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Formulation and evaluation of herbal anti-diabetic capsule containig *Trigonella foenum-graecum* L. and *Musa paradisiaca* L.

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Abstract

Aim of current study to formulate and evaluate antidiabetic capsules containing *Trigonella foenum-graecum* L. (fenugreek seeds) and *Musa paradisiaca* L. (raw banana). Both drugs were subjected to powder microscopic analysis. Fenugreek seeds were extracted with hydro-alcoholic solvent and raw banana fruits with peels were extracted with alcohol. Both the extracts were tested by phytochemicals tests, Quercetin in raw banana and diosgenine in fenugreek was identified by HPTLC fingerprint analysis,drug –drug and drug-excipient compatibility was studied using FT-IR. Preformulation study was performed on prepared granules; capsules were prepared and evaluated for weight variation, disintegration and in-vitro alpha amylase assay for antidiabetic activity. Both drugs showed the presence of phytochemicals like steroids, flavonoids, tannins, phenols, and saponins. HPTLC fingerprint analysis confirms the presence of quercetin and diosgenine in raw banana and fenugreek respectively. Both extracts and drug excipients were found to be compatible after the IR study. Capsules containing both extracts were found to have good alpha-amylase activity.From all the results it can be concluded that the current formulation presents significant challenges in terms of manufacturing and optimizing therapeutic outcomes because the actual quantities of phytochemicals in the extracts are not standardized.

Key words : Herbal capsule, HPTLC finger print analysis, quercetin, diosgenine, alpha amylase assay.

The efficacy of herbal remedies in treating various ailments is widely acknowledged. Because synthetic medications frequently have unwanted side effects, researchers that

study natural products have concentrated a great deal of effort on developing anti-diabetic medications derived from natural plants. Modern healthcare offers new opportunities for novel drugs from traditional medicine. However, the anti-diabetic benefits of phyto-pharmaceuticals and less toxic medicinal plants are not yet sufficiently supported by scientific research. Combining herbal medications is preferred because it increases the intended pharmacological activity and is thought to be less harmful and free of unwanted side effects than synthetic medications. The development of an affordable and secure antidiabetic medication is necessary. Methi, or fenugreek (*Trigonella foenum graecum*), seeds are rich in phytochemicals such as alkaloids, phenols, steroids, flavonoids, and saponins.They are members of the Fabaceae family. Asthma, gastric lesions, Parkinson's disease, diabetes mellitus, cancer, and rheumatoid arthritis can all be treated with it[1]. The banana, scientifically referred to as Musa paradisiaca, belongs to the Musaeae family and is a member of the tannin, alkaloids, and flavonoid (quercetin, gallocatechin) families of phytoconstituents. These phytoconstituents have therapeutic applications and can be used to treat bronchitis, diarrhea, menorrhagia, diabetes mellitus, and dysentery[2,3]. In an attempt to advance this research, the current study assessed the pharmaceutical quality of the herbal capsules that were prepared using the dried ethanol and hydroethanolic extracts of *Trigonella foenum graecum* L. and *Musa paradisiaca* L.

Phytochemical investigation[4] *:*

Fenugreek seeds and raw banana fruits with peels were extracted with hydro-alcoholic and alcoholic solvents, respectively, and tested for phytochemicals such as alkaloids, carbohydrates, flavonoids, tannins, saponins, steroids.

HPTLC fingerprint analysis :

Preparation of a quercetin standard solution A stock solution of standard quercetin (110 µg/ml) and diosgenin (100 µg/ml) was prepared in methanol.

Sample preparation :

Sample solutions of *Trigonella foenum-graecum* and *Musa paradisiaca extracts* were also prepared in methanol, and 20 µl and 30 µl of this solution were used as a test solution for HPTLC analysis.

Sample application : 20 µl of raw banana extract test solution and 2 µl of standard quercetin solution were loaded as 8 mm band length in the 100 x 100 mm Silica gel 60F 254, TLC plate using a Hamilton syringe and CAMAG LINOMAT 5 instrument. Plates were activated by washing with methanol and heating at 105 °C for 20 minutes. Plates were kept in a chamber with mobile phase formic acid: ethyl acetate: toluene: water (0.5:6.0:3.0:0.250 v/v/v).

By following the same conditions 20 µl of test solution of fenugreek seed extract and 2 µl of standard diosgenin solution were loaded.. Plates were kept in a chamber with mobile phase n-Propanaol: Water (7:2 v/v).

