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Phytochemical Quantification and Characterization of Anti-Diabetic Potential of Polyherbal Formulation by FT-IR and GC-MS Analysis

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Abstract

The main objective of the present study was to develop and evaluate anti-diabetic potential of polyherbal formulation (PHF) using *Nigella sativa*, *Cinnamomumverum*, *Allium sativum*, *Zingiberofficinale*, *Curcuma longa* and *Trigonellafoenumgraecum* for management of diabetes. The PHF was investigated by advanced analytical techniques. The proximate analysis of PHF revealed all parameters were within the limits indicating no adulteration and contamination. In addition, gas chromatography-mass spectrometry (GC-MS) and Fourier transform-infrared (FT-IR) spectroscopy analysis showed the presence of bio-active phytochemicals including phenolic compounds, antioxidants, anti-inflammatory and anti-diabetic constituents that are good therapeutic potential for prevention and management of diabetes. The efficacy of PHF was evaluated by dividing into four groups (PHF 1.5 g, PHF 3.0 g, metformin 500 mg and placebo) of newly diagnosed type 2 diabetic patients for 90 consecutive days and monitored on a monthly basis. PHF 3.0 g dose showed a significantly higher anti-diabetic effect as compared to PHF 1.5 g while comparable results in relation to metformin 500 mg. The phytochemical characterization of PHF will ensure its quality and safety. Moreover, the anti-diabetic efficacy of PHF is comparable with anti-diabetic efficacy of metformin. PHF has the potential to achieve glycemic control in type 2 diabetic patients with a diabetic diet prescribed.

Keywords: Polyherbal formulation, Phytochemical analysis, GC-MS, FT-IR, Type 2 diabetic patients

Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder affecting 463 (9.3%) million people across the globe and is expected to increase to 578 (10.2%) million people by 2030 and 700 million by 2045 [1]. According to the 2nd National Diabetes Survey of Pakistan (NDSP) 2016-2017, approximately 26.3% (27.4 million) people in Pakistan are suffering from diabetes [2]. Diabetes is escalating worldwide due to an imbalance between oxidative stress and antioxidant levels which increases inflammation, insulin resistance and hyperglycemia in the human body. Poor glycemic control causes the formation of reactive oxygen species (ROS), inflammation, delayed wound healing, micro and macro-

vascular complications in diabetic patients [3]. In addition, prolonged use of modern medications in diabetes management not only incurs a high cost but also results in serious side effects including weight gain, hypoglycemia, gastrointestinal problems, kidney and liver toxicity [4].

Modern medicines including various types of insulin are successful in the management of diabetes, however, their cost, complications, limited tolerability and side effects are the factors responsible for decreasing their general acceptance [5]. Hence, people are increasingly turning their focus toward nutrition therapy for diabetes due to its efficacy, safety, lesser toxicity, fewer side effects, and accessibility at reasonable prices. Herbs and their herbal formulations are generally considered effective, safe, low cost and almost 80% of the global population depends on herbs for treatment, cure and prevention and are mostly used without prescription [6]. Using more than one herb in a product (polyherbalism) is a better way to attain enhanced therapeutic action and decrease the concentration of single herbs, by reducing herbal side effects and complications. Moreover, the polyherbal formulations work synergistically on the root causes of diabetes and its complications [7]. Herbs used in our daily diets are good sources of anti-diabetic properties and could be a better choice for herbal formulation.

Quality is the main concern of consumers in relation to herbs and herbal formulations. Poor quality control in herbal formulations may result in intentional or unintentional adulteration, substitution, contamination and many other ways that may decrease the quality of herbal products and may lead to hazardous impacts on the health of consumers [8]. Thus quality assessment of herbal formulations is essential to control the quality to bring them into the modern system of medicine for the betterment of mankind. So, various tools and techniques must be applied to ensure the quality of herbal products, is always appreciated by the world health organization (WHO) in developing countries [9]. Moreover, quality assessment of herbal formulations based on the concentration of their active principle is also required by industry and government agencies [10].

