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Formulation and evaluation of safe and effective polyherbal antidiabetic drug

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Abstract: Diabetes is a commonly spreading disease in the world with Pakistan having prevalence levels of 30% approximately. The objective of this research study is to manage the growing problem of increasing diabetic levels in Pakistani population with a safe and effective herbal remedy, as the synthetic anti-diabetic medicines are generating intolerable side effects which could be reduced by this study outcome. Four plants i.e. *Fagonia arabica, Moringa oleifera, Nigella sativa* and *Trigonella foenum-graecum* were selected for formulation. The raw material was obtained from certified suppliers and underwent quality testing using standardized methods, then thoroughly mixed in the best combination and filled using capsule filling machine. The capsules were then tested for quality through phytochemical, physicochemical and phytopharmaceutical testing, FTIR assay, weight variation and disintegration test following standard methods. The safety was tested via acute oral toxicity test, while efficacy was tested through oral glucose tolerance test and anti-diabetic assay. The result indicated that the newly formulated capsules are effective against high blood sugar levels, comply with the pharmacopeial standards, and are safe enough as an oral drug. Further work on stability testing and toxicological studies are in progress which upon successful completion will open doors for clinical trials.

Keywords: Antidiabetic; polyherbal drug; *Fagonia arabica, Moringa oleifera, Nigella sativa, Trigonella foenum-graecum*

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INTRODUCTION

Diabetes is the most commonly spreading disease in the world (Cole J B *et al*, 2020). The global prevalence of diabetes in 2021 is 536 million people suffering from diabetes (de Paula, D *et al* 2023).

The prevalence of diabetes mellitus in Pakistan in 2022 is 26.7% in adults according to the report of International Diabetes Federation. Azeem *et al* in 2022 reported in his article prevalence of diabetes in 2016 is 11.7%, in 2018 is 16.98%, and in 2019 is 17.1%, which indicates that diabetes is increasing day by day (Azeemi *et al*, 2022).

Al-Khayri, *et al*. stated in his article about the large no of medicines that are available in the market for the treatment of diabetes; many marketed products show several drawbacks like cost and for long use causes serious side effects. Thus, natural medicinal plants are good alternates to treat diabetes without inclusion of these drawbacks (Al-Khayri *et al* 2023).

The purpose of this research work is to manage the increasing diabetic levels in the Pakistani population with a safe and effective herbal remedy, as the synthetic anti-diabetic medicines are generating intolerable side effects which could be reduced by this study outcome. Four plants *Moringa oleifera, Fagonia arabica, Trigonella foenum-graecum* and *Nigella sativa* were selected for formulation after a thorough literature search.

Moringa oleifera, commonly named as sohanjna phali, belonging to the family Moringaceae is used for health issues worldwide. Due to its health benefit, this plant is also called Miracle tree (Golkar AA *et al* 2020). Plant extract of *Moringa oleifera* is used as anti-inflammatory, anti-hypertensive, diuretic, antiulcer, anti-neoplastic, anti-hyperlipidemic and for several other health issues. The leaves extract of moringa is used to treat hyperglycemic and dyslipidemic conditions (Pareek *et al*, 2023; Ahmed *et al*, 2019).

Fagonia arabica, commonly named as Dhamasa booti, is used as anti-inflammatory, analgesic, antimicrobial, antioxidant and to treat other miscellaneous health problems (Iftikhar N *et al* in 2021). The genus of *Fagonia* is used traditionally in the treatment of different diseases like asthma, fever, pain, tooth pain and diabetic condition. It is also reported to possess anticancer effects (Fatima *et al*, 2025). *Fagonia arabica* contains many important phytochemicals such as anthraquinone, phenols, flavonoids, saponins, tannins and terpenes (Kanwal *et al*, 2021).

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Nigella sativa is commonly named as Kalonji. Prophet Mohammed S.A.W. claimed that black seed is a Panacea. In old era black seeds were used to treat different diseases like fever, cough, back pains, chest congestion, dizziness, infertility, inflammations, diarrhea and dysentery. The seeds of *Nigella sativa* helps in management of diabetes, cholesterol, lipid profile and weight loss (Ahmad, M.F *et al*. 2021). The seeds of *nigella sativa* contain iron, vitamins, carbohydrates, protein and crude fibers (Albert *et al*, 2024). It contains many active phytoconstituents like thymoquinone, pinene, thujene, carvacol, isocaryophillene, but thymoquinone is the active compound responsible for anti-diabetic activity. A researcher has reported that different organic extracts of *Nigella sativa* decrease glucose levels in blood by different mechanism (Shafodino *et al* 2022). It also exhibits anti-oxidant, anti-inflammatory, immunomodulatory, anti-histaminic, anti-microbial and anti-tumor activities (Dalli, M. *et al* 2021).

Trigonella foenum-graecum is commonly named as Methi. It belongs to the family Fabaceae. The seed of fenugreek plant is bitter in taste (Sun *et al*, 2021). In Fenugreek seeds possess different activities like anti-diabetic activity, anti-oxidant activity and anti-hyperlipidemic activity (Geberemeskel, *et al* in 2019). Some literature also mentioned some important pharmacological activities of fenugreek seeds i.e. antitumor, carminative, expectorant, anti-inflammatory, de-obstruent, emollient, restorative, parasiticide, febrifuge, hypoglycemic, burning sensation, uterine tonic and anti-cholesterolemic. The phytoconstituents present in fenugreek seeds are fiber, alkaloids, gum, flavonoids, saponins, volatile oil and some other constituents (Varshney *et al*, 2023).

