European Journal of Molecular & Clinical Medicine

ISSN 2515-8260 Volume 10, Issue 1, Winter 2023

In-vitro Anti-diabetic Effects of Ethanolic Extracts of *Verbascum thapsus, Trigonella foenum graecum, Ficus semicordata* and *Cocos nucifera*

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ABSTRACT

In-vitro synergistic anti-diabetic effects of alcoholic plant extracts of *Vervascum thapsus* leaves (AEVT), *Trigonella foenum graecum* seed (AETFG), *Ficus semicordata* leaves (AEFS) and *Cocos nucifera* husk (AECNH) was evaluated. A successive solvent extraction method was used to prepare ethanolic extracts of the plant's material (first Petroleum ether followed by benzene and ethyl alcohol). Alcoholic extracts of each plant were taken for experimental purposes. First, the individual plant extracts were tested for in-vitro anti-diabetic activity using alpha-amylase by DNSA color reagent. Based on individual results, the anti-diabetic activity of combined ethanolic plant extracts were determined for synergistic effects. Alcoholic extracts were also evaluated for efficiency to prevent glucose diffusion through a dialysis membrane. The observation indicated each plant extracts possess ant-diabetic activity to varying degrees. When they were combined plant extracts produces a synergistic effect.

Keywords: Anti-diabetic, polyherbal, *Verbacum thapsus, Trigonella foenum graecum, Ficus semicordata, Cocos nucifera* husk

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder that occurs due to high glucose levels in the blood.[1] Diabetes mellitus is caused by due to partial or complete deficiency of insulin, due to which acute and chronic complications and hyperglycemia occur.[2] Although this is not a contagious disease, diabetes is known as the major cause of death besides causing other health issues like stroke, kidney failure, heart disease, and high blood pressure.[3] According to IDF (International diabetes federation) 2019, around the world 463 million people tolerated diabetes mellitus & this data was estimated to increase to 700 million by 2045. Diabetes mellitus is a very

expensive and health-deteriorating disease having a very large burden on the economy of the country.[4,5] Because of serious side effects and the unsuitability of synthetic drugs in pregnancy herbal drugs are preferred in the treatment of diabetes mellitus.[6] Apart from the conventional treatment of diabetes mellitus, as per many researches medicinal plants employed in traditional formulations have been used in the treatment of diabetes mellitus.[7,8] More than 400 medicinal plants are documented for the treatment of diabetes.[9,10,11] Due to systematic and scientific study now a day's many medicinal plants have been recorded for many medicinal activities. As per the Ayurvedic system of medicine, drug formulations are depending on 2 principles i.e. use of one drug& use of drugs more than one.[12] Formulation containing two or more herbs is known as polyherbal formulation. The polyherbal formulations have better biological activity than plant extracts that have individual plant extracts.[13] However active phytoconstituents of the individual plant have been well established, they normally occur in low amounts and are insignificant to gaining desired therapeutic activity. So broad scientific studies have shown that medicinal herbs of diversified strength when mixed, may give more effects, in contrast, to the use of the individual medicinal herbs and also sometimes the total of their effect recorded. This situation of positive herb-herb interactivity is called synergism. Definite therapeutic activities of active phytoconstituents of herbs are important only when increased by that of other phytoconstituents but do not produce desired effects when used individually.[14] *Verbascum thapsus* belonging to the Scrophulariaceae family is a well-known herbaceous plant that is distributed all around Europe, North America, and temperate regions of Asia. Numerous pharmacological activities have been reported as anti-inflammatory, antiviral, antimicrobial, anticancer, antihyperlipidemic, and antihepatotoxic.[15] Fenugreek (it is an annual herb), a member of the family Leguminosae (Fabaceae). It is distributed to North America, Southeastern Europe, and central asia.[16] Antidiabetic activity in alloxan-induced type-2 diabetic rats was reported in an aqueous extract of Fenugreek seeds.[17]*Ficus semicordata* (Family-Moraceae), commonly known as bhue goolar, khaina, and khanayo, is a kind of fig. More than 750 species of trees, shrubs, and climbers are growing in the subtropical and tropical parts of the entire world.[18] Various species of Ficus showed antidiabetic, antioxidant, antileprosy, anti-ulcer activities, etc.[19] *Cocos nucifera* belongs to the family Arecaceae, due to considerable linking between polysaccharides, lignins, and phenolics. Mesocarp becomes fibrous and hard. In northeastern Brazil, coconut husks have been traditionally used to cure arthritis and diarrhea. Antiviral, free radical scavenging activity, antibacterial activity, anti-leishmaniasis, and antinociceptive activities have been reported in the aqueous extract of coconut husk.[20] Chemical constituents reported in *Cocos nucifera* husk are Catechins, epicatechins pentosans, cellulose, lignin, and Condensed tannins (B-type procyanidins).[20]

