed kernel extract of *Mangifera indica*

Alpha glucosidase inhibitory activity of extract was carried out according to the method of (35)). 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 200µl of 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.

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

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

Statistical analysis

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

RESULTS AND DISCUSSION

Table 1: Phytochemical analysis of aqueous seed extract of *Momordica charantia* and seed kernel extract of *Mangifera indica*

Flavonoides are more concentrated in *Momordica charantia* than in *Mangifera indica*. Alkaloids are equal in concentration in *Momordica charantia* and *Mangifera indica*. Terpenoids are more concentrated in *Mangifera indica* than in *Momordica charantia*. Saponins are equal in concentration in *Momordica charantia* and *Mangifera indica*. Steroids are equal in concentration in *Momordica charantia* and *Mangifera indica*. Carbohydrates are equal in concentration in *Momordica charantia* and *Mangifera indica*.

Phytochemicals	<i>Momordica charantia</i>	<i>Mangifera indica</i>
Flavonoides	++	+
Alkaloids	+	+
Terpenoides	+	++
Saponins	+	+
Steroids	+	+
Carbohydrates	-	+

Table 1: Phytochemical analysis of aqueous seed extract of *Momordica charantia* and seed kernel extract of *Mangifera indica*

Antioxidant potential of *Momordica charantia*, *Mangifera indica* and its combination

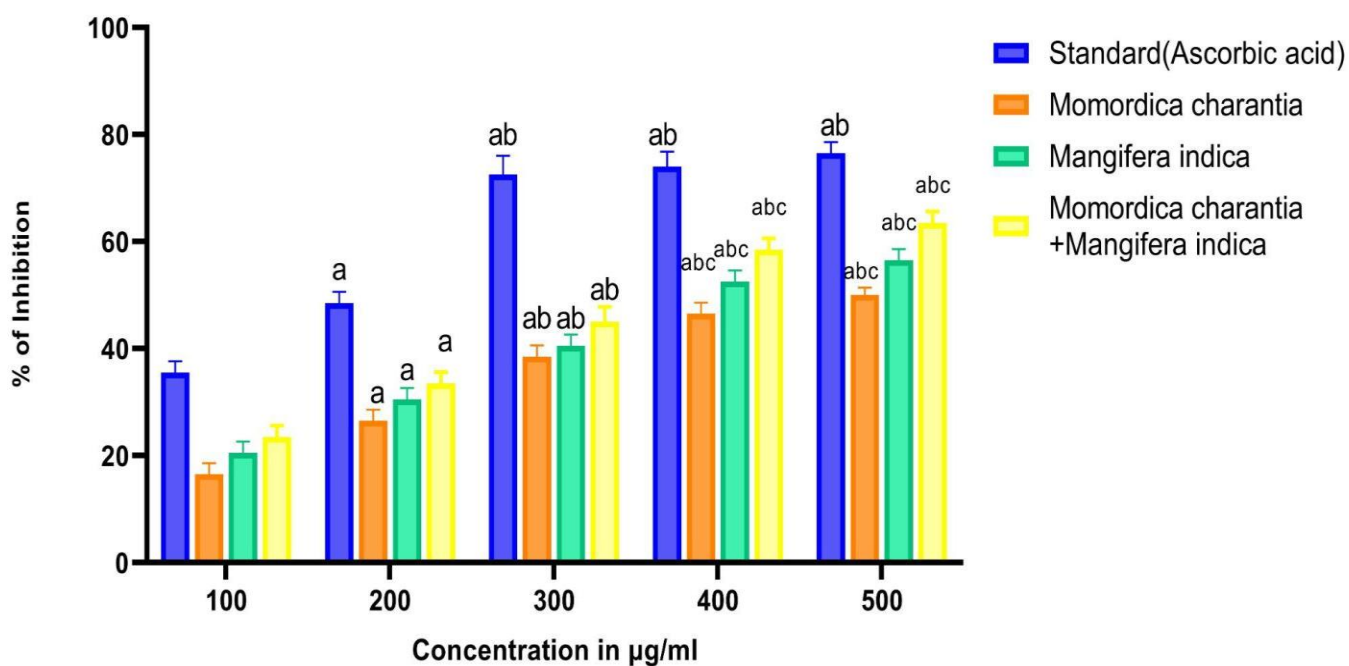


Figure 1: Represents antioxidant potential of aqueous seed extracts of *Momordica charantia*, seed kernel extract of *Mangifera indica* and its herbal formulation against the standard Ascorbic acid- DPPH Assay. X axis represents the concentration in µg/ml and Y axis represents the inhibitory potential of the extracts. Blue bar represents standard Ascorbic acid, orange bar represents aqueous seed extracts of *Momordica charantia* green bar represents seed kernel extract of *Mangifera indica* and yellow bar represents its herbal formulation. Each bar represents Mean \pm SEM of 3 independent observations. Significance at $p \leq 0.05$. X axis represents the concentration in µg/ml and Y axis represents the inhibitory potential of the extracts.

