IJPSR (2018), Volume 9, Issue 3 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 31 May, 2017; received in revised form, 15 September, 2017; accepted, 30 September, 2017; published 01 March, 2018

COMPARATIVE ANALYSIS OF CHEMICAL AND PHYTOCHEMICAL COMPOSITIONS OF FOUR SELECTED MEDICINAL PLANTS AND EVALUATION OF ANTIDIABETIC PROPERTIES OF AQUEOUS EXTRACTS OF *TRIGONELLA FOENUM-GRAECUM* AND COMBINATION OF *TRIGONELLA FOENUM-GRAECUM* - *MOMORDICA CHARANTIA*

Md. Golam Mukit, Md. Salim Raza, Asad Ud-Daula, Bably Sabina Azhar and A. T. M. Mijanur Rahman *

Department of Applied Nutrition and Food Technology, Islamic University, Kushtia - 7003, Bangladesh.

Keywords:

Medicinal plants, Proximate composition, Phytochemicals, Polyherbal therapy, Antidiabetic activity

Correspondence to Author:
A. T. M. Mijanur Rahman

Associate Professor,
Department of Applied Nutrition and Food Technology, Islamic University, Kushtia - 7003, Bangladesh.

E-mail: mijananftiubd@gmail.com

ABSTRACT: The present study was designed to compare the chemical and phytochemical compositions of *Momordica charantia*, *Azadira chtaindica*, *Trigonella foenum-graecum* and *Phyllanthus emblica* and subsequently, antidiabetic activities of *T. foenum-graecum* and a combination of polyherbal extract of *T. foenum-graecum* and *M. charantia* were evaluated on alloxan induced diabetic mice. The proximate and phytochemical compositions of the plants were quantitatively determined using standard methods. In order to evaluate antidiabetic activity, normal and alloxan induced diabetic mice were orally administered 0.5 mL aqueous extract of *T. foenum-graecum*and combination of *T. foenum-graecum* - *M. charantia* once per day for 7 days. These two plants were selected for antidiabetic activity based on their high phytochemical contents. A near normal blood glucose value of 98.5 ± 0.2 mg / 100mL and 97.02 ± 0.55 mg / 100 mL was observed in the 7th and 5th day experimental animals after administration of *T. foenum-graecum* and *T. foenum-graecum* - *M. charantia*, respectively and remained at the same level even after further oral administration. Consequently, it is evident that compared with single extract of *T. foenum-graecum*, the combined polyherbal extract of *T. foenum-graecum* - *M. charantia* promptly lowered blood glucose and maintained a relatively steady level over the study period. However, there was no change in blood glucose levels of control animals subsequent to administration of either extracts of *T. foenum-graecum* or *T. foenum-graecum* - *M. charantia*.

INTRODUCTION: The use of plants with medicinal properties for the treatment of different diseases is as old as human civilization [1]. Over the past decade, herbal medicine has become a topic of global importance, making an impact on both world health and international trade.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.9(3).1307-16

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.9(3).1307-16

It is estimated by the World Health Organization that approximately 80% of the world's population depend on traditional medicine for their primary health care [2].

Herbal medicine has been used continuously by a large proportion of the population in the developing countries like Bangladesh largely due to the high cost of western pharmaceuticals and healthcare. Traditional medicines are also becoming more accepted in these countries because of their ready availability, as well as their cultural and spiritual points of view [3].

Bangladesh is considered rich in medicinal plants genetic resources by virtue of its favorable agroclimatic condition and seasonal diversity. With productive soils, a tropical climate, and seasonal diversity, Bangladesh contains about 6500 plants species among them, 500 plant species have medicinal values [4]. Almost 80% people of are dependent on natural resources (*e.g.*, medicinal plants) for their primary healthcare [5], with herbal medication remaining a popular and accepted form of treatment [6].

Medicinal plants can be used to treat various diseases along with diabetes mellitus which is considered as the third major cause of death after cancer and heart disease [7]. The International Diabetes Federation (IDF) estimated that 8.7 million or 4.9% of people living in Bangladesh had diabetes in 2014 and by 2030, that number is expected to grow to 13% of the population [8]. This explosion in diabetes prevalence will place Bangladesh among the top ten countries in terms of the number of people living with diabetes in 2025. Although various medicinal plants used in the treatment of diabetes, an ethnobotanical survey revealed that *Momordica charantia*, *Azadirachta indica*, *Trigonella foenum - graecum* and *Phyllanthus emblica* are most frequently used for the management of the disease in Bangladesh [9].