The object of the current study is to explore the individual as well as the synergistic antidiabetic potential of polyherbal combination containing ethanolic extracts of *Vervascum thapsus* (leaves)*, Trigonella foenum graecum* (seeds), *Ficus semicordata* (leaves) *and Cocos nucifera* (husk), which would be used for the development of this combination of medicinal plants as a polyherbal mixture for the treatment of diabetes mellitus type-II.

MATERIALS AND METHODS

Chemicals & Reagents

α-amylase and dialysis membrane were obtained from Hi media laboratories Pvt. Ltd. Mumbai (India). Other reagents and chemicals, and solvents used for research were of analytical grade and procured locally from Moradabad, Uttar Pradesh, India.

Plant material

The plant materials were collected locally from Moradabad (Fenugreek and Coconut husk), *Verbascum thapsus,* and *Ficus Semicordata* from Uttrakhand, identified and authenticated by Dr. Sunita Garg (Former Chief Scientist, Head RHMD) and Mr. R.S. Jayasomu (Chief scientist, head RHMD) in CSIR-NIScPR Delhi. Plant materials were separately screened for any foreign particles, dried, and finely powdered with the help of a grinder. The dried, powdered plant materials after extraction with petroleum ether were subjected to successive solvent extraction with benzene followed by ethyl alcohol (90%) till the solvent becomes colorless. All the extracts were separately concentrated by a rotatory evaporator and stored at -20 °C until use.

Assay for α-amylase inhibition (DNSA method)

The following steps were followed for assay:[21, 22]

Step 1: DMSO (10%) was used to dissolve the extracts and 6 concentrations of plant extracts were prepared using sodium phosphate buffers, 200, 400, 600,800,1000, 1200 µg/ml. Step 2:200 µl plant extract and 200 µl 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) containing 2 units/ml alpha amylase solutions (Concentration 200µl/ml) and incubation was done for ten min at 25 °C temperature.Step3: 200µl of one percent starch solution in 0.02 M Sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) was pre-incubated and added to each tube at 5 second intervals and then incubated for ten minutes at the temperature of 25 °C. Step 4: To stop the reaction one ml of color reagent DNSA (3,5-Dinitrosalisylic acid) was added. Step 5: Incubation of each test tube was done in a boiling water bath for five min and then cooled at room temperature. Step 6: 10 ml D. water is added to the reaction mixture for dilution. Step 7: Absorbance of the mixture was taken at 540 nm. The calculation was done as per the following formula:

$$\% \text{ Inhibition} = A_{540} \text{ Control} - A_{540} \text{ Extract} / A_{540} \text{ Control} \times 100$$

Glucose diffusion inhibitory study

Step 1: One ml of ethanolic extract of the crude drug was taken and transferred to a 12000 MW dialysis membrane along with glucose solution (0.22mMin 0.15M NaCl). Step 2: Thread was used to tie the dialysis membrane at both ends and dipped in a beaker (containing 40 ml of 0.15 M Sod. Chloride and ten ml of D. water). Step 3: The solution containing one ml of 0.15M NaCl, 22 mM glucose, and one ml of D. water was used as a control. Step 4: At room temperature beakers were transferred to the orbital shaker. The motion of the glucose solution to the external solution was recorded each half an hour. 3 readings were taken for three hours.[10,11, 22]

Polyherbal combination of AETFG, AEVT, AEFS, and AECNH

Ethanolic extracts of all the drugs were individually subjected to in-vitro anti-diabetic activity (α-amylase inhibitory activity by DNSA method and glucose diffusion inhibitory assay). A combination of all four ethanolic plants extract was taken in the same ratio to prepare a polyherbal mixture and antidiabetic synergistic effects were contrasted to the individual plant extract and standard drug acarbose.

