RESEARCH ARTICLE

Bioguided Fractionation and Isolation of Natural Antidiabetic Agents from Polyherbal Tablet

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ABSTRACT

Polyherbal antidiabetic tablets were formulated, evaluated and compressed by combining the powder extract of *Nigella sativa*, *Trigonella foenum*, and *Glycyrrhiza Glabra*. A number of tests were carried out, including a uniformity weight test, an assessment of the tablets' hardness and friability, an ash value, moisture content, an extractive value that is soluble in water or alcohol, density (bulk and tapped), repose angle, Carr's index, disintegration time with preliminary phytochemical screening. The tablet also had *ex-vivo* antidiabetic performance to enzymes alpha-amylase as well as glucosidase. To ensure the presence of active ingredients, thin-layer chromatography was performed. Column chromatography as performed using silica gel G as a stationary phase. The column of material was created using acetic acid, toluene, and ethyl acetate. As follows: (5.0:4.2:0.8) for 10 ml. The mobile phase of the sample, which contains toluene, ethyl acetate, acetic acid (50:42:8) and fractions, was isolated, collected and sent to IIT Bombay for GCHRMS analysis. In GCHRMS, the chemical constituent was found to be 2 H-Pyran-2-one, tetrahydro-4-hydroxy-4-methyl-, Tetrahydro [2 , 2'] bifuranyl-5-one, 1H-Benzimidazole, 2-(1-methylethyl)-, Bifenthrin, Propionamide, 2,2-diphenyl-N-(2-pyridinyl)-, Dihydromyristicin, 2-Dodecanone, di-p-Tolyl sulfone. The tablets showed promising results for alpha-amylase and alpha-glucosidase inhibition activities.

Keywords: Antidiabetic, evaluation, compression, polyherbal tablets, alpha amylase, alpha glucosidase, active constituents, GCHRMS

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INTRODUCTION:

Numerous aetiologies contribute to the dangerous, chronic, and complex metabolic condition known as diabetes, which has both immediate and long-term negative effects.[1] Numerous organs suffer long-term damage, malfunction, and failure as a result of diabetes. Diabetes is commonly known by dehydration, increased urination, vision issues and decreased weight.[2,3] Within next 25 years, it is anticipated to rank among the major disablers and killers in the globe. Diabetes is brought on by a number of conditions, which are referred to as predisposing or risk factors. Environmental factors that boost the risk of diabetes include diet, obesity, and a sedentary lifestyle.[4] Currently, a lot of diabetes healer medications are being developed to manage glycemic conditions.[5]

MATERIAL AND METHODS

Plant Matter

Parched pip of *Nigella sativa*, *Tigonella foenum*, and *Glycyrrhiza glabra* were obtained from the neighborhood market (Figure 1). The seeds were recognized and confirmed by the Department of Botany and Research Centre at Padmashree Vikhe Patil College, Loni.

Pharmacognosy and microscopy of Root and Seeds

Fresh plant material was used to study pharmacognostic and microscopic characters.

Extraction and physicochemical screening[6,7] of plant material

The *G. glabra, Trigonella foenum*, and *N. sativa* plant materials the beans were dried, a powdery substance and then processed with an 80 mesh screen. Methanol was the extraction solvent utilized for powdered seed extraction, and the extraction process took 4 hours.

Determination of Ash value

Ash value is useful for assessing the purity and quality of crude drugs.

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Figure 1: Dry seeds of *N. sativa, T. foenum*, and *G. glabra*

Total ash: beneficial for discouraging the purity and authenticity of the sample

Acid insoluble ash: Sandal matter is measured in order to evaluate how soluble an acid is.

Water soluble ash: The residue that is created after the drug's complete ash value has been processed weighs differently than the drug's entire ash.

Determination of extractive value: By dividing the drug's weight by the amount of solvent used, one can calculate the volume of solvent that was utilized.

Alcohol soluble extractive value: in extractive value that is alcohol soluble using ethanol as a solvent

Water soluble extractive value: with water solubility extract function water is used as solvent

Loss on drying: LOD (%) = (M2-M3) / (M2-M1)

In which,

M1= Mass of empty china plate

M2= Mass of china plate having remedy at 0h

M3= Mass of china plate following 6h

Phytochemical Screening

Nonnutritive plant chemicals known as phytochemicals are made up of curative and preventive medicinal substances. Standard screening procedures were used to determine for different plant components.[8] Test for saponins, phenols, glycosides, flavonoids, alkaloids, tannins, amino acid[9], Carbohydrate, diterpenes, lipids, steroids and terpenoids was done.[4]

Preparation and Evaluation Test of Polyherbal Antidiabetic Tablets

Formulation of antidiabetic herbal tablets

200 gm of seed powder from *G. glabra*, *T. foenum*, and *N. sativa* were initially extracted, and the extract was then utilized. Two compositions of polyherbal tablets in the ratios of 1:1:1 and 0.5:0.5:0.5 were prepared. For the (1:1:1) formulation, 1 gm. of the methanolic extract of *G. glabra*, *T. foenum*, and *N. sativa* powder was used, and for the (0.5:0.5:0.5) formulation, 0.5 gm. of powder was used for each tablet. The materials for the direct compression method were run through sieve number 60 (Table 1).[8]

Evaluation test of polyherbal Antidiabetic tablets, Color and appearance, Angle of repose, the tapped density hausner ratio, compressibility index, hardness, friability[6], determination disintegration time was done.