Fruit of *M. charantia* is a very popular and versatile Bangladeshi vegetable. It can serve as an excellent source of protein, carbohydrate, minerals, vitamins and other nutrients [10] which are essential for human health. It also contains various significant phyto-chemicals like saponins, tannins, flavonoids, alkaloids, and phenolic compound which can act as preventive agents in many other disorders including hyperglycemia [11]. Aqueous extracts of *M. charantia* reduced blood glucose level when it was treated with streptozotocin induced diabetic rats [12]. *Azadirachta indica* has also a long history of use in folk medicine as a treatment against various ailments [13]. Water extract of *A. indica* was found to significantly lower the elevated blood glucose in glucose loaded and alloxan induced diabetic rats [14].

Fenugreek seeds contain various phytochemicals including alkaloids, flavonoids, saponins, tannins and caumarin and minerals and Vitamins [15]. An experimental study revealed that water extracts of fenugreek seeds contain a bioactive compound which has a capability of lowering blood glucose level when it is administered in alloxan induced diabetic rabbits intermittently [16]. *Phyllanthus emblica* is highly nutritious and is one of the richest sources of Vitamin C, amino acids, minerals and rich in proximate composition like moisture, protein, carbohydrate, fibre *etc*. It contains several chemical constituents like tannins, alkaloids and phenols which are reported to possess biological activity [17]. The aqueous extract of *P. emblica* fruit has been reported to have hypoglycemic potential as well as antidiabetic activity [18].

There are many kinds of medicinal plants available in Bangladesh which are rich in nutrients. However, due to the ignorance and lack of proper knowledge of the people, they do not know the nutritive value of most of these medicinal plants. Moreover, these plants have also been used as a source of therapeutics because of their biodiversity and affluent of phytochemicals and secondary metabolites [19]. However, there has been no report of studying the chemical and phytochemical compositions of the four most commonly used medicinal plants in Bangladesh, which are very important for the management of diabetes mellitus.

A single medicinal plant cannot be as effective as the polyherbal therapies or treating severe diseases. Because polyherbal therapies, the combination of various types of agents from different plant sources, have the synergistic, potentiative, agonistic / antagonistic pharmacological agents within themselves, which work together in a dynamic way to produce therapeutic efficacy with minimum side effects. In combined form, the extracts tend to complement each other thereby producing the desired normoglycemia [20]. Although *T. foenum-graecum* and *M. charantia* are being used for the management of diabetes for a long time, to the best of our knowledge, no study has investigated the anti-diabetic activity of the combined aqueous extract of *T. foenum-graecum* and *M. charantia*.

The object of the present study is to examine the proximate and phytochemical compositions of *M. charantia*, *A. indica*, *T. foenum-graecum* and *P. emblica*. An additional experiment was also conducted on alloxan induced diabetic mice to evaluate the antidiabetic properties of a single

medicinal plant extract of *T. foenum-graecum*, and a combination of polyherbal extract of *T. foenum-graecum* and *M. charantia*.

MATERIALS AND METHODS:

Chemicals: Ethanol, methanol, chloroform, formaldehyde and gallic acid were purchased from Merck (KGaA 64271 Damstadi, Germany) and diethyl ether from BDH Laboratory (Supples Poole, BH15. 1TD. England). 3, 5-dinitrosalicylic acid used as a crosslinking agent and aluminium chloride, potassium acetate, potassium sulphate were obtained from Labochemie Pvt. Ltd., (107 Wondehouse Road, Jehangir Villa, Mumbai - 400005, India). Quercetin, protein standard and all other chemicals used in this study were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

Collection of Plant Materials: Matured fresh fruits of *M. charantia* and seeds of *T. foenum-graecum* were collected between November and December, 2015 respectively from the Sheikhpara bazar adherent to the Islamic University Kushtia, Bangladesh. Leaves of *A. indica* was collected from the botanical garden of Islamic University Kushtia, Bangladesh and fruits of *P. emblica* was collected from the herbal market of Kushtia, Bangladesh, A plant taxonomist, Dr. Nur-Es-Saba Homyra from the Department of Botany of Kushtia Government College identified all the plants.