RESULTS

Assay for α-amylase inhibition (DNSA method)

The results were given in table 1 and figure 1. Ethanolic extracts of all four plants showed Different effects on the utilization of glucose. At All the concentrations, AETFG (Alcoholic extract of *Trigonella foenum graecum* seeds) exhibited the highest inhibition of α-amylase at the highest value of 45.25 % which was seen at 1200 µg/ml concentration. AEVT (alcoholic extract of *Verbascum thapsus* leaves) exhibited the next higher value at a concentration of 44%at 1200µg//ml. AEFS (Alcoholic extract of *Ficus semicordata* leaves) exhibited the third higher value of 40.5 % which was seen at a concentration of 1200 µg//ml and AECNH (Alcoholic extract of *Cocos nucifera* husk) showed minimum inhibition of α-amylase with the higher value of 30% observed at 1200 µg//ml. Polyherbal combination showed 60 % α-amylase inhibition at a concentration of 1200 µg//ml, Which was highest when compared to other drugs tested including standard drugs.

Table 1: Alpha amylase% Inhibition brought about by ethanolic plant extracts of different concentrations of *Trigonella foenum graecum* seeds (AETFG), *Verbascum thapsus* leaves (AEVT), *Ficus semicordata* leaves (AEFS), *Cocos nucifera* husk (AECNH) and polyherbal combination as recorded with respect to Acarbose.

Concentration(µg/ml)	Standard (Acarbose)	AETFG %	AEVT %	AEFS %	AECNH %	Polyherbal combination%
0	**0**	**0**	**0**	**0**	**0**	**0**
200	10	8.5	7	6.5	4	12
400	18	17	15.5	14	10	20
600	28	27	25.5	22.5	15.5	29.5
800	36	34.5	30	27.5	20	38
1000	46	44.5	40	38	25	50.5
1200	50	45.25	44	40.5	30	60