α Amylase potential of *Momordica charantia*, *Mangifera indica* and its combination

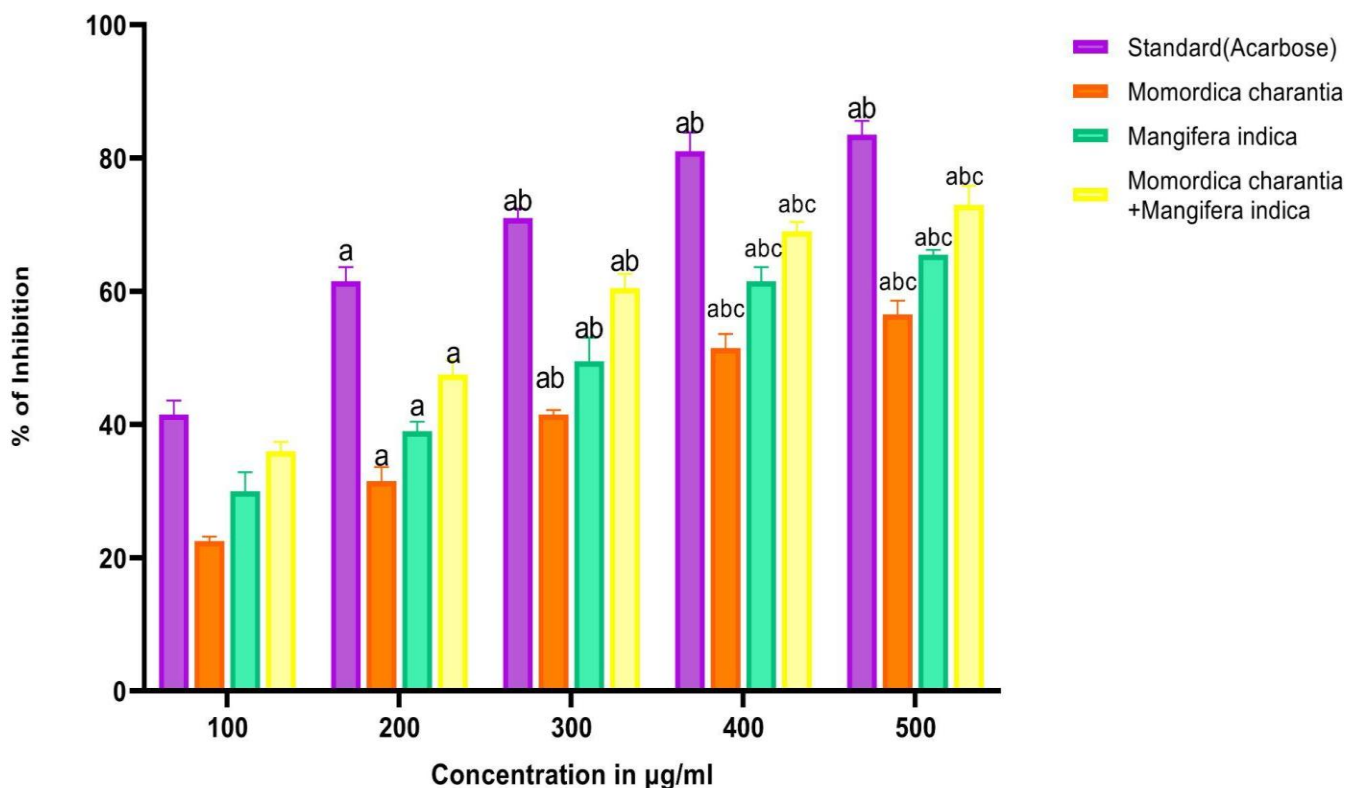


Figure 2: Represents Alpha Amylase inhibitory potential of aqueous seed extracts of *Momordica charantia*, seed kernel extract of *Mangifera indica* and its herbal formulation against the standard Acarbose. X axis represents the concentration in µg/ml and Y axis represents the inhibitory potential of the extracts. Purple bar represents standard Acarbose, orange bar represents aqueous seed extracts of *Momordica charantia* green bar represents seed kernel extract of *Mangifera indica* and yellow bar represents its herbal formulation. Each bar represents Mean \pm SEM of 3 independent observations. Significance at $p \leq 0.05$.