Preliminary Processing of Plant Materials: The fruits of *M. charantia* and *P. emblica* and leaves of *A. indica* were cleaned with water to remove soil, dust and foreign materials. Subsequently, the seeds dissected fruits (pericarp) and leaves were chopped into smaller pieces with a sharp knife and then air dried for five days. On the other hand, the seeds of *T. foenum-graecum* were sieved to remove foreign particles and subsequently, properly cleaned seeds were air dried for five days. The dried fruits, leaves and seeds then separately ground into powder with the aid of an electrical grinder. These pulverized samples were thereafter packed in air-tight plastic containers and stored in the refrigerator (2 - 8 °C), from where aliquots were withdrawn and used for individual analysis.

Chemical Analysis: The proximate compositions (moisture, ash, crude protein and crude fibre) were determined based on the standard methods of the association of official analytical chemist [21], while lipid content by the method of Bligh and Dyer [22]. The total percentage carbohydrate content was determined by the difference method as reported by Edeogu *et al*., [23]. Total soluble sugar and starch were estimated through following anthrone reagent method [24]. The concentration of reducing sugars was determined according to methodology described by Miller [25]. The total soluble protein content was estimated by using method of Lowery *et al*., [26] The quantitative estimation of bioactive chemical constituents of the four medicinal plants under study were carried out in aqueous extracts using the standard procedures as described by Harborne [27], Trease and Evans [28] and Sofowara [29].

Experimental Animals: Healthy mature Swiss-albino mice with bodyweight ranging from 25 to 30 grams were collected from the Department of Biotechnology and Genetic Engineering of Rajshahi University, Bangladesh used in the present study. The animals were acclimatized to standard laboratory conditions (temperature 24 ± 1 °C, relative humidity 55 ± 5%) and a 12 h photoperiod in suspended wire meshed galvanized cages (4 - 6 mice/cage) for one week before the commencement of the experiment. During the entire period of study, the mice were supplied with a semi-purified basal diet and water *ad libitum*.

Extraction of Aqueous Plant Materials: Aqueous extract of *T. foenum-graecum* seeds powder and mixture of *T. foenum-graecum* - *M. charantia* powder was prepared by grinding 300 mg of dried seeds of *T. foenum-graecum* and *M. charantia* fruits in 3 mL of glass distilled water. 0.5 mL of this solution was administered once per day to each set of ten animals. Freshly prepared extracts were administered.

Induction of Diabetes and Study Design: The blood glucose levels of normal male mice were determined and allowed to fast overnight. A single intra-peritoneal injection of alloxan monohydrate with a dosage of 120 mg/kg body weight in physiological saline was given [30]. This dosage was prepared because it produced maximum glucose levels. Mice with glucose levels ranging between 200 mg/dl and 350 mg/dl were considered severely diabetic and use for estimations of blood glucose at 1st, 3th, 5th, and 7th day after administration of

alloxan. The animals were divided in to six groups of five each.

Group I: Control mice with normal saline (5 mL/animal/day).
Group II: Mice with oral administration of *T. foenum - graecum* extract (50 mg/animal/day).
Group III: Mice with oral administration *of T. foenum - graecum - M. charantia* extract (50 mg/animal/day).
Group IV: Alloxan induced diabetic mice (120 mg/kg body weight).
Group V: Alloxan induced diabetic mice (120 mg/kg body weight) with *T. foenum-graecum* seeds extract (50 mg/animal/day) after alloxan treatment.
Group VI: Mice treated with a mixture of *T. foenum-graecum - M. charantia* extract (50 mg/ animal/day) after alloxan treatment.

The dosage to be most effective was 50 mg/animal (0.5 ml of extract) [31]. Animals were segregated after 1st, 3th, 5th, and 7th day after orally administration of seed and fruit extracts and the samples were collected. The same procedure was followed for alloxan induced diabetic animals. 1mL of peripheral blood (PB) was collected from the mice in sterile screw capped glass vials containing EDTA by using sterile disposable syringes. For blood glucose values were expressed as mg / 100mL.

Statistical Analysis: The results generated from the analysis were subjected to statistical analysis using the Statistical Package for Social Sciences (SPSS) Version 15. All data is expressed as Mean ± standard deviation (mean of three determinations).