As all the four plants are reported to possess hypoglycemic activity. These indigenous herbs can be used in combination for the management of diabetes mellitus. The aim of this research work was to develop a new formulation free from side effects and is effective for the management of diabetes.

MATERIALS AND METHODS

Collection and authentication of plant material

All four herbs were purchased from a local herbal market and were identified by Professor of Department of Pharmacognosy and samples were deposited in the Departmental Herbarium of University of Karachi. Specimen voucher no. issued were *Fagonia arabica* FAH-01-19*, Moringa olifera* MOL-12-24*, Nigella sativa* NSS-13-24 and *Trigonella foenegraceum* TFS-14-24.

Dosage Form Development: The four herbs were mixed an optimal combination determined through preliminary testing. The selected ratio for *Moringa olifera, Fagonia arabica, Trigonella foenum-graecum* and *Nigella sativa* was 2:1:1:1 respectively. The ratio was decided after detailed literature search keeping in mind the reported medicinal properties and toxicity of each herb. The antidiabetic assay results are in concordance with selected ratio. Powder of four herbs was thoroughly mixed in the above-mentioned ratio and filled in capsules according to the standard reported method (Archer *et al*, 2020).

Powdered microscopy

Powder microscopy of raw materials separately and mixed materials was conducted for quality check after slide preparation and observation under an electronic microscope for major diagnostic element identification (Liu, Z *et al* 2022).

Phytochemical analysis

Phytochemical analysis The preliminary phytoconstituents testing was conducted to identify the different phytoconstituent present in raw material and capsule which is described by Insanu, M *et al*. (2021).

Fourier transform infrared spectroscopy

The extract of capsules was prepared and analyzed for infrared spectra using 670-IR spectrometer (Agilent, *USA*) following the method reported by Kozlaowicz *et al* (2020). Solvents used were of analytical grade (Sigma-Aldrich). The specifications used are mentioned in table 1 below:

Table 1: Specifications of FTIR Spectroscopy

	Parameter	Specification
1	Attenuated Total Reflectance	ZnSe crystal (truncated at 45°)
2	Spectral measurements region	700 to 3800 cm^{-1}
3	Resolution	1 cm^{-1}
4	Temperature	23 °C
5	Software	Grams/AI software

Pharmaceutical analysis

Weight Variation: weight variation is performed by weighing 20 capsules one by one with the help of analytical balance, after weighing capsules the average weight was calculated and standard deviation was observed. The weight variation must comply BP limits which is ± 10% for capsule weighing ≥ 500mg (Archer *et al*, 2020).

Disintegration Test: Disintegration test of capsules was performed up to 20 minutes. Six capsules were placed in disintegration apparatus basket. After 20 minutes basket removed from test residue and at the end capsules were observed. In the case of 1 or 2 capsules not completely disintegrated, the procedure will be repeated for 12 capsules (Archer *et al*, 2020).

Phyto pharmaceutical analysis of mixed powder

Phyto pharmaceutical analysis of capsule powder material was performed as per described method. Flow characteristic is defined as the uniformity of powder material which is important for stability and efficacy of a drug. Different tests like angle of repose, porosity, bulk density, tapped density, compressibility and Hausner ratio were performed following the method reported (Adhithyakrishna *et al*, 2024).

Physico chemical analysis of powder parameters

Physico chemical analysis is used to evaluated the quality of powdered drug. Physico chemical analysis are determined by following parameters:

Fluorescence analysis

Capsules powder i.e. mixed material of all four herbs in selected ratio was taken in 10 ml of each organic solvent (chloroform, ethanol, ethyl acetate, hexane, hydrochloric acid) and water then under ordinary and ultraviolet lights were used to observe the color reaction (Adhithyakrishna *et al*, 2024).

Alcohol and Water-soluble extractives

Alcohol soluble extract was calculated by dissolving 2g of raw material in 20ml alcohol. Four vials were taken for *Moringa oleifera, Fagonia arabica, Nigella sativa* and *Trigonella foenegraceum.* Continuous shaking on electric shaker was done for 6 hours and kept macerated over night. Next day solution was filtered and dried at 105°C to calculate alcohol soluble extractives. The same procedure was repeated for water soluble extractive keeping solvent water instead of alcohol (Gaykhe *et al*, 2018).

Pre-clinical experiments

Ethical Approval

The study was approved from Animal Ethical Committee f Ziauddin University letter No. 2024-06/IK/FoP dated 26 Feb 2024.

Animal selection

The Wistar albino rats were used in research work. The adult. Healthy Wister Albino rats aged between 6-8 weeks of both sexes were selected for the study. Research animals were procured from the animal Resource Research Center, Faculty of Pharmacy and Pharmaceutical Sciences of Ziauddin University, Karachi, Pakistan. The weight range of rats was between 120g-170g. The animals were kept in quarantine for 10 days under a climate control environment (temperature 25 °C and humidity 65±10%) and allowed standard diet and water *ad libitum* (Maor-Sagie E *et al* 2023).

Study design of preclinical experiments

Three types of Preclinical assays were carried out including Acute oral toxicity test, Oral Glucose Tolerance Test and Anti-Diabetic Assay. All experiments were conducted as per standard reported methods.