Culinary herbs and spices have immense potential for the treatment of diabetes and its complications which is supported by scientific studies in the literature. Turmeric (*Curcuma longa)* is known as “haldi”, have been reported for its medicinal properties utilized either alone or in the form of formulations to reduce hyperlipidemia due to the presence of polyphenols having antioxidants and anti-inflammatory properties [11]. Fenugreek (*Trigonellafoenumgraecum)* or methi dana contains immune controlling polysaccharides, a protector of β cells in type 2 diabetic patients [12]. Black seed (*Nigela sativa)* or kalonji possesses both hypoglycemic and hypolipidemic properties, and provides protection against β cell destruction [13]. Cinnamon (*Cinnamomumverum)* or dalchini, contains pro-cyanidin oligomers which are responsible for anti-diabetic activity in patients with diabetes [14]. In addition, garlic (*Allium sativum)* popularly known as “lehsun”, contains antioxidants which promote catalase and peroxidation activities which prevent inflammation and oxidative stress in diabetic patients [15]. Ginger (*Zingiberofficinale)* or “adrak”, lowers glucose and lipid levels due to the presence of antioxidants. Besides, it acts as immune-modulatory, anti-inflammatory and antiapoptosis [14]. The objective of the present study was to develop, characterise and evaluate the anti-diabetic potential of polyherbal formulation by advanced analytical techniques and assess its efficacy and

antidiabetic potential by administrating it in newly diagnosed type 2 diabetic patients.

Materials and Methods

Formulation Development

For effective development of herbal formulation, selection of herbal spices (*Nigella sativa*, *Cinnamomumverum*, *Allium sativum*, *Zingiberofficinale*, *Curcuma longa* and *Trigonellafoenumgraecum*) was carried out on the basis of anti-diabetic efficacy and herbs were procured from local market and authenticated by Herbarium Medicinal Botanic Centre, PCSIR Laboratories, Peshawar, Pakistan (Table 1). Herbs were washed with distilled water to take away dust, dirt and parasites and dried in the open air at room temperature. Then dried herbal spices were powdered using a commercial spices grinder and sieved through 80 meshes separately for uniformity in size. The powdered herbs were mixed with a commercial mixer to prepare the polyherbal formulation. All the ingredients were mixed in equal amounts or a ratio of 1:1, respectively.

Table 1. **Enumeration of herbs species used in polyherbal formulation for treatment of diabetes.**

Common name	Botanical name	Parts used	Family	Quantity
Garlic	*Allium sativum*	Root	Liliacae	1 part
Cinnamon	*Cinnamomumverum*	Bark	Lauraceae	1 part
Ginger	*Zingiberofficinale*	Root	Zingiberaceae	1 part
Black cumin	*Nigella sativa*	Seeds	Ranunculaceae	1 part
Turmeric	*Curcuma longa*	Root	Zingiberaceae	1 part
Fenugreek	*Trigonella-foenumgraecum*	Seed	Leguminosae	1 part

Standardization of Polyherbal Formulati on

Standardization of herbal products is a vital step to establish a biochemical profile and ensuring the quality of the herbal products.

Quantitative Phytochemical Analysis

The standard methods of the Association of Official Analytical Chemists (AOAC) were used to perform proximate analysis of PHF to determine crude protein, crude fat, crude fiber, moisture, ash content and nitrogen free extracts [16]. Moreover, polyphenolic content was determined by the Folin Ciocalteu method to assess the total phenolic content in PHF powder [17]. Flavonoid content was assessed by the Aluminium chloride method to estimate total flavonoid content in PHF extract [18]. The antioxidant activity was determined by DPPH assay. The DPPH (2,2-diphenyl-1-picrylhydrazyl radical) is a stable free radical solution which changes its color with the addition of extract of PHF (antioxidants). The proposed method by Brand-Williams 1995 was used in this assay [19]. Micronutrients in the polyherbal formulation were determined by the proposed method of Hussain et al. [20]. The samples of PHF were subjected to digestion by concentrated nitric and perchloric acid. The micronutrient concentration of Zn and Fe of PHF was carried out by atomic absorption spectrometer (Perkin Elmer AA Analyst 700).