RESULTS AND DISCUSSION:

Analysis of the Proximate Composition: Table 1 presents the result of the proximate chemical composition of *M. charantia*, *A. indica*, *T. foenum-graecum* and *P. emblica*. In comparison with the other plants, the value of moisture content was highest in *P. emblica* (85.4 ± 0.30%).

This high moisture content of *P. emblica* has also been reported by a previous study (81.2%) [32]. On the other hand, the low level of moisture content of *M. charantia* (7.90 ± 0.12%), *T. foenum-gracum* (7.71%), and *A. indica* (9.20 ± 0.10%) has been supported by other studies which were 10.74 ± 2.29%, 9.2%, and 10.30 ± 0.28% [33, 34, 35], respectively.

TABLE 1: PROXIMATE COMPOSITION OF *M. CHARANTIA, A. INDICA, T. FOENUM-GRAECUM* AND *P. EMBLICA*

	Medicinal plants			
Parameters (%)	***M. charantia***	***A. indica***	***T. foenum-gracum***	***P. emblica***
Moisture (wet wt.)	7.90 ± 0.4	09.20 ± 0.7	07.71 ± 0.09	85.4 ± 1.80
Ash (dry wt.)	7.40 ± 0.4	07.30 ± 0.3	5.33 ± 0.83	3.4 ± 0.70
Total protein (DW)	25.30 ± 0.7	11.93 ± 0.07	23.00 ± 0.4	1.04 ± 0.04
Fat (DW)	5.2 ± 0.2	4.37 ± 0.43	8.00 ± 0.1	0.51 ± 0.04
Carbohydrate (DW)	32.31 ± 0.31	44.71 ± 0.21	46.25 ± 0.25	15.91 ± 0.91
Total sugar (DW)	0.23 ± 0.02	9.00 ± 0.4	5.51 ± 0.2	10.35 ± 0.45
Reducing sugar (DW)	0.07 ± 0.01	03.00 ± 0.71	5.80 ± 0.05	4.40 ± 0.60
Crude fibre (DW)	12.50 ± 1.0	14.41 ± 0.35	13.10 ± 0.25	4.00 ± 0.40
Starch (DW)	0.82 ± 0.02	1.4 ± 0.50	6.5 ± 0.30	0.2 ± 0.06

*Data presented as mean value ± SD of triplicate determination

The results from the table indicate that the ash content of *M. charantia* in dry basis was highest (7.40 ± 0.01%) followed by *A. indica*, *T. foenum-graecum* and *P. emblica*. The values of *M. charantia* and *P. emblica* found in the present study were almost exactly the same as that of other studies [33, 36] which were 7.36 ± 0.52% and 3.76 ± 0.43%, respectively. However, there were only small differences between the previously reported values and the present values of total ash of *A. indica* and *T. foenum-graecum* [35, 37] which were 8.31 ± 1.52% and 7.71 ± 0.2%, respectively.

As can be observed in the table above, *M. charantia* contained the highest (25.30 ± 0.01%) amount of protein followed by *T. foenum-graecum* (23.00 ± 0.09%), *A. indica* (11.93 ± 0.31%), and *P. emblica* (1.04 ± 0.10%). These data are in agreement with previous reports that have shown that the total protein content of *M. charantia*, *A. indica*, *T. foenum-graecum* and *P. emblica* were 27.88 ± 3.75%, 8.93 ± 0.31%, 26.00 ± 0.2%, and 0.5%, respectively [33, 35, 38, 32]. Among the four different medicinal plants evaluated in this study, *T. foenum-graecum* contained the highest (8.00 ±

0.17%) amount of fat, followed by *M. charantia* (5.2 ± 0.02%), *A. indica* (4.37 ± 0.57%), and *P. emblica* (0.51 ± 0.10%). In a previous study, Zohary *et al*., [38] reported the total fat content in *T. foenum-graecum* was 8.00 ± 0.2 which is exactly the same value as in the experiment. In comparison with the other plants, the value of total carbohydrate content was highest in *T. foenum-graecum* (46.25 ± 0.22%). This high carbohydrate content of *T. foenum-graecum* has also been reported by a previous study (47.50 ± 2.20%) [36]. The total reducing sugar content was highest in *T. foenum-graecum* seeds (5.80 ± 0.05%) which is slightly lower than the previous report (7.71 ± 0.20%) [37]. The other data are also similar to findings with previous studies [39, 35, 40].