Gchrms Analysis[10-13]

The sample of the methanolic fraction was sent to IIT Bombay for GCHRMS analysis following the completion of thin layer chromatography and column chromatography employing elution solvent methylbenzene: ethyl acetate: acetic having proportion (5.0:4.2:0.8).

In-vitro Alpha Amylase Inhibition Assay of Tablets (0.5:0.5:0.5) and (1:1:1) Tatio[14-16]

Procedure

The Patil SB method was applied to study the inhibitory effects of in vitro amylase. In simple terms, 500 L of the test chemical Sample - Methanol extraction Tablets (0.5:0.5:0.5) and 500 L of 0.1M monosodium phosphate of pH 6.9 with 0.5% -amylase catalyst were allowed to react. 500 L of 1% starch soluble, extra pure (Loba Chemie) with 0.1M monosodium phosphate of pH 6.8 was incorporated after a 10-minute incubation at 25 degrees Celsius. Again incubated for a ten-minute period at 25°C. The identical procedure was used for the controls, however, instead of the enzyme, 500 L of buffer was used. Once incubation completed, 1000 L of DNS (Loba Chemie) has been added to the test and control groups. Acarbose is used as a standard drug.

Inhibition (%) = Absorbance 540 (control) – Absorbance 540 (extract) 100

Absorbance 540(control)

Invitro Alpha Glucosidase Inhibition Test of Tablets (0.5:0.5:0.5) and (1:1:1) Ratio [17-19]

Procedure

The preparation of a blank involved mixing the sample solution with all the reaction ingredients except the catalyst

Table 1: Formulation table for tablet preparation

Ingredients	*F1 (1:1:1) (mg)*	*F2 (0.5:0.5:0.5) (mg)*
G. glabra extract	1	0.5
T. foenum extract	1	0.5
N. sativa extract	1	0.5
Mannitol	150	150
MCC	50	50
Talc	1.75	1.75
Flavoring agent	1.75	1.75
Magnesium stearate	1.75	1.75

(α-glucosidase) solution[20, 21]. Process was stopped by addition of soda crystals (50 µL, 0.2 M). At 400 nm, immersion of sample and void was measured.

Inhibition (%) = control - Test / Control x100

RESULT AND DISCUSSION

Despite significant advancements in the knowledge and treatment of diabetes, the illness and its complications continue to spread unchecked. Parallel to this, recent improvements in our understanding of the pathophysiology of illness have created a number of fresh opportunities for finding and creating cutting-edge treatments to tackle the diabetic epidemic. Phytochemicals discovered in conventional herbal remedies are also offering a fascinating prospect for the creation of novel therapies. This has increased efforts worldwide to cultivate medicinal plants that contain significant amounts of potential phytochemicals that have been shown to have many positive benefits in the cure of hyperglycemia and its associations.

Microscopy shows the presence of oil globules, aleurone grains, mucilage, fibers, endodermis etc (Figures 2-4).

3.6% moisture was discovered to be present in laboratory formulations. Glazyrrhiza glabra was found to have a water extractive value of 0.17%, trigonella foenum of 0.11%, and nigella sativa of 0.19%. Glazyrrhiza glabra, trigonella foenum, and nigella sativa all had extractive values of 0.03%, 0.04%, and 0.09% for alcohol, respectively (Table 2). Various secondary metabolites were found to be present in preliminary phytochemical tests as shown in Table 3. The tablets' disintegration was discovered to be caused by 12 mines. Tablets didn't disintegrate in more than 30 mines. Hardness was 4 (kg/cm^2), brittleness was 2.17%, and weight fluctuation was within acceptable ranges for medicinal use (Table 4).