Acute oral toxicity study

For the Acute Oral Toxicity Test, 15 albino Wistar rats of both sexes were randomly divided into five groups (n=3). The animals of all groups were orally administered with the following scheme:

Group 1 (Control Group) 1ml of Distilled water
Group 2 (Test Group) 5 mg/kg of the test drug
Group 3 (Test Group) 50 mg/kg of the test drug
Group 4 (Test Group) 300 mg/kg of the test drug
Group 5 (Test Group) 2000 mg/kg of the test drug

After the treatment, the rats were observed for any sign of toxicity like tremors, lacrimation, excess urination, diarrhea, dizziness, sleep etc. for 6 hours at regular intervals and further observed for delayed toxicity or mortality for 14 days (Iswar *et al*, 2019).

Oral glucose tolerance test

For oral glucose tolerance test, 20 Wistar rats of both sexes were taken. Before starting the experiment, the animals were deprived of food and only free access to water was provided. After 12 hours rats were randomly divided into four groups (n=5).

Group 1 (Negative Control Group) 1ml of Distilled water was orally administered
Group 2 (Test Group 1) 250 mg/kg of the test drug was orally administered
Group 3 (Test Group 2) 500 mg/kg of the test drug was orally administered
Group 4 (Positive Control Group) 0.6 mg/kg Glibenclamide was orally administered

Blood Glucose levels were noted using one touch glucometer in all treated animals and after 30 minutes, 2g/kg glucose load was administered orally to all the treated animals. Blood glucose was then tested immediately after glucose load (0 minutes) and then the procedure was repeated at regular time intervals of 30, 60, 120 and 240 minutes after the administration of glucose load (Chaimum-aom, N *et al* 2017).

Anti-Diabetic assay

For the antidiabetic assay, 30 Wistar rats of both sexes were taken. The animals were randomly divided into five groups (n=6). Group 1 was Normal control without induction of diabetes, while Group 2-5 were kept to induce diabetes. All the animals of group 2-5 were induced diabetes after a fasting period of 12 hours through intraperitoneal injection of alloxan monohydrate at a dose 150mg/kg. The animals were checked for diabetes through monitoring of blood glucose levels after 72 hours and animals having levels >250mg/dl were considered diabetic.

Group I (Normal control): was given 1ml normal saline orally
Group II (Negative control): Diabetic induced rats were given 1ml normal saline orally
Group III (Positive control): Diabetic induced rats were given Glibenclamide 5mg/kg orally

Group IV (Test group): Diabetic induced rats were given test drug 250 mg/kg orally
Group V (Test group): Diabetic induced rats were given test drug 500 mg/kg orally After a week of oral administration of tested drug, the blood glucose levels were checked by glucometer. At the end of study, the animals were sacrificed for histopathological analysis of vital organs (Kinara *et al*, 2016).

STATISTICAL ANALYSIS

One-way analysis of variance ANOVA was used to calculate statistical significance of all the values. The values are represented as Mean ± standard errors (SE) in triplicate values. The probability $p \leq 0.05$ is used to demonstrate that all the values are statistically significant at a 5% level. Tukey's b test was conducted for statistical analysis with subset of alpha=0.05.

RESULTS

Powdered microscopy
Powdered microscopy was carried out for raw material individually before formulation and then again checked after the preparation of the final drug. figs. 1-4 show the major diagnostic elements of *M. olifera, F. arabica, T. foenum-graecum* and *N. sativa* powders, while fig. 5 shows the powdered microscopy of capsule material.

Preliminary phytochemical analysis was carried out as described above. The phytochemicals found in all four herbs are presented in table 2.

Standardization of Capsules
Phyto pharmaceutical analysis of powder
Physico-chemical analysis of powder
Before formulation, the powdered raw material was observed in UV light at 254 and 366nm for colorimetric reaction. The sample preparation is already mentioned above in the experimental section. The colors observed at two different wavelengths are represented in table 4.

FTIR Testing of capsule powder
FTIR analysis of capsule powder was carried out for standard practice as per procedure described in experimental section. The FTIR spectra is exhibited in fig. 6 below:

Acute oral toxicity
Acute oral toxicity was performed to find out the safety of drug for oral use. After performing the test of all animals were healthy and alert except two animals were lethargic at higher dose for a period of 15 minutes. No mortality was recorded. If the animals of higher are alive and healthy for 14 days, then the test compounds qualify the acute toxicity test and it is safe enough to be used as an oral drug.

Oral Glucose Tolerance Test
In OGTT Assay, the glucose tolerance capacity was tested in albino Wistar rats. The results were compared to control group which are expressed in table 5 below and comparative analysis presented in Figure 7.

Anti-Diabetic activity
Anti-Diabetic Assay
In antidiabetic activity, the diabetic effect of drugs in different groups was detected in diabetic rats by treating with test and standard drugs. The results were compared to control group which are expressed in table 6 below and comparative analysis is expressed in Figure 8 below:

DISCUSSION

Herbal medicines are gaining importance in modern world due to the adverse effects of synthetic medicines. It has been noticed that after long term use of synthetic medicines, many vital organs are affected. Therefore, search for natural compounds to replace synthetic medicines is crucial. There are several traditional medicines claiming cure of diabetes, but still scientific evidence is lacking. Further regulatory policies for herbal products are relaxed which results in substandard herbal drugs. The purpose of this research work is to add some scientific evidences and to find a way to produce herbal products of good quality using standardized methods. Although the research work is in process, but enough work is done to be reported. The background and methodologies are described in previous sections.