Moreover, the amount of total crude fibre was highest (14.41 ± 0.35%) in *A. indica* leaves followed by *T. foenum-graecum* (13.10 ± 0.25%), *M. charantia* (12.50 ± 1.13%) and *P. emblica* (4.00 ± 0.10%). These results are in agreement with previous studies [33], [35, 37, 40]. *T. foenum-graecum* seeds had the highest (6.5 ± 0.30%) amount of starch, while *P. emblica* had the lowest (0.2 ± 0.10%). In a previous study [38], the starch content in *T. foenum-graecum* was found to be 06.00 ± 0.30%. The total amount of starch content present in other two medicinal plants was 0.82 ± 0.02% in *M. charantia*, and 1.4 ± 0.50% in *A. indica*, while previous report indicated that it was 0.74 ± 0.01% [39] and 1.1 ± 0.4% [41] respectively. The little differences observed in the nutritional composition of the studied medicinal plants compared with previous studies might be due to different growth conditions, genetic factors, and geographical variations in the level of soil fertility.

Many traditional plants remedies are known in folk medicine and used for treatment and management of diabetes mellitus [42], and some have been validated by scientific studies to actually exert biological action against diabetes or its complications. In addition to their role played in human and animal nutrition, knowledge of proximate, micronutrients and phytochemical composition is fundamental to the understanding of modes and mechanisms of action of medicinal plants in general. Nutrients are necessary for life and good health; these may be found in a number of different foods.

The general functions of nutrients include fuel (energy) expressed in kcal, building materials for body structures and regulation and control of body processes. The proximate analysis shows that these medicinal plants are good sources of carbohydrate and protein; these may serve as source of energy and nutrients for the body metabolic activities in addition to its medicinal properties. The carbohydrates and proteins present in the plant may be a conglomerate of bioactive sugars, glyco-proteins or proteins which gives the plant its medicinal potency against certain diseases. Some plants are known to contain certain sugars which are biologically active against some diseases [43, 44].

Also, some plant proteins such as momorcharins (isolated from seeds of *M. charantia*), momorcochin (isolated from tubers of *Momordica cochinchinensis*) have been reported to exhibit abortifacient, antitumor, ribosome inactivating and immunomodulatory properties [45, 46]. The total ash content of the plants indicated that those plants are rich in minerals. The crude fat may add to the caloric value extractable from the plant for metabolic activities. The study also shows that these plants contain considerable amount of fiber, this could be beneficial when consumed. Dietary fibre is important for lowering blood cholesterol and blood sugar. It is known to reduce the risk of diseases such as obesity, diabetes, breast cancer, hypertension and gastrointestinal disorder [47].

Determination of Total Phenolic Content: The total phenolic content for aqueous extracts were estimated by Folin Ciocalteu's method using gallic acid as standard. Thegallic acid solution of concentration (0 - 7 mg/L) conformed to Beer's Law at 765 nm with a regression co-efficient (R^2) = 0.996. The plot has a slope (m) = 0.1403 and intercept = 0.0353. The equation of standard curve is y = 0.1403x + 0.0353 **Fig. 1**.

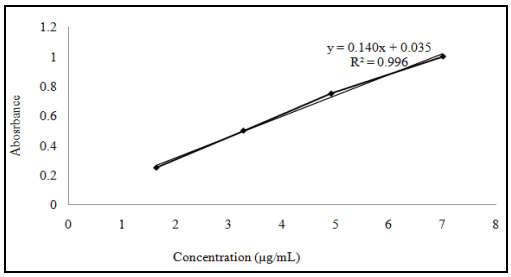

FIG. 1: TOTAL PHENOLIC CONTENT FOR STANDARD GALLIC ACID

It can be observed from **Fig. 2** that the value of total phenolic content was highest in *M. charantia* (565 ± 0.44 µg/mg) followed by *A. indica* (95.5 ± 1.20 µg/mg), *P. emblica* (32.05 ± 3.20 µg/mg), and *T. foenum-graecum* (6.50 ± 0.02 µg/mg). The high total phenolic content of *M. charantia* has also been reported by a previous study (561 ± 0.54 µg/mg) [48].