Spot development : The sample-loaded plate was developed in the same corresponding mobile phase while it was held

in a TLC twin-trough developing chamber that was over saturated with solvent vapour.

Photo-documentation : Solvents were removed from the developed plate by using hot air to dry it. The plate was stored in a CAMAG REPROSTAR 3(photo-documentation chamber) and was used to take pictures at UV 254 nm and UV 366 nm[5].

Derivatization : For raw banana extract, the developed plate was sprayed with a spray reagent containing 0.5 g of 2 aminoethyl-diphenyl borinate mixed with 200 ml of cool ethyl acetate. Heated at 100 °C for 3 minutes. For fenugreek seeds extract The developed plate was sprayed with spray reagent anisaldehyde sulphuric acid reagent containig 0.5ml anisaldehyde + 10ml Concentrated sulphuric acid + 20ml glacial acetic acid + 170ml cool methanol heated at 100 °C for 3 minutes[5].

FTIR :

The primary uses of a FTIR Fourier Transform Infrared Spectrophotometer are in the identification of chemical bonds and functional groups found in compounds. The concept that molecules tend to absorb specific light frequencies that are indicative of their corresponding structures is used by the IR spectroscopy theory. The chemical bonds within a molecule can be determined from the absorption spectrum of infrared light. Dry powder made from extracts of each plant material was used for FTIR analysis. In order to prepare the sample, 100 mg of potassium bromide pellets were packed with 2 mg of the dry extract powder. An FTIR spectroscope (Shimadzu, IR) was used to scan the powdered sample of each plant extract at a resolution of 4 cm, within the 400–4000 cm–1 range[6].

Polyherbal granule formulation :

The wet granulation method was used to create the granules containing *Trigonella foenum graecum* and *Musa paradisiaca* extracts which were combined with various excipient ratios, as indicated in Table-1.

Table-1. Trial batches

Sr.No	Ingredient	T1	T2	T3	T4	Role of each ingredient
1	*Musa paridisiaca*	388	388	**388**	388	API
2	*Trigonella foenum - graecum*	194	194	**194**	194	API
3	Lactose	143	118	**118**	118	Diluent
4	Magnesium Stearate	8	8	**8**	8	Lubricant
5	Starch paste	12	12	**12**	12	Granulating agent
6	Sodium methyl paraben	5	5	**5**	5	Preservative
7	Disintegrant					
a.	Without disintegrant					
b.	**Sod. Starch glycolate**			**25**		**Super Disintegrant**
c.	Cross carmelose				25	Disintegrant
D.	Starch		25			Disintegrant
	TOTAL	750	750	**750**	750	-

Pre-formulation study :

It includes assessment of different parameters like bulk and tap density, Hausner's ratio, Carr's index, angle of repose in order to determine the flow properties of both extracts[7,8] and for disintegration time.

Evaluation of capsules:

The parameters like organoleptic characters, average weight, weight variation, disintegration time, moisture content and invitro antidiabetic activity of selected trial batch were determined in order to evaluate the capsules of polyherbal granules[8]

In-vitro antidiabetic α-amylase inhibition assay :

Capsules were open and granules were triturated to convert it into fine powder. Various concentration ranging from 100 to 500 µg/mL were made. To 0.5 ml of each sample 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing alpha amylase (0.5mg/ml) solution was added and was incubated at 25 °C for 10 minutes.Each tube received 0.5 ml of a 1% starch solution in a 0.02 M sodium phosphate buffer (pH 6.9). Following a 10-minute incubation period at 25 °C, reaction mixtures were stopped with 1.0 mL of 3, 5-dinitro salicylic acid (DNSA) and incubated for 5 minutes on boiling water then cooled. The reaction mixture was diluted by adding 10.0 mL distilled water and absorbance was measured at 540 nm using UV-Visible spectrophotometer. The control samples represent 100% enzyme activity and were prepared without any plant extract.Same procedure was repeated for standard acarbose.[9]

% Inhibition was determined using the formula below.

$$\% \text{ Inhibition} = \frac{(\text{Control absorbance} - \text{sample absorbance}) \times 100}{\text{Control absorbance}}$$

Phytochemical investigation : both extracts showed presence of alkaloids, carbohydrates, flavonoids, tannins, saponins and steroids *etc*.