In the GCHRMS study, the bioactive constituent was found to be 2 H-Pyran-2-one, tetrahydro-4-hydroxy-4-methyl-,

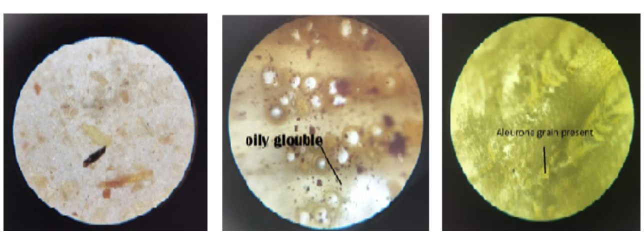


Figure 2: Microscopy of *G. glabra*

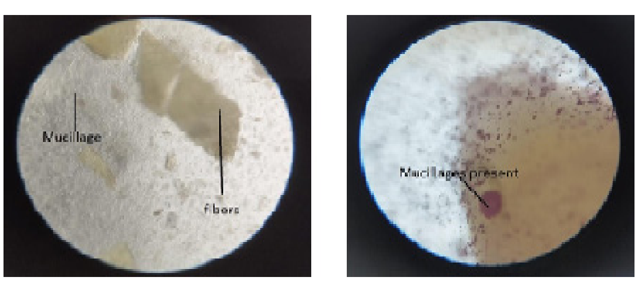


Figure 3: Microscopy of *T. foenum*

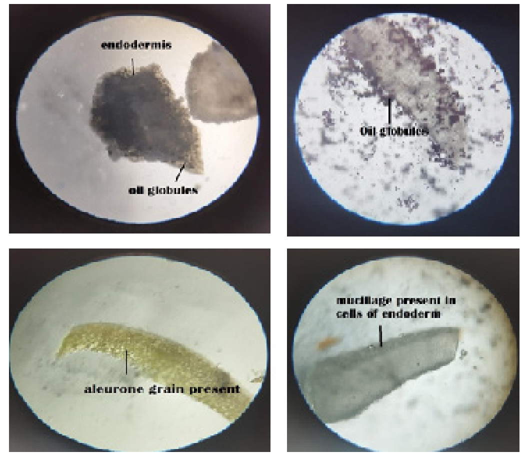
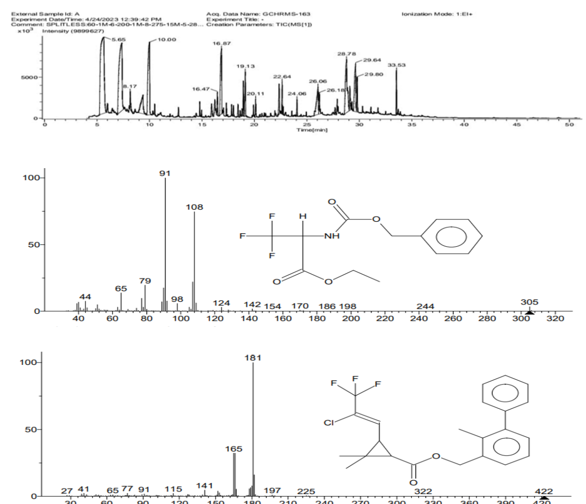
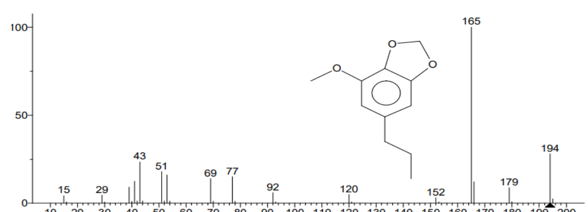


Figure 4: microscopy of *N. sativa*



N-(Benzyl oxy carbonyl)-3,3,3-trifluoroalanine ethyl ester Bifenthrin



Dihydromyristicin

Figure 5: GC-HRMS chromatogram - glycerrihiza glabra, *T. foenum*, *N. sativa* of methenolic extraction

Tetrahydro [2 , 2'] bifuranyl-5-one, 1H-Benzimidazole, 2-(1-methylethyl)-, Bifenthrin, Propionamide, 2,2-diphenyl-N-(2-pyridinyl)-, Dihydromyristicin, 2-Dodecanone, di-p-Tolyl sulfone. The tablets showed promising results for antidiabetic activity due to the presence of listed chemical constituents (Table 5, Figure 5).

The given sample of compound Sample – Methanol extraction Tablets (0.5:0.5:0.5) Sample – Methanol extraction Tablets (1:1:1) was showed good inhibition of amylase enzyme

Table 2: Physicochemical standard test

Phytochemical tests	*G. glabra (% w/w)*	*T. foenum (% w/w)*	*N. sativa (% w/w)*
Ash value	0.36	0.47	0.26
Acid unsolvable ash	0.27	0.38	0.30
Water unsolvable ash	0.91	0.67	0.89
Alcohol soluble extractive value	0.09	0.04	0.03
Water soluble extractive value	0.17	0.11	0.19
Loss on drying	0.38	1.37	0.25