α Glucosidase inhibitory potential of *Momordica charantia*, *Mangifera indica* and its combination

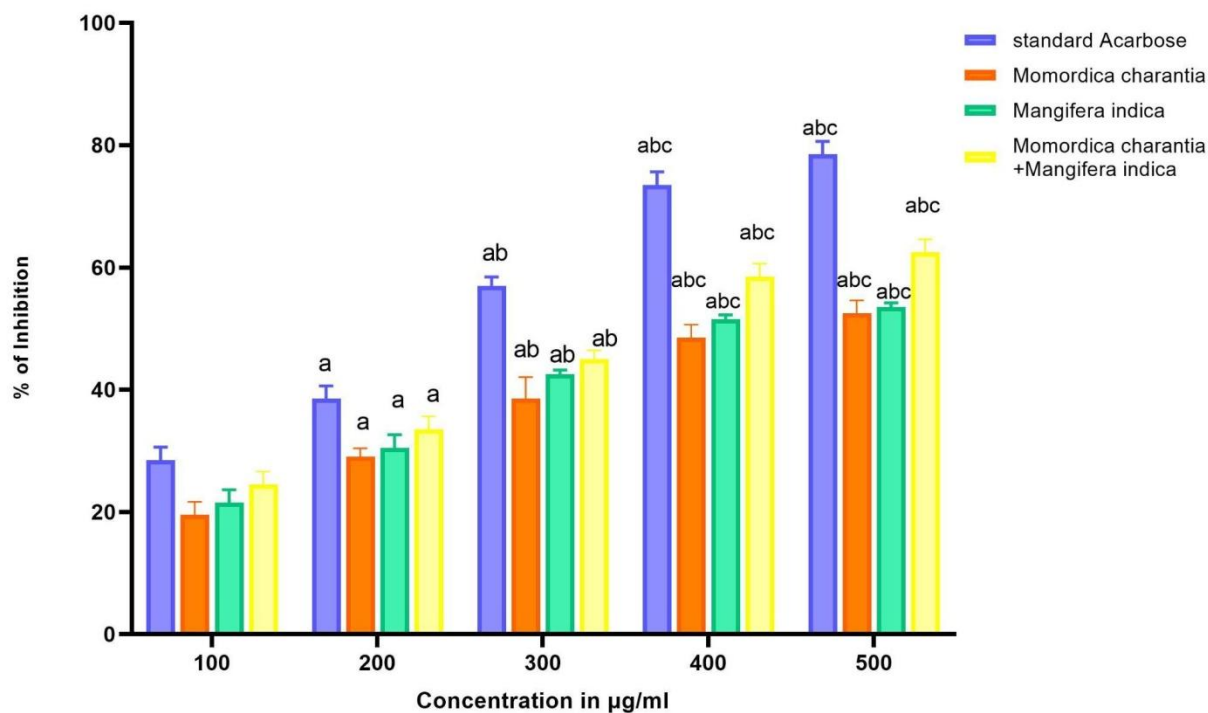


Figure 3: Represents Alpha Glucosidase inhibitory potential of aqueous seed extracts of *Momordica charantia*, seed kernel extract of *Mangifera indica* and its herbal formulation against the standard Acarbose. X axis represents the concentration in µg/ml and Y axis represents the inhibitory potential of the extracts. Blue bar represents standard Acarbose, orange bar represents aqueous seed extracts of *Momordica charantia* green bar represents seed kernel extract of *Mangifera indica* and yellow bar represents its herbal formulation. Each bar represents Mean ± SEM of 3 independent observations. Significance at $p \leq 0.05$.

The results revealed a strong presence of phytochemicals such as alkaloid, flavonoids, terpenoids, saponins and steroids in both the seed extract of *Momordica charantia* and seed kernel extract of *Mangifera indica* (Table:1). Phytochemicals are secondary metabolites which

are present only among plants. They possess various biologically active compounds that protect and help in normal functioning of the human body. The presence of phytochemicals like alkaloids, terpenoids, saponins, steroids and flavonoids indicate that the extract has potential for further *in vitro* analysis like antioxidant activity, antidiabetic activity.