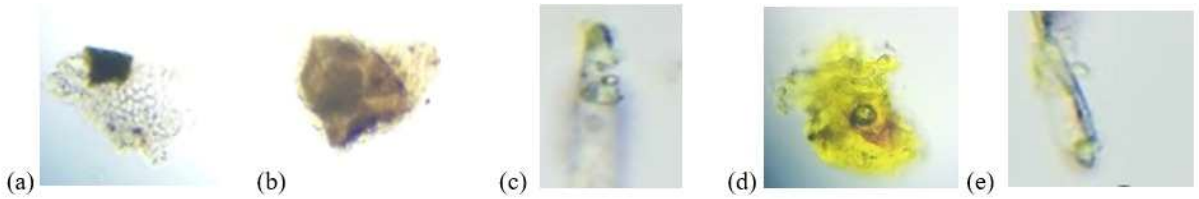
Phytochemical analysis of powders revealed that alkaloids, phenols, saponins, flavonoids, glycosides and carbohydrates were present in all four powders, while tannins and steroids were present in all except Nigella, terpenoids were present in all except Fagonia. The constituents responsible for antidiabetic activity and possible mechanism of action are discussed later in this section.

Powdered Microscopy exhibited some important pharmacognostic features with major diagonostic elements as shown in fig. no. 1-5 in the result section. These special cells and trichomes are helpful in identification and standardization of powdered herbal material (Singh *et al*, 2018). The various microscopic elements present in these herbs will be helpful for quality check of raw material.

The polyherbal drug standardization is a difficult task but for production of a quality medicine, it is necessary to evaluate the raw material and then the finished product. Therefore, quality tests were conducted after procurement of raw material through organoleptic, physicochemical, phytochemical and powdered microscopy. Then the powdered drugs were mixed thoroughly in a particular ratio and filled in capsules.

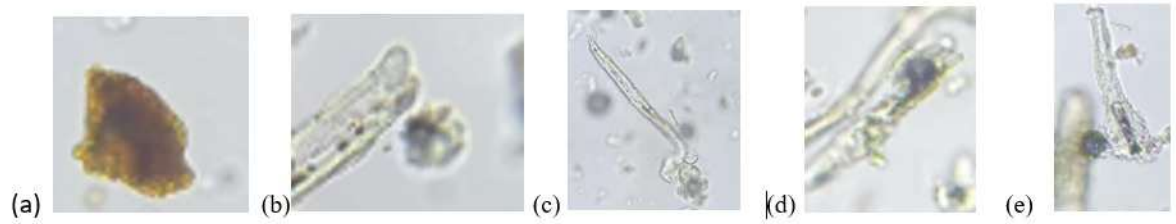
Table 2: Phytochemical Analysis

Phyto constituent	*Fagonia*	*Moringa*	*Black seed*	*Fenugreek*
Alkaloids	+	+	+	+
Phenols	+	+	+	+
Tannin	+	+	-	+
Terpenoid	-	+	+	+
Saponin	+	+	+	+
Flavonoids	+	+	+	+
Steroids	+	+	-	+
Carbohydrate	+	+	+	+
Glycosides	+	+	+	+



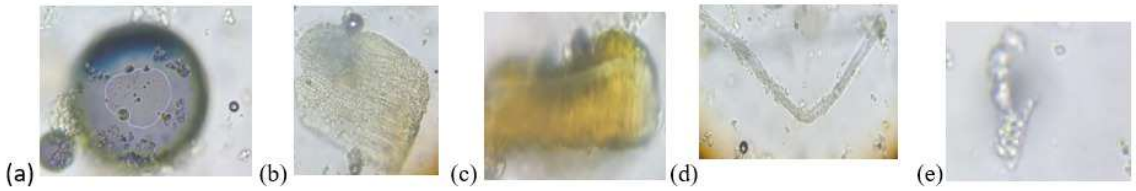
a. Parenchymatous cells b. Lignified cells c. Ca-Oxalate crystals d. Oil globules e. Trichomes

Fig. 1: Powdered microscopy of *Moringa oleifera* Moringa



a. Lignified Cells b. Sclereids c. Trichome d. Pitted vessels e. Starch grains

Fig. 2: Powdered microscopy of *Fagonia arabica*

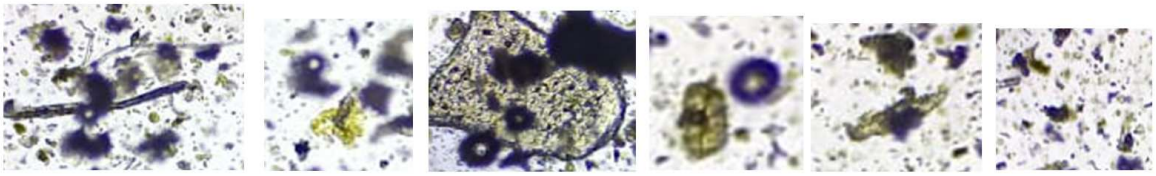


a. Oil globules b. Parenchymatous Cells c. Lignified cells d. Trichome e. Ca-Oxalate Crystals

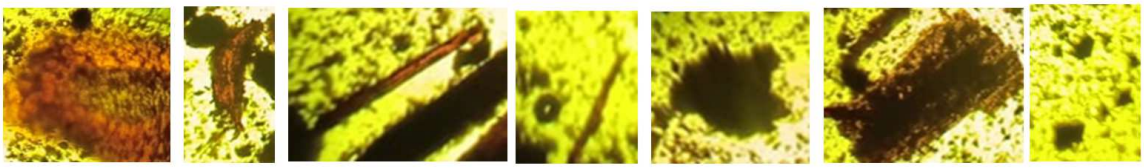
Fig. 3: Powdered microscopy of *Trigonella foenum-graecum*

a. Oil globules b. Lignified Fibers c. Starch grains d. Tannins e. Rectangular Epidermal Cells