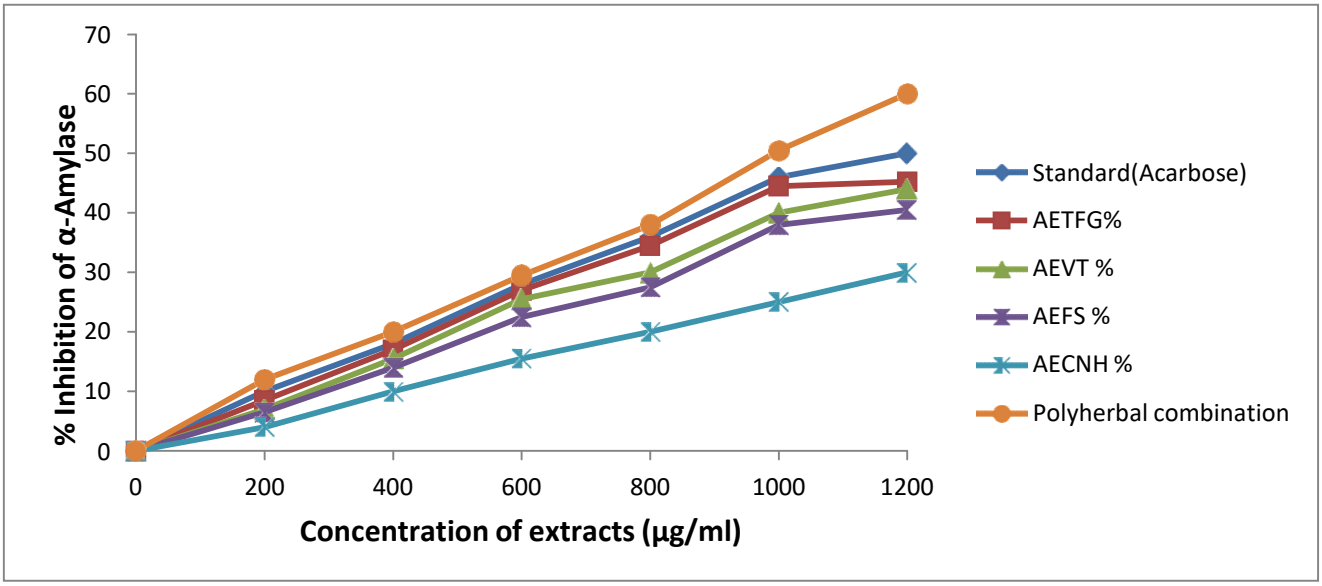

Figure 1: Effects of ethanolic extracts of *Trigonella foenum graecum* seeds (AETFG), *Verbascum thapsus* leaves (AEVT), *Ficus semicordata* leaves (AEFS), *Cocos nucifera* husk and polyherbal combination at varying concentrations on alpha amylase activity as compared to acarbose.

Inhibitory study for Glucose diffusion

The results obtained from the inhibitory study of glucose diffusion are shown in table 2, figure 2, and 3. The comparison is shown in figure 2 and figure 3. Ethanolic extracts of all the plants showed significant glucose diffusion inhibitory activity. AECNH showed the highest inhibition to the diffusion of glucose and AEVT showed minimum inhibition to the glucose diffusion.

Table 2: Effect of ethanolic extract (100 g/l) of AETFG, AEVT, AEFS, AECNH on glucose diffusion through dialysis membrane over 30 hrs.

Groups	Glucose Concentration (mg/dl) at different hrs					
	3	6	12	18	24	30
Control	0.007±0.0003	0.03±0.0015	0.117±0.0058	0.118±0.0064	0.117±0.0074	0.118±0.0076
AETFG	0.005±0.0016	0.025±0.0017	0.095±0.0087	0.1±0.002	0.119±0.0043	0.12±0.0047
	(79.00)	(81.00)	(82.50)	(83.54)	(86.25)	(87.55)
AEVT	0.0065±0.0018	0.0163±0.0024	0.00179±0.0018	0.0575±0.0063	0.117±0.0027	0.1599±0.0067
	(66.00)	(68.22)	(70.50)	(73.53)	(75.46)	(80.99)
AEFS	0.065±0.0033	0.0128±0.0030	0.0209±0.0090	0.036±0.002	0.058±0.0044	0.0959±0.0029
	(55.25)	(64.55)	(65.33)	(67.88)	(69.33)	(70.25)
AECNH	0.0044±0.0018	0.0138±0.0028	0.0148±0.0015	0.0165±0.0018	0.0279±0.0039	0.0536±0.0014
	(50.55)	(52.44)	(53.50)	(58.59)	(60.50)	(65.59)

Relative movement% is presented in the bracket.
Values are means±SEM for group of 3 observations.