Table 3: Phytochemical screening test

Phytochemical test	*G. glabra*	*T. foenum*	*N. sativa*
Saponin	+	-	+
Phenol	+	-	-
Glycosides	+	+	+
Flavonoids	+	+	+
Alkaloids	+	+	-
Tannin	+	-	+
Amino acid	-	-	-
Carbohydrate	-	+	-
Lipids	+	+	-
Steroids and terpenoid	+	-	+

(+)Present, (-) Absent

Table 4: Evaluation test of tablet preparation

Evaluation test	*F1*	*F2*
Color	Yellowish brown	Yellowish brown
Shape	Round	Round
Odor	Characteristics Odour	Characteristics Odour
Taste	Pleasant taste	Pleasant taste
Density - Bulk	0.46	0.41
Density - Tapped	0.52	0.51
Carr's index	15.9	18.3
Hausners ratio	1.13	1.24
Angle of repose	30.96(excellent)	30.96 (excellent)
Hardness	4.2	4.0
Thickness	4.4	4.1
%Friability	2.17	2.15
Disintegration time (minutes)	12.20	12.22

Table 6: alpha amylase retardation assesment

Sample code	*mass*	*Absorbance (540nm)*	*Mean*	*%Retardation*
Control normal	---	0 .73 0 .67 0 .70	0.70	---
Standard Acarbose	1 mg/mL	0.20 0.21 0.23	0.21	70
Sample– Methanol extraction Tablets (0.5:0.5:0.5)	1 mg/mL	0.56 0.50 0.52	0.52	25.71
Sample– Methanol extraction Tablets (1:1:1)	1mg/mL	0.36 0.30 0.30	0.32	54.28

Table 7: alpha glucosidase retardation assesment

S. No	*Sample code*	*mass (µg/ml)*	*Absorbance (400nm)*	*% retardation*
1	CONTROL	-	0.54	-
2	Acarbose	100	0.11	79.62
3	Sample-Methanol extraction Tablets (0.5:0.5:0.5)	100	0.25	53.70
4	Sample – Methanol extraction Tablets (1:1:1)	100	0.37	31.48

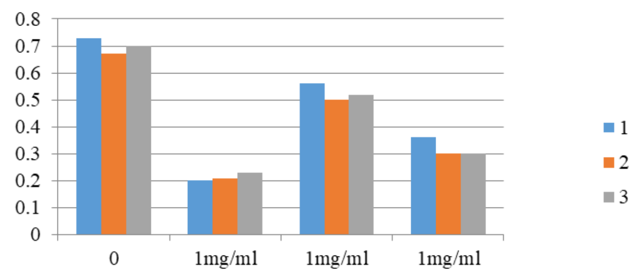


Figure 6: alpha amylase inhibition assay

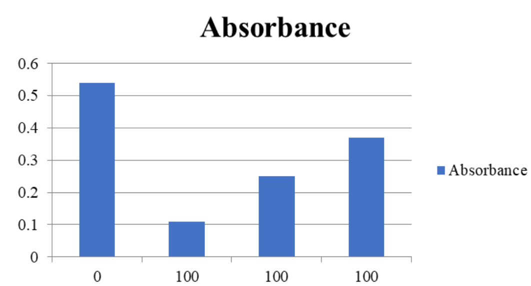


Figure 7: alpha glucosidase inhibition assay

Table 5: Interpretation of GC-HRMS study

S. No	*Chemical constituent*	*Mol. formula*	*Mol. weight*	*Time*	*Area*
1	N-(Benzyl oxy carbonyl)-3, 3, 3-trifluoroalanine ethyl ester	C13H14F3NO4	305	10.00	102535209.82
2	Bifenthrin	C23H22CLF3O2	422	19.13	12699787.44
3	Dihydromyristicin	C11H14O3	194	20.11	24686473.69

at the concentration of 1mg/ml when compared to acarbose standard. According to table7, samples at the concentration 100 µg/ml showed Good activity against glucosidase enzyme (Tables 6,7; Figures 6,7).

CONCLUSION

The given sample of compound Sample – Methanol extraction Tablets (0.5:0.5:0.5) Sample – Methanol extraction Tablets (1:1:1) was showed good inhibition of amylase enzyme at the concentration of 1mg/ml when compared to acarbose standard. According to the table, samples at the concentration 100 µg/ml showed Good activity against glucosidase enzyme. In the GCHRMS study, the bioactive constituent was found to be 2 H-Pyran-2-one, tetrahydro-4-hydroxy-4-methyl-, Tetrahydro [2 , 2'] bifuranyl-5-one, 1H-Benzimidazole, 2-(1-methylethyl)-, Bifenthrin, Propionamide, 2,2-diphenyl-N-(2-pyridinyl)-, Dihydromyristicin, 2-Dodecanone, di-p-Tolyl sulfone. The tablets showed promising results for antidiabetic activity due to the presence of listed chemical constituents.

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