Fig. 4: Powdered microscopy of *Nigella sativa*



a. Trichomes b. Ca-oxalate Crystals c. Parenchymatous cells d. Oil Globules e. Lignified Fibers f. epidermal cells



g. Parenchymatous Cells h. Lignified Cells i. Trichomes j. Oil Globules k. Tannins l. Epidermal Cells m. Starch grains

Fig. 5: Powdered microscopy of *Capsule Powder*

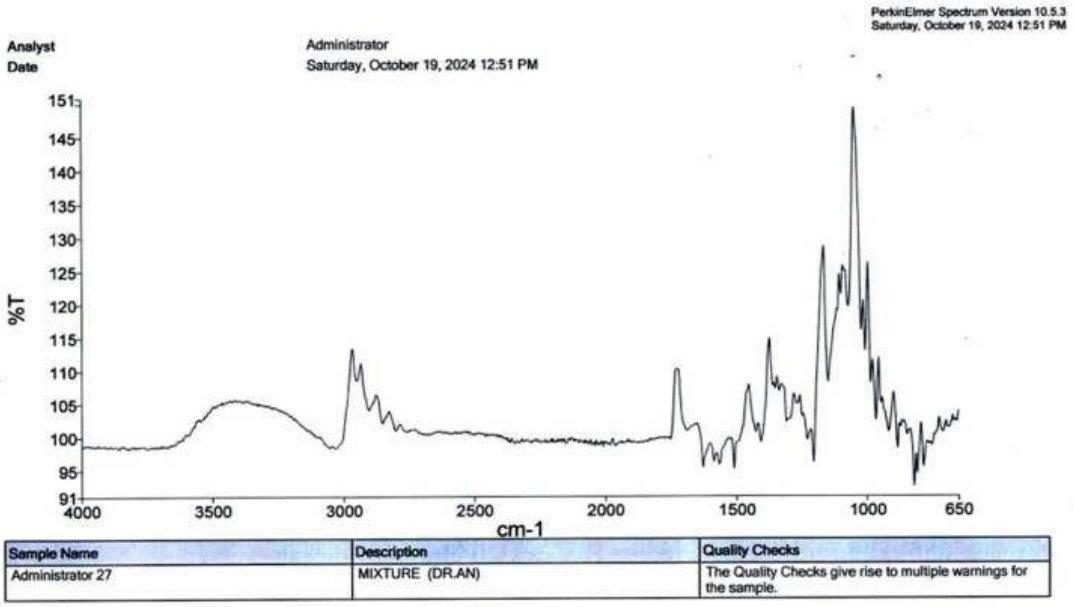


Fig. 6: FTIR Spectra of Capsule material

After batch production, the capsule powder was evaluated through phytopharmaceutical testing, in which the flow characteristics of mixed powder, organoleptic evaluation and fluorescence testing was conducted. Angle of repose was found to be 7.61, porosity 22%, tapped and bulk density 0.52 and 0.41 respectively while compressibility index 0.219 and Hausner ratio 1.18. This data shows that mixed capsule powder has good flow properties which will be helpful in capsule filling (Stranzinger *et al*, 2017). The capsule powder was coarse, dull, greenish-yellow with herbal smell and astringent taste. Colorimetric observation of powdered material through Fluorescence Test with different organic solvents at 254 and 366 nm exhibited different colors i.e. for *Fagonia arabica* it exhibited different shade of yellow and brown colors, for moringa different shades of green and black, for nigella yellow, green and brown shades and for fenugreek shades of green, brown black and orange colors. The detail is mentioned in table 5. These tests are conducted to find adulteration in herbal material (Stefan *et al*, 2023). No adulterated material was found in test drug as per the data obtained after colorimetric florescence testing. The four raw materials were tested for solubility in water and alcohol and were found to be soluble in both solvents ranging from 5-12% in alcohol and 5-18% in water. Fagonia and fenugreek powders showed high solubility in water as compared to alcohol while moringa and nigella exhibited higher solubility in alcohol than water (table 6).

Table 3: Flow characteristics of combined powder parameters of Capsule material

Angle of repose	7.61
Porosity	22 %
Tapped density	0.52
Bulk tapped	0.41
Compressibility index	0.219
Hausner ratio	1.18