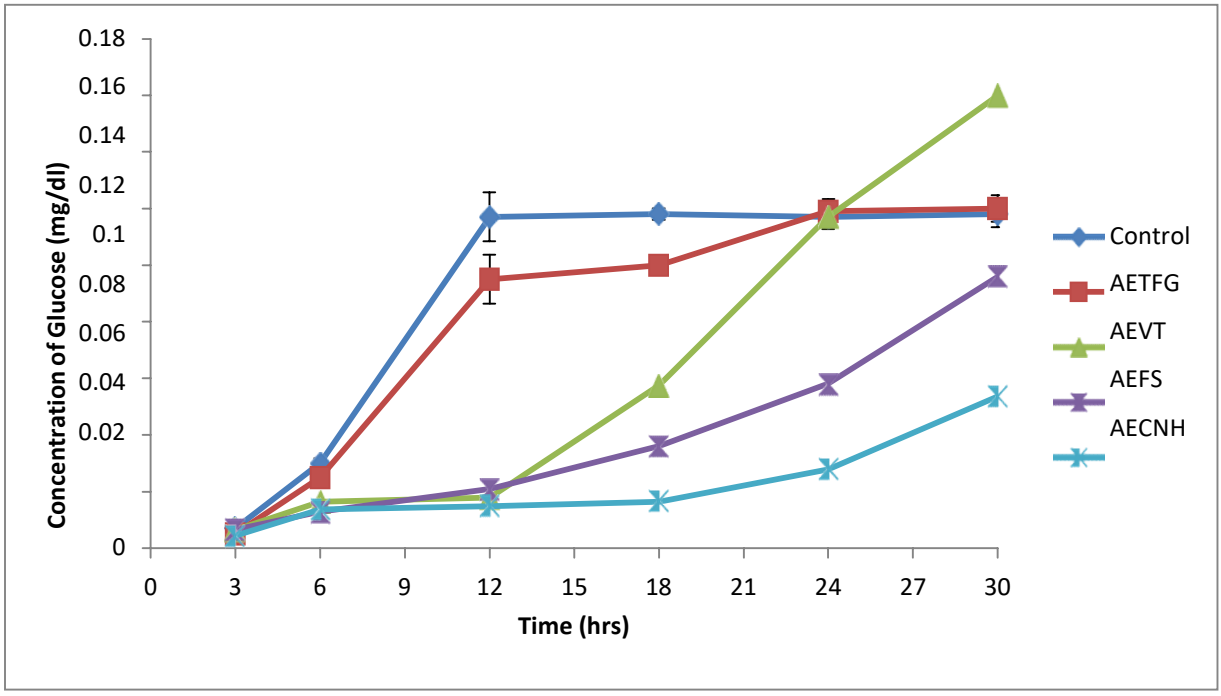

Figure 2: Effects of ethanolic extracts of *Trigonella foenum graecum* seeds (AETFG), *Verbascum thapsus* leaves (AEVT), *Ficus semicordata* leaves (AEFS) and *Cocos nucifera* husk on glucose diffusion through dialysis membrane as compared to aqueous control.

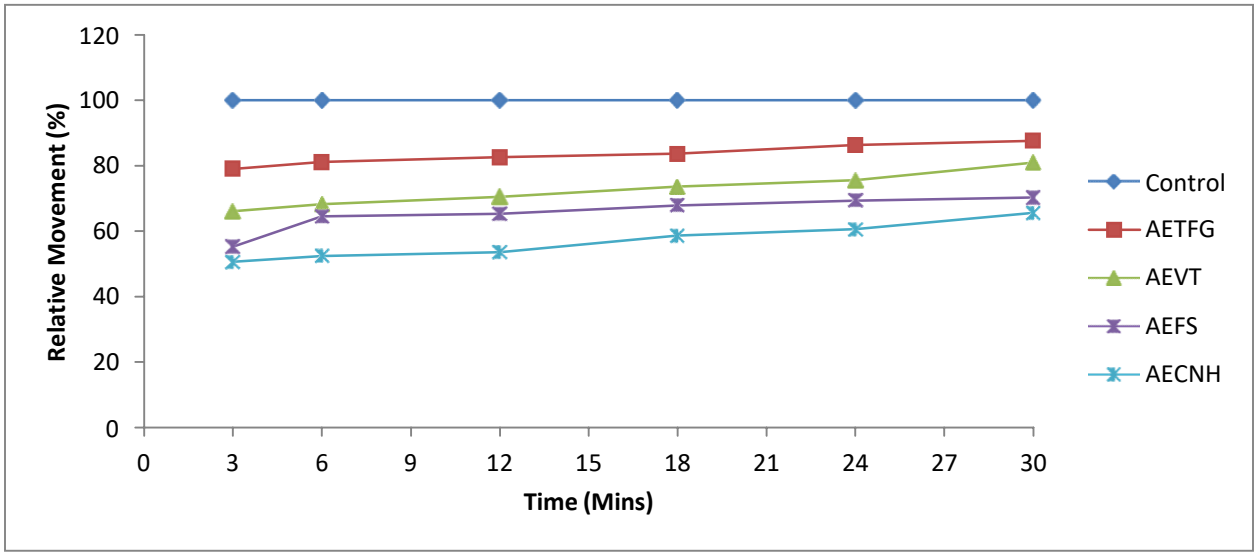

Figure 3: Relative movements (in %) of glucose through dialysis membrane concerning aqueous control under the influence of ethanolic extracts of *Trigonella foenum graecum* seeds (AETFG), *Verbascum thapsus* leaves (AEVT), *Ficus semicordata* leaves (AEFS) and *Cocos nucifera*husk. Values are percentages with their standard errors.