Determination of Total Flavonoid Content: The total flavonoid content for aqueous extracts were measured with the aluminium chloride colorimetric assay using quercetin as standard. The quercetin solution of concentration (1 - 5 µg/mL) conformed to Beer's Law at 510 nm with a regression co-efficient (R^2) = 0.9991. The plot has a slope (m) = 0.214 and intercept = - 0.0184. The equation of standard curve is y = 0.214x + (-0.0184) **Fig. 2**.

As can be seen from **Fig. 4**, *T. foenum-graecum* seeds contained the highest (610 ± 3.3 µg/mg) amount of total flavonoid followed by *A. indica* (30.35 ± 1.50 µg/mg), *M. charantia* (15.5 ± 0.32 µg/mg) and *P. emblica* (5.4 ± 5.50 µg/mg). These data are in agreement with previous reports that have shown that the total protein content of *T. foenum-graecum*, *A. indica*, *M. charantia* and *P. emblica* were 607.0 ± 3.6 µg/mg, 32.50 ± 1.95 µg/mg, 17.7 ± 0.72 µg/mg, and 3.69 ± 6.55 µg/mg, respectively [49, 50, 48, 40].

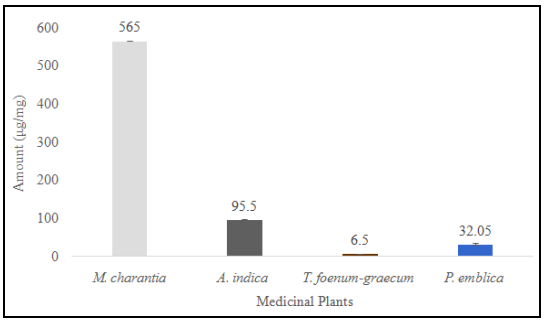

FIG. 2: TOTAL PHENOLIC CONTENT OF FOUR STUDIED MEDICINAL PLANTS. ERRORBARS REPRESENT STANDARD DEVIATIONS

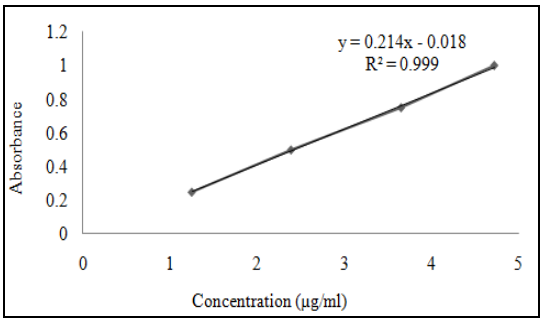

FIG. 3: TOTAL FLAVONOID CONTENT FOR STANDARD QUERCETIN

Fig. 5 shows a comparison of phenolic and flavonoid compounds present in the four studied medicinal plants. The above figure clearly illustrates that *M. charantia* contained the highest amount of phenolic content whereas *T. foenum-graecum* contained the highest amount of flavonoid. Since *T. foenum-graecum* and *M. charantia* contained the highest amount of phyto-chemicals, therefore, the water extracts of *T. foenum-graecum* and a mixture of *T. foenum-graecum* - *M. charantia* were administered into alloxan induced diabetic mice in order to evaluate their hypoglycemic activity.