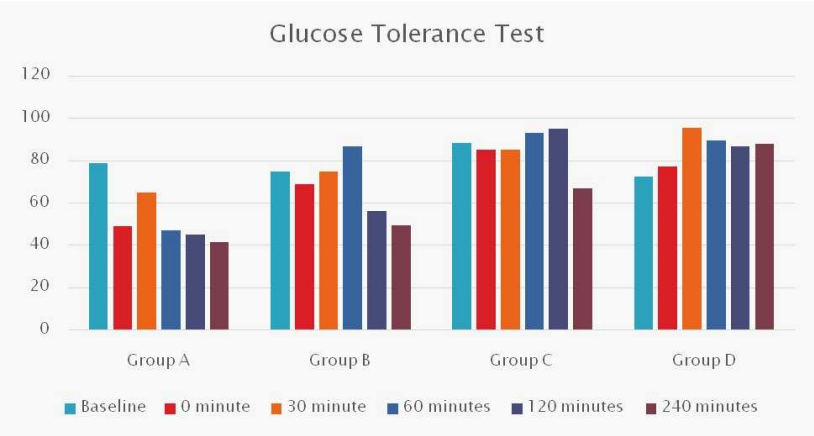


Fig. 7: Oral Glucose Tolerance Test

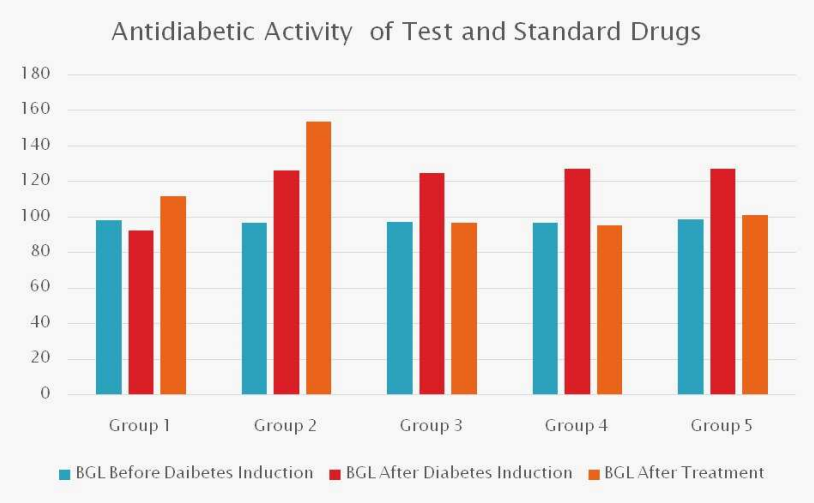
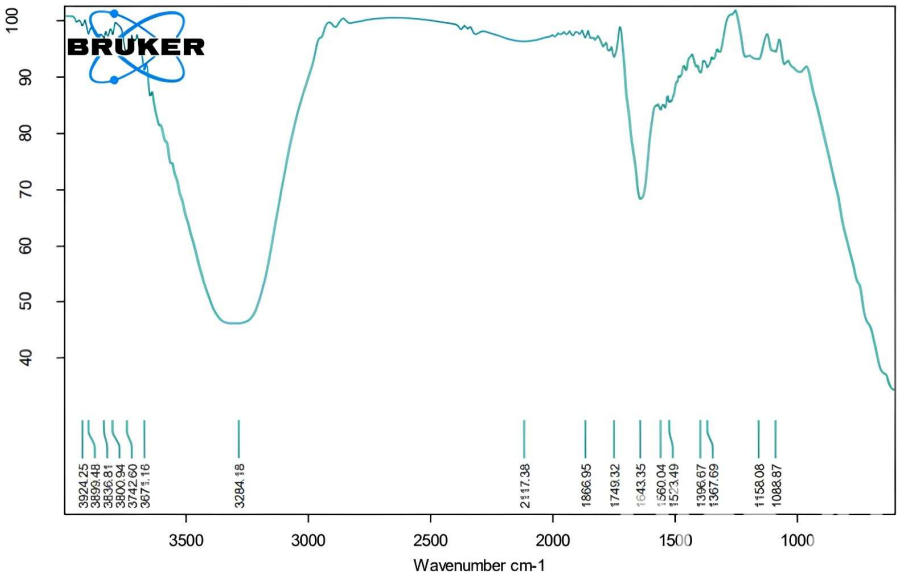
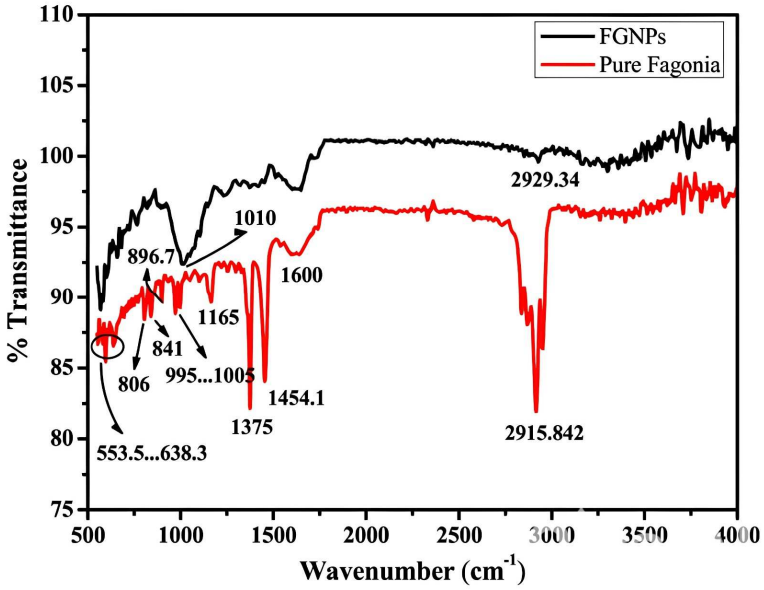