HPTLC fingerprint :

The HPTLC fingerprint was used for the identification of flavonoid and saponin compounds present in the raw banana and fenugreek extract, respectively. The HPTLC profile of the extracts was recorded by the run performed along with the standard flavonoid (quercetin) and saponin (diosgenin). The identities of flavonoid (quercetin) and saponin (diosgenin) in the respective extracts were confirmed by overlaying the UV absorption spectra of the sample with those of the reference standard. The bands of the raw banana and fenugreek extracts revealed the presence of flavonoid quercetin and saponin (diosgenin) in the respective samples (Fig. 1 and 2). The standard quercetin and the raw banana had Rf values of 0.585 and 0.600, respectively, as shown in Figure 1, and the standard diosgenin and fenugreek extract had Rf values of 0.704 and 0.748 respectively.

FTIR:

Fenugreek seeds : FTIR is a useful tool for analyzing the structural bonding and identifying the functional groups of a compound. FTIR spectra of dry fenugreek seed extract (Figure 3) depicted bond stretching ranged

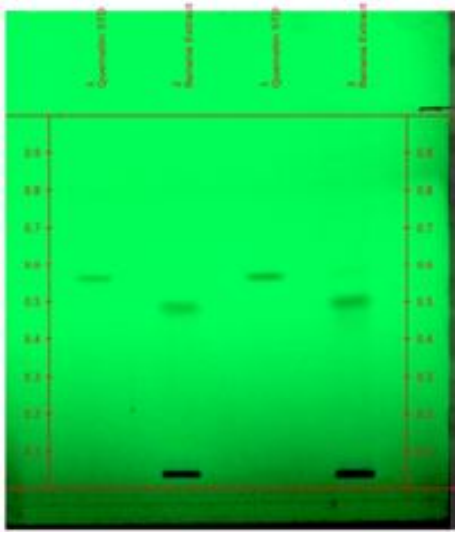

Figure 1. Chromatogram of Quercetin and banana extract at 254 nm

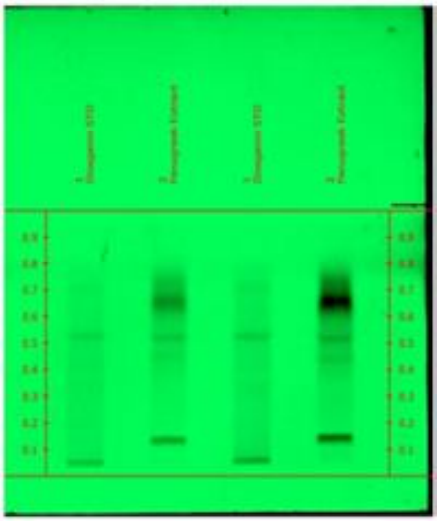

Figure 2. Chromatogram of Diosgenin and fenugreek extract at 254 nm

from 4000 cm^{-1} to 400 cm^{-1}. The peak at 3323.35cm^{-1} has been attributed to the presence of O – H group which shows the existence of phenolic compounds carbohydrates in the extract. The peaks at 2924.09 cm^{-1} could be associated to –CH stretching corresponds the presence of saponin glucosides,1739.79 C=O stretching. Peaks at 1641.42-, 1581.63, 1282.66 cm^{-1} correspond to C=C, C=O, CH_2 and CH_3 which can be attributed to the presence of aromatic ring, aldehyde, carboxyl, phenols, flavonoids, saponin glycosides, and amino acids [29,30]. The peaks at 1035.77: C–O,C=O, C–H stretching, amine C–N (stretch) also represents the presence of glycosidic bonds which indicates the existence of sugar chains belonging to saponins.[10,11]

Raw banana : Peak at 3296.3 is assigned to the stretching vibration of the O–H groups in phenolic acid compounds and carbohydrates.2926.0 for C-H ,Peak at 1649.14 associated with C=O,1409.9 responsible for bend CH2, 1074.35 for C - C. [100]. A sharp long band at 1602.48 cm^{-1} corresponding to (N–H Stretch) of amines, a nitro group at 1514.40 cm^{-1}, O–H (stretch) of phenols at 1350.62 cm^{-1}. Additional bands located at 1092.21 and 1061.21 cm^{-1} signify amine C–N (stretch)[12]. As shown in figure 4.