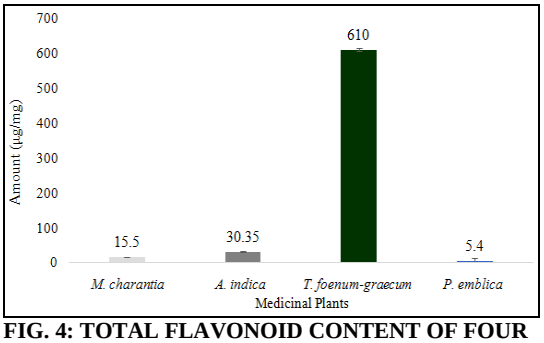

FIG. 4: TOTAL FLAVONOID CONTENT OF FOUR STUDIED MEDICINAL PLANTS. ERRORBARS REPRESENT STANDARD DEVIATIONS

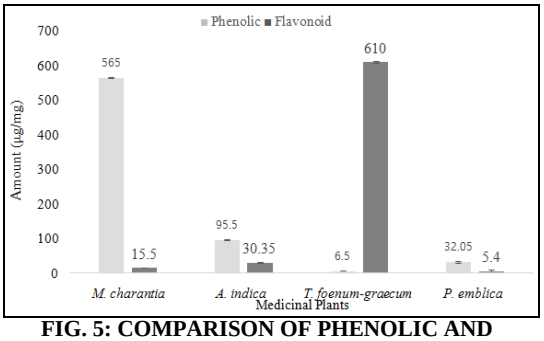

FIG. 5: COMPARISON OF PHENOLIC AND FLAVONOID CONTENT OF FOUR MEDICINAL PLANTS. ERRORBARS REPRESENT STANDARD DEVIATIONS

In group IV, the blood glucose level rose significantly to 253.98 ± 0.03 mg/100 mL in the 1st day experimental animals and to 389.03 ± 0.02 mg/100 mL in the 7th day experimental animals, when alloxan was administered to them **Table 2**. Alloxan has been used to induce diabetes following previous authors. The administration of alloxan resulted in the steady increase in the blood glucose

level during seven days of experimental period, indicating hyperglycemia. These observations are similar to those of previous studies [51, 52] that have used alloxan to induce diabetes in a variety of species. However, when *T. foenum-graecum* extract was orally administered to them, the blood glucose level dropped to 114.22 ± 0.01 mg/100 mL in the 1st day experimental animals. Subsequently, there was a gradual reduction in the 3rd day and 5th day experimental animals. A near normal value of 98.5 ± 0.02 mg/100mL was observed in the 7th day experimental animals. It is evident the administration of *T. foenum-graecum* extract has brought down the blood sugar level significantly. These results are in agreement with a previous study [53].

TABLE 2: THE EFFECT OF *T. FOENUM-GRAECUM* SEEDS EXTRACT AND A COMBINATION OF *T. FOENUM-GRAECUM* AND *M. CHARANTIA* EXTRACT ON BLOOD GLUCOSE LEVEL IN NORMAL AND ALLOXAN INDUCED DIABETIC MICE

	Blood glucose (mg/100mL)					
Experimental Periods	**Group I**	**Group II**	**Group III**	**Group IV**	**Group V**	**Group VI**
1st day	97.5 ± 0.2	96.5 ± 0.3	99.5 ± 0.2	253.98 ± 0.6	120.12 ± 0.1	121.12 ± 0.3
3th day	99.0 ± 0.4	97.0 ± 0.2	98.0 ± 0.5	305 ± 0.8	114.22 ± 0.1	105.81 ± 0.7
5th day	98 ± 0.08	96.0 ± 0.3	94.0 ± 0.1	345 ± 0.5	105.11 ± 0.25	97.02 ± 0.55
7th day	98.5 ± 0.4	98.0 ± 0.1	95.0 ± 0.35	380 ± 0.4	98.5 ± 0.2	97.22 ± 0.45

On the other hand, when the polyherbal extracts of *T. foenum-graecum* - *M. charantia* were orally administered, the blood glucose level dropped to 105.81 ± 0.07 mg/100 mL in the 1st day experimental animals. Subsequently, there was also a gradual reduction in the 3rd day and 5th day experimental animals. A near normal value of 97.02 ± 0.07 mg / 100 mL was observed in the 5th day experimental animals and remained at the same level even after further oral administration. Therefore, it is evident that compared with single extracts of *T. foenum-graecum*, the combined extract (*T. foenum-graecum* - *M. charantia*) promptly lowered blood glucose and maintained a relatively steady level over the study period.

This result is in agreement with other investigation that have shown that combined extracts effect on blood glucose appears a positive synergy [55], hence more beneficial than individual treatments. In combined form, the extracts tend to complement each other thereby producing the desired normoglycemia. However, the administrations of extracts of *T. foenum-graecum* and mixture of *T. foenum-graecum* - *M. charantia* did not show any particular change in blood glucose level of control animals.