Fig. 8: Comparative analysis of Antidiabetic Activity of test and standard drugs



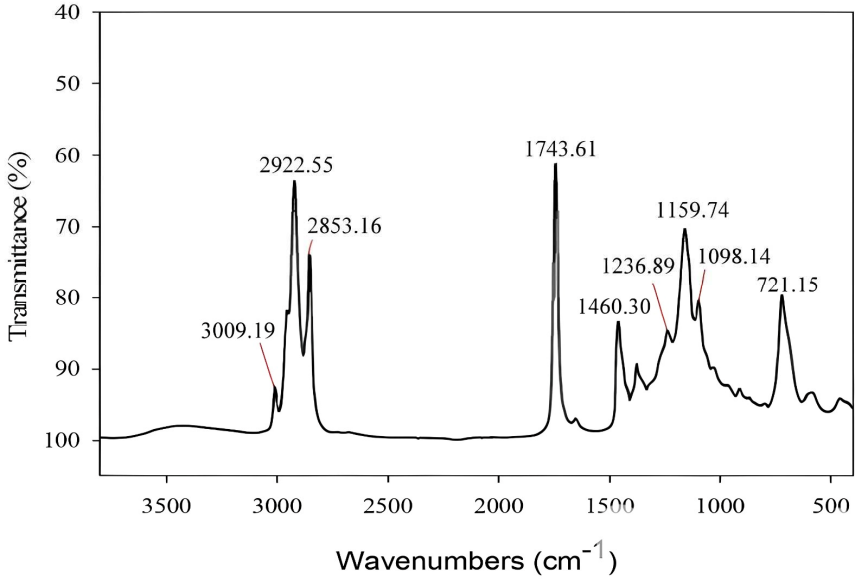
FTIR Spectra of *Moringa oleifera* leaves reported by Maheshwari et al, (2023) show multiple peaks at 3248.14, 2117.38, 1866.95, 1749.32, 1643.35, 1560.04, 1523.49, 1396.67, 1367.69, 1158.08 and 1088.87 cm-1 out of which some prominent peaks match our combined spectra.

Fig. 9: FTIR spectra of *Moringa oleifera* leaves



FTIR spectra of *Fagonia arabica* reported by Mariam et al, (2021) show clear peaks at 553.5, 806, 841, 1165, 1375, 1454.1, and 2915.824 cm^{-1} which are also evident in our FTIR spectra.

Fig. 10: FTIR spectra of *Fagonia Arabica*



FTIR Spectra of *Trigonella foenum-graecum* seeds reported by Akbari et al (2019). The prominent peaks at 721.15, 1159.74, 1743.61, 2853.16 and 2922.55 are visible in our spectra of capsule powder too.

Fig. 11: FTIR Spectra of *Trigonella foenum-graecum* seeds

The finished capsules were also checked for weight variation and disintegration tests. The average weight of capsules was found 502.33±0.2334 and all capsules were disintegrated within 20 minutes. This shows that capsules comply BP limits and are acceptable for oral intake. FTIR spectrum of our test drug is presented in fig. 6. It was compared to FTIR spectra of different researchers reported spectra of individual herbs.

Acute Oral Toxicity is a standard test conducted to find out the safety of drug to be used orally. After performing the test, all animals were found alive, active and healthy without any sign of toxicity indicating that the test drug is safe enough to be administered orally (Iawar *et al*, 2019).

Table 4: Organoleptic Evaluation of Capsule Material

Parameters	Capsules
Color of powder	Greenish yellowish
Color of capsules	Greenish
Odor	Herbal smell
Appearance	Coarse dull
Taste of powder	Astringent taste

Table 5: Colorimetric observation of powdered material through Fluorescence Test

Solvent	Wavelength	*Fagonia Arabica*	*Moringa Olifera*	*Nigella Sativa*	*Trigonella foenegracium*
Water	254nm	Yellowish green	Blackish	Yellow	Dull greenish
	366nm	Brownish	Dull black	Green	Black
Ethanol	254nm	Dark yellow	Black	Light green	Greenish
	366nm	Lemon yellow	Parrot green	Light brown	Brown
chloroform	254nm	Yellowish green	Blackish	Greenish	Greenish
	366nm	Mustard	Black	Brownish	Dull brown
Ethyl	254nm	Yellowish	Light green	Yellowish green	Greenish
acetate	366nm	Mustard yellowish	Sea green	Brownish	Orange
Hexane	254nm	Yellowish green	Dark green	Yellowish	Green
	366nm	Light brown	Black	Dull brown	Brownish red
HCl	254nm	Reddish brown	Green	Dark green	Blackish
	366nm	Dull brown	Black	Dark brown	Black