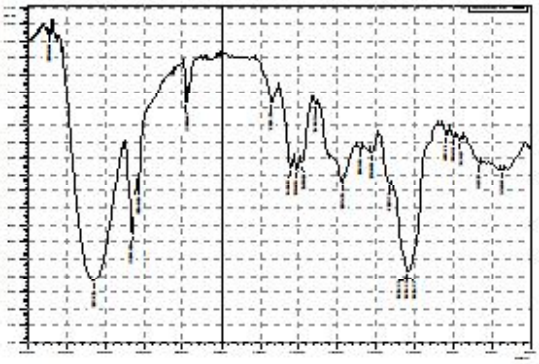

Figure 3. FTIR spectrum of Fenugreek seeds

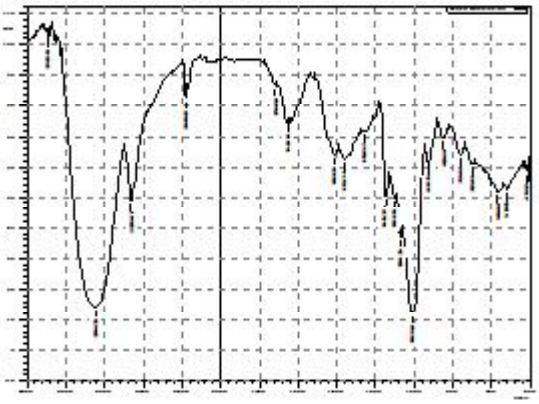

Figure 4. FTIR spectrum of raw banana

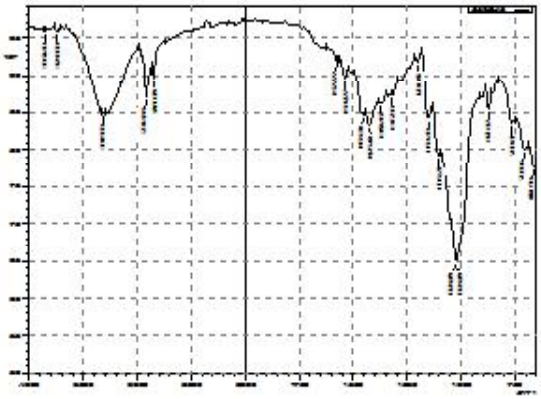

Figure 5. FTIR spectrum of Granules

Polyherbal granule formulation :

FTIR spectrum of Granules (figure 5) showed no drug drug or drug excipients interactions The blended powder was turned into granules because of the gelatinous nature of fenugreek seeds and raw banana fruit with peel extracts, which prevented the powder from flowing.

Preformulation study :

Table-2. Pre-formulation study on prepared granules

Batch	Bulk density (g/ml)	Tapped density (g/ml)	Hausner's ratio	Carr's index (%)	Angle of repose (Ø°)	Disintegration time (Minutes)
T1	0.436	0.464	1.064	7.03	22	10
T2	0.479	0.509	1.062	5.8	26	12
T3	0.500	0.532	1.064	6.01	27	8
T4	0.478	0.508	1.062	5.90	20	9

From preformulation study (Table-2) batch T3 was found to be having good flow property and faster disintegration time, hence batch T3 was selected as final batch and filled in capsule shells and assessed for the evaluation of the polyherbal granule capsules involved the determination of parameters such as moisture content, disintegration time, average weight, weight variation, and organoleptic characteristics.

Evaluation of capsules :

All capsules from selected batch has shown satisfactory evaluation results as shown in table-3. good in vitro alpha amylase antidiabetic activity as shown in table-4.

Table-3. Evaluation of capsules

Sr. No.	Parameters	Observations
1	Organoleptic characters	Capsules are of green coloured cap and green body filled with granules
2	Size	00
3	Average weight (mg)	750
4	Disintegration time (min)	8.3+- 0.12
5	Moisture content (%)	3.01 +_ 0.22
6	pH	7.21+-0.23

Table-4. In-vitro antidiabetic α-amylase inhibition assay.

Sr. No	Concentration (µg/mL)	Acarbose	Capsule
1	100	50±0.06	50.±0.09
2	200	63.42±0.22	55±0.04
3	300	68.01±0.21	57.±0.06
4	400	72.14±0.22	60±0.13
5	500	80±0.21	65±0.11
IC_{50} (µg/ml)	-	57.05	88.57

Antidiabetic herbal capsules containing extracts of *Trigonella foenum-graecum* L. (fenugreek seeds) and *Musa paradisiaca* (raw banana) fruits with peels, which contain phytochemicals such as alkaloids, carbohydrates, flavonoids, tannins, saponins, and steroids, were prepared and evaluated. The presence of diosgenin in fenugreek and quercetin in raw bananas was confirmed by HPTLC fingerprint analysis. There was no incompatibility between drugs and excipients after IR analysis. The prepared capsules showed satisfactory evaluation parameters with good in vitro alpha-amylase antidiabetic activity.

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