There are many traditional herbal remedies that have been used to treat diabetes in Asia and other developing countries [54, 55, 56]. *M. charantia* is one of the plants that have been investigated thoroughly for the treatment of diabetes [57]. *M. charantia* and its various extracts and components are believed to exert their hypoglycemic effects *via* different physiological, pharmacological and biochemical modes [58, 59, 60]. The possible modes of the hypoglycemic actions of *M. charantia* and its various extracts and compounds are its hypoglycemic effect [61], stimulation of peripheral and skeletal muscle glucose utilization [62, 63], inhibition of intestinal glucose uptake [64], inhibition of adipocyte differentiation [65], suppression of key gluconeogenic enzymes [66, 67], stimulation of key enzyme of HMP pathway [66], and preservation of islet β cells and their functions [68]. Today, over 140 different studies worldwide have investigated anti-hyperglycemic and hypoglycemic effects of the different extracts and ingredients of *M. charantia* in both human and animal models [69, 70, 58].

The use of Fenugreek seeds (*Trigonella foenum-graecum*) for the treatment of diabetes has long been described in the Greek and Latin pharmacopoeias. In the recent past, several studies have demonstrated hypoglycemic properties of fenugreek seeds in both animal and human studies, thus, lending support to its traditional use [71, 72]. The hypoglycemic effect of fenugreek is believed to be largely due to its high content of soluble fiber, which acts to decrease the rate of gastric emptying thereby delaying the absorption of glucose from the small intestine. Another possible mechanism for the efficacy of fenugreek is its content of a specific amino acid, hydroxyisoleucine, which represents 80% of the free amino acids in fenugreek seeds, may possess insulin-stimulating properties [73].

Fenugreek is also known to contain compound like trigonelline and coumarin with reported hypoglycemic properties [74]. There are many medicinal plants, which are effective and commonly studied in relation to diabetes and its associated complications [75]. A single medicinal plant cannot be as effective as the polyherbal therapies for treating severe diseases. Therefore, there is a need to develop effective formulations using indigenous medicinal plants, subjecting them to pharmacological experiments and clinical trials. The antidiabetic efficacy of a single plant extract of *T. foenum-graecum*, and a combined extracts from two medicinal plants, *T. foenum-graecum* and *M. charantia* due to the presence of higher amounts of phytochemicals as compared to the others, were evaluated. These plants have been used traditionally for the treatment of diabetes mellitus and all of them are scientifically evaluated for their potency individually [76, 77].

Many traditional healers rely on herbal preparations, often consisting of complex ingredients and with very specific preparations, to treat their patients' illnesses, rather than just employing single plant extracts. A large number of plants used in traditional healing are employed in often sophisticated mixtures, rather than as individual plants. This is why traditional herbal mixtures, with their wealth of compound fragments and new compounds originating in the preparation process, could well yield new clues to the treatment of a wide variety of disease.

CONCLUSION: It could be concluded that all four medicinal plants studied contain appreciable amount of nutrients and can contribute to the nutrient and energy requirement of human being. The results obtained in the present investigation clearly support that administration of aqueous extracts of a single plant of *T. foenum-graecum* and a polyherbal extracts from *T. foenum-graecum* - *M. charantia* on alloxan induced diabetic mice have brought blood glucose level back to normal level. However, the polyherbal extract of *T. foenum-graecum* - *M. charantia* had brought the blood glucose level down to normal level more quickly compared with single extracts of *T. foenum-graecum*. On the other hand, there was no change in blood glucose levels of control animals subsequent to administration of either extracts. Further study is required to clarify aspects pertaining to the safety of *T. foenum-graecum* and *T. foenum-graecum* - *M. charantia* extracts being a potential alternative medicine for diabetes.

ACKNOWLEDGEMENT: The study has been supported from a research project of the University Grant Commission (UGC), Bangladesh. The experiments were conducted in the laboratory of Food Biotechnology under the Department of Applied Nutrition and Food Technology at Islamic University, Kushtia, Bangladesh.

CONFLICT OF INTEREST: The authors have no conflict of interests to declare regarding the publication of this paper.

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How to cite this article:
Mukit MG, Raza MS, Ud-Daula A, Azhar BS and Rahman ATMM: Comparative analysis of chemical and phytochemical compositions of four selected medicinal plants and evaluation of antidiabetic properties of aqueous extracts of *Trigonella foenum-graecum* and combination of *Trigonella foenum-graecum* - *Momordica charantia*. Int J Pharm Sci & Res 2018; 9(3): 1307-16. doi: 10.13040/IJPSR.0975-8232. 9(3).1307-16.