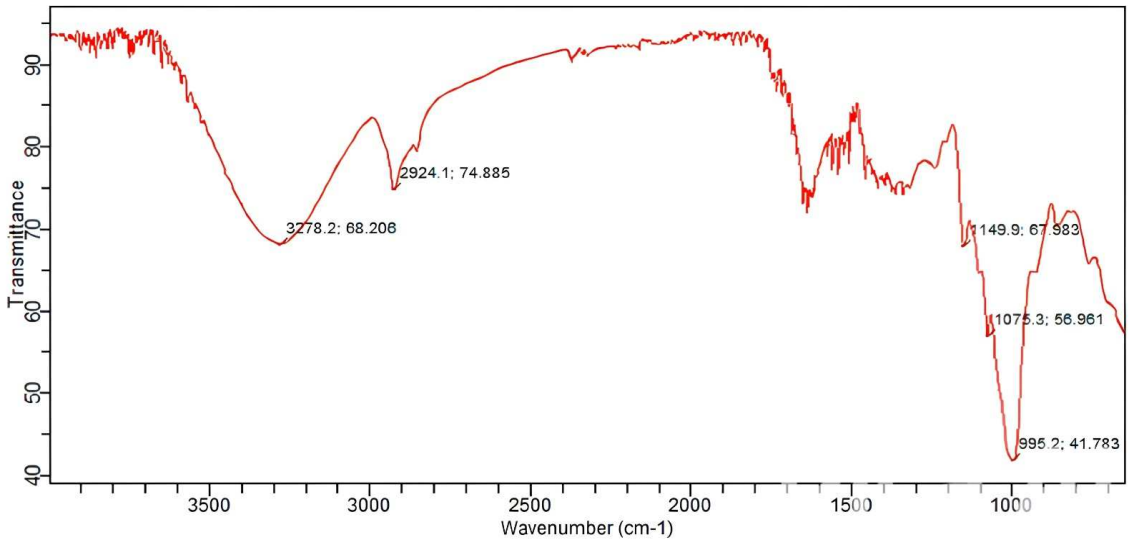
Table 6: Solubility testing of extract

Parameters	Trigonella	Black seeds	Fagonia	Moringa
Alcohol soluble extract	5%	12%	5%	10%
Water soluble extract	17%	5%	18%	5%

Table 7: Oral Glucose Tolerance Test

Groups	Body Weight (g)	Dose	Time -30	0	30	60	120	240
A	154.33±4.509	5mg	79± 7.810	49± 7.000	65± 4.358	47±1 1.532	45± 6.557	41.67± 12.447
B	157.67±2.516	500 mg/kg	75± 6.982	69± 6.557	75± 12.767	86.67± 17.097	56.33± 18.147	49.67± 10.017
C	162.33±2.516	250 mg/kg	88.33± 9.712	85.33± 5.033	85± 7.937	93± 10.535	95± 3.00	67±6.244
D	158.67±2.081	1ml	72.33± 2.081	77.33± 5.131	95.33± 9.0185	89.67± 22.478	86.67± 11.372	88± 3.605

$p \leq 0.05$ is considered for all the values to be statistically significant



FTIR spectra of *Nigella sativa* seeds reported by Ali *et al* (2022). The peaks are mentioned at 3278^{-1} cm, 924 cm^{-1}, 1149cm^{-1}, 1075 cm^{-1} and 995 cm^{-1}shows that similar peaks are present in our spectra (fig. 6).

Fig. 12: FTIR spectra of *Nigella sativa* seeds

Table 8: Antidiabetic activity of test and standard drugs

S. No	Groups	Avg BW	Blood glucose level		
			Glucose before induction	Glucose after induction	Glucose after treatment
1.	Control I	164.6±2.4908	98.2 ± 3.114	92.338±6.693	112±6.204
2.	Control II	159.8±5.118	96.6 ±3.3615	126.186±5.932	154±3.937
3.	Test I	162.6±3.435	97.4 ±5.813	124.969±4.8476	97.0 ±3.535
4.	Test II	166.4±2.701	96.8 ± 3.962	127.058±5.2915	95.4±6.348
5.	standard	163.4±3.0495	98.6±3.911	127.487±4.4384	101±2.915

Oral Glucose Tolerance Test was performed to assess the antidiabetic potential of test drug. The OGTT assay was performed on Wistar albino rats as per the standard protocol presented in experimental section. The test drug exhibited highly significant ability to decrease blood sugar levels in dose dependent manner. The standard drug Glibenclamide decreased blood glucose levels from 79 ± 7.810 to 41.667 75±6.982±12.447 while our test drug at 500 mg /kg dose lowered blood glucose levels from to 49.666 ± 10.017 and at 250 mg / kg dose from 88.333 ± 9.712 to 67 ± 6.244 as shown in table 7. The comparative data can be analyzed in fig. 7.

This is concordant with antidiabetic potential of four herbs reported earlier. According to researchers, Moringa maintain the insulin in our body and producing hyperglycemic effect in both types of diabetes. It is traditionally consumed for management of diabetes producing its action by inhibiting the activity of α-amylase and α-glucosidase and also enhancing the secretion of insulin (Gopalakrishnan *et al* 2016; Ahmad *et al* 2019). A considerable decrease in blood glucose level could be due to the presence of phytochemicals anthraquinone, anthocyanins, hemlock tannin, and glycoside and a phenolic steroid in leaves of moringa (Zainab *et al* 2020). The genus of *Fagonia* is used traditionally in treatment of different disease like asthma, fever, pain, tooth pain and diabetic condition. Nigella sativa contains many active phytoconstituents but thymoquinone is the active compound responsible for anti-diabetic activity. A researcher ha Shafodino (2022) reported that different organic extracts of *Nigella sativa* decrease glucose levels in blood by different mechanism (Shafodino *et al* 2022). Fenugreek seeds contain anti-diabetic activity along with many other medicinal properties (Geberemeskel *et al* in 2019).

Antidiabetic assay was conducted for further confirmation. The diabetic rats were treated with test and standard drugs and compared with control group. A highly significant dose dependent antidiabetic effect was found in test compound (fig. 8).

This highly significant hypoglycemic activity indicates that our test drug could be used to manage type 2 diabetes in a safe and effective manner. Further studies are required i.e. clinical trials so that the drug could be marketed after regulatory approvals.

CONCLUSION

A suitable formulation with anti-diabetic activity is formulated and tested for quality and stability using standard reported methods. This formulation is expected to be effective and safe for long-term management of diabetes after clinical studies.

Conflict of interest

This is an original article, all the authors have gone through the manuscript and the Authors have no conflicts of interest to disclose.

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