Gas Chromatography-mass Spectrometry (GC-MS) Analysis of Formulation

Polyherbal formulation was diluted in 100 mL of methanol for 48 h; the filtrate was obtained and concentrated on water bath. Then the methanol extract of PHF was subjected to analysis by using a Shimadzu GCMS-QP5050A (Kyoto, Japan) The capillary column DB-5/RTX-MS was used for the separation (30 m length and 0.25 mm diameter consisting of 95% dimethyl polysiloxane). Helium was used as carrier gas at the flow rate of 1 mL/min with linear velocity of 37.2 cm/sec and 1 μL injection volume. For sample analysis, column was

maintained 90 °C for 1 min after injection. The injection temperature was elevated to 200 °C with a 10 °C increase every minute. The final temperature was elevated to 250 °C with a 10 °C increase in temperature/min for 15 min. The temperature of the detector was maintained at 300 °C while the injector at 250 °C. An electron ionization system was used for the detection of ionization energy of 70 eV. Pressure was maintained at 60.0 kPa and a sample was run for 60 min. A scan rate of 0.50 s (cycle time: 0.2 s) was applied, which covers a mass ranging from 35 to 600 amu [21]. Interpretation on mass spectrum GC-MS was carried out by comparison of the retention times and MS data to the National Institute of Standards and Technology (NIST) library.

Fourier Transform-infrared Spectroscopy Analysis of Formulation:

Fourier transform-infrared (FTIR) spectroscopy is a tool for the identification of different kinds of chemical bonds or functional groups in herbal medicine in order to obtain the FTIR spectra (fingerprint) of polyherbal formulation powder [22]. For FTIR analysis, the samples of PHF were powdered finely, mixed with KBr and pelletized to prepare the specimen [23]. Then PHF samples were loaded in FTIR-8400 spectrophotometer (Shimadzu, Japan) having a scanning range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}.

Antidiabetic Effect of Polyherbal Formulation on Type 2 Diabetic Patients

Formal approval was acquired from the tertiary care hospital Ethical Review Committee to conduct research on diabetic patients. Moreover, informed consent was obtained from participants in the study in order to affirm their willingness. The polyherbal formulation was packed in sterilized polyethylene packs in the form of sachets. A total of 256 participants with T2D were randomized in the study (Figure 1). The 152 newly diagnosed type 2 diabetic (T2D) patients of both genders (BMI>23 kg/m2) aged between 30-70 years were selected according to inclusion/ exclusion criteria for efficacy analysis and divided into four groups (n=38 each) and administered with two doses of PHF, metformin and placebo for 90 days consecutively and biochemically evaluated on monthly basis. The participants were distributed randomly into three treatment groups and placebo:

1. Group- PHF 1.5: Polyherbal formulation (1.5 g/day) with a diabetic diet.
2. Group- PHF 3.0: Polyherbal formulation (3.0 g/day) with a diabetic diet.
3. Group- Metformin: Metformin tablets potency of 500 mg thrice a day with a diabetic diet.
4. Group- Placebo: Control group, powdered wheat bran sachets with a diabetic diet.

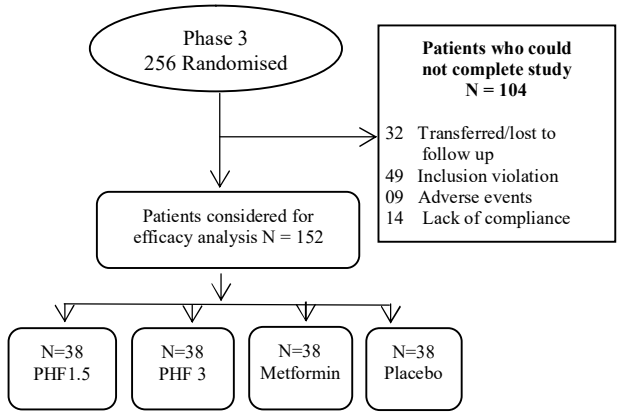

Figure 1. **Flow diagram of study participants**

Biochemical Analysis of Blood Glucose Levels in T2D Patients

Fasting, random glucose and glycated Hemoglobin (HbA1c) levels were estimated

by Cobas 6000 c501 Chemistry analyzer at PEMH Laboratory, Rawalpindi. After centrifugation at 2000 rpm for 10 min, plasma was obtained. The hexokinase enzymatic technique was used as a reference method. Each test was replicated 2-3 times.