Antioxidant activity of aqueous seed extract of *Momordica charantia* and seed kernel of *Mangifera indica* was determined by DPPH free radical scavenging assay. Free radicals / molecules possessing an unpaired electron leads to oxidative stress. Phenolic compounds have great importance in free radical scavenging activity. Similar findings by G. Leela Prakash et al suggest a significant total antioxidant activity possessed by both the aqueous and methanol extract of *Momordica charantia*. Samba Fama Ndoye et al, 2018, has analysed the total antioxidant activity by DPPH radical scavenging activity. The effects of the antioxidants on DPPH free radical scavenging was considered to be due to their hydrogen donating ability. The results obtained in the study show that aqueous seed extract of *Momordica charantia*, seed kernel extract of *Mangifera indica* and its herbal formulation exhibit significant antioxidant activity with IC_{50} of 450, 410 and 350 $\mu\text{g/ml}$ respectively as compared with the standard Vitamin C (Figure 1). Herbal formulation was found to exhibit a significantly more antioxidant potential than the individual extracts. Further studies may be needed to find out the potential health benefits of the extracts in prevention and scavenging of free radicals.

In the study, *in vitro* α -amylase inhibitory activity and α -glucosidase inhibitory activity of aqueous seed extracts of *Momordica charantia*, seed kernel extract of *Mangifera indica* and its formulation were studied. Results revealed a dose dependent increase in % of inhibitory activity. The extracts showed potent anti diabetic activity in a dose dependent manner, with a IC_{50} for α -amylase inhibitory activity was found to be 380, 400, 360 $\mu\text{g/ml}$ respectively (Figure 2) and IC_{50} for α -glucosidase inhibitory activity was found to be of 420, 400, 320 $\mu\text{g/ml}$ respectively (Figure 3) and increased in a dose dependent manner as compared to the standard. Acarbose is a standard drug used for treating diabetes, and used for the comparison of anti diabetic activity of the extract. The extracts might be used as starch blocker since it prevents or slows the absorption of starch into the body, mainly by blocking the hydrolysis of glycosidic linkage of starch. There is a positive relationship between phytoconstituent content and ability to

inhibit α -glucosidase and α -amylase. Outcome of this study indicates that aqueous extracts of *Momordica charantia*, *Mangifera indica* and its herbal formulation could be used as potential antidiabetic agents. Similar to antioxidant potential, formulation exhibited an increased anti-diabetic potential compared to its individual extract.

The increased antioxidant and anti-diabetic potential of the herbal formulation compared to the individual extract can be because of the synergistic effect. More studies need to be done to check the medicinal value of polyherbal formulations. Many indigenous medicines come as a formulation and research is needed to check and document the role of each extract and the synergism shown by the herbal extracts when made into a formulation. Only a proper research on this avenue can help spread awareness on the importance of polyherbal drug preparation and its potential in curing various ailments.

In future, further purification and toxicity studies followed by in vivo studies on the herbal formulation needs to be done. Exploring natural drugs by various research can replace synthetic drugs and its side effects. Prevention can be done even before getting diagnosed with the disease. As there is no significant research done on anti-diabetic properties of seed extract of *Momordica charantia*, seed kernel extract of *Mangifera indica* and its formulation, the current can be a feasible option for further research for drug formulation and diabetic cure.

CONCLUSION:

From the obtained results, it can be concluded that herbal formulation of aqueous seed extract of *Momordica charantia*, seed kernel extract of *Mangifera indica* possessed a significant antioxidant and antidiabetic potential than the individual extract. In future, more research is needed to throw light on the synergistic role of herbal extracts in a formulation to create awareness on the rich medicinal value of the folk and hereditary medicines.

Acknowledgement:

The authors would like to thank Saveetha Dental College and Hospitals, SIMATS, Saveetha University for providing laboratory facilities to conduct the study.

Statement of conflict of interest:

There is no conflict of interest as declared by the author.

SOURCE OF FUNDING:

The present study was supported by the following agencies

- Saveetha Dental College,
- Saveetha Institute of Medical and Technical Science,
- Saveetha University
- The Kalpana Indane Gas Agency.
- **NOTE:**
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- The study highlights the efficacy of "traditional medicine" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.
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