In the present study, AETFG, AEVT, AEFS, and AECNH crude extracts exhibited optimum α-amylase inhibitory activity and glucose diffusion inhibitory activity across the dialysis

membrane; hence these extracts were combined to prepare polyherbal combinations to know synergistic potential produced on antidiabetic properties when performing *in-vitro*. The α-amylase inhibitory activity of extracts as a polyherbal combined mixture when compared to that of individual AETFG, AEVT, AEFS, and AECNH. The results obtained showed that the polyherbal mixture showed better α-amylase inhibitory activity and glucose diffusion inhibitory activity.

DISCUSSION

When abnormal absorption of glucose occurs in the blood it causes type–II diabetes because the concentration of glucose rises in the blood. Due to the rise in blood glucose level serious obstacle for organs like the kidney, heart, retina, and brain, also occurs in patients.[28,29,30] One of the most important ways to minimize glucose absorption is by inhibiting the digestion of carbohydrates. Two enzymes are involved in the digestion of carbohydrates i.e. pancreatic α-amylase and intestinal α-glucosidase to release absorbable glucose. Inhibition of both the enzyme (α-glucosidase and α-amylase) may play an important role to minimize the digestion of intestinal carbohydrates so that managing type –II diabetes mellitus.[31,32]

The present study proved the anti-diabetic potential of alcoholic extracts of *Trigonella foenum graecum* seeds, *Verbascum thapsus* leaves, *Ficus semicordata* leaves, and *Cocos nucifera* husk. The above extracts when combined showed synergistic in-vitro anti-diabetic activity.

Cocos nucifera husk was investigated to have α-amylase inhibitory activity in methanol extract and its ethyl acetate fraction.[23]Alcoholic extract of *Trigonella foenum graecum* seeds reported for their in-vivo antidiabetic activity.[24,25]Anti-diabetic activity of *Ficus semicordata* (ethanol extract of the plant) in streptozotocin-induced diabetic rats was reported.[26] Leaves of *Verbascum thapsus were* evaluated for antihyperglycemic activity (in alloxan-induced diabetic rats)& found that ethanolic extract of the whole plant showed significant antidiabetic activity.[27]Thisstudy is the first combined *in-vitro* study of selected plants for a synergistic effect.

Alcoholic extract of AETFG seeds showed the highest α-amylase inhibition. AETFG too exhibits a powerful inhibition of the movement of glucose through a dialysis membrane. AEVT exhibit the next best alpha-amylase inhibitory action but exhibited the lowest inhibition of glucose diffusion over a dialysis membrane. It successfully inhibited glucose diffusion only for 180 min. AEFS exhibits a powerful alpha-amylase inhibitory action and powerful inhibition towards the flow of glucose through a dialysis membrane. It exhibited a very powerful anti-diabetic activity in both the tests performed. AECNH showed the least inhibition of α-amylase but in the glucose diffusion inhibitory test, it showed the highest inhibition effect on the glucose diffusion over a dialysis membrane.

From the present study, it is concluded that ethanol extract of *Trigonella foenum graecum* seeds was found to have maximum anti-diabetic activity when compared to other individual drugs tested in this study. But when combined ethanolic extracts of the selected plants were tested for α-amylase inhibitory activity test and glucose diffusion inhibitory test the practical effect is greater than the theoretical effect proving that the above plant extracts showed a synergistic effect. These medicinal plants can be again analyzed to develop anti-diabetic drugs free from injurious side effects.

CONCLUSIONS

In this present study, all four ethanolic extracts of AETFG, AEVT, AEFS, and AECNH actively showed anti-diabetic activity *in-vitro*. The combined extracts of these medicinal plants exhibited better anti-diabetic activity when comparison was done to the individual plant part extracts. Concisely, because of the various mechanisms of anti-hyperglycemic activities given by the poly herbal extracts, it may be again scope to develop a possible treatment for type-2 diabetes mellitus. As the present research serves as a pioneer in recording the synergistic anti-diabetic activity of polyherbal combination between AETFG, AEVT, AEFS, and AECNH, again preclinical trials are required to prove its anti-diabetic action and potential compounds responsible for the inhibitory action of alpha-amylase enzyme.

ACKNOWLEDGMENTS

We acknowledge Principal Teerthanker Mahaveer College of Pharmacy, Teerthanker Mahaveer University, Moradabad U.P. India for providing all the facilities to carry out this research work.

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