Statistical Analysis

All analytical experiments were conducted in triplicates and findings were expressed as Mean ± standard error of mean (SEM). This study was a three-month randomized placebo-controlled design. Data was statistically analyzed by means of SPSS software (version 23) and STATISTIX-8. Two factor factorial two way ANOVA was applied to check the interventional groups with time. Means were compared using LSD and a significant value of $P < 0.05$ was fixed to determine significant parameters.

Results and Discussion

The prepared polyherbal formulation was developed and evaluated for essential phytochemicals present.

Physicochemical Studies of Polyherbal Formulation

The proximate composition of PHF powder revealed the highest nitrogen free extract (NFE) content of 67.33±0.06% and protein content of 11.24±0.09%, moderate moisture content of 09.63±0.05% and low concentration of crude fat of 05.13±0.07%, crude fiber content of 04.52±0.04% and ash content of 2.15±0.02% as shown in Table 2. Moreover, the phytochemical profile of formulation revealed a phenolic content of 412.0 mg CE/100g, flavonoids content of 873.0 mg QE/100g, antioxidant activity of 56.23%, iron content of 08.46 mg and zinc content of 03.21 mg, respectively as depicted in Table 3.

Table 2. **Nutritional composition of PHF on dry weight basis (Mean of values).**

S. No	Nutrients	Composition
1	Crude Protein	15.75 ±0.09
2	Moisture	09.63±0.05
3	Crude fat	10.13 ±0.07
4	Crude fiber	10.52 ±0.04
5	Ash	04.15 ±0.02
6	NFE*	49.82 ±0.06

*NFE = Nitrogen free extract

Table 3. **Nutraceutical Potential analysis of PHF (Mean of values).**

S.No.	Nutrients	Composition
1	Phenol (mg CE/100g) +	412.0
2	Flavonoids (mg QE/100g)*	873.0
3	Antioxidant activity (%) **	56.23
4	Zinc (mg /100 g)	03.21
5	Iron (mg /100 g)	08.46

CE = Catechol equivalent*QE= Quercetin equivalent ** free radical scavenging activity

The most important parameters for the quality control of polyherbal formulation are NFE, protein content, moisture content, crude fat, crude fiber and ash content in order to identify adulteration and contamination. Besides, its proximate composition indicates the authenticity and purity of PHF [24]. PHF also contains polyphenols, antioxidant activity and flavonoids [25]. PHF showed the presence of iron and zinc which are within normal limits and no heavy metals (cadmium, lead and mercury) were detected which ensures the safety of formulation [26]. In addition, the phytochemical study of PHF indicated the existence of phenol and revealed that PHF is an important source of antioxidants and free radical scavengers [27]. In diabetes, hyperglycemia is caused by increased production of free radicals which in turn lead to rise of oxidative stress level [28]. The antioxidant potential of poyherbal formulation will probably cause reduction in oxidative stress in prediabetic and diabetic patients. The presence of poly phenols in PHF enhances antioxidant activity in diabetics [29].

Identification of Bioactive Compounds by GC-MS Analysis

The GC-MS chromatograms of PHF extracts are presented in Fig. 2 created on retention

time, peak area, molecular weight and molecular formula. The extracts contained a complex mixture of different compounds as listed in Table 4. The major bioactive compounds were identified were: propanoic acid in garlic (1.18%, antioxidant, hypoglycemic), cinnamaldehyde a dominant phenolic compound of cinnamon (0.42%, hypoglycemic), α curcumene (2.48% anti-inflammatory, anti-diabetic and anti-oxidant), naphthalene (3.92%), caryophyllene (4.92 hypoglycemic), ar-tumerone from turmeric (8.22%), palmitic acid and caryophylline from fenugreek (6.56 anti-diabetic, anti-cancer, antioxidant and anti-inflammatory) and gingerol (hypoglycemic) shogaol acetate, gingerol and copaene from ginger (antioxidant, anti-inflammatory, 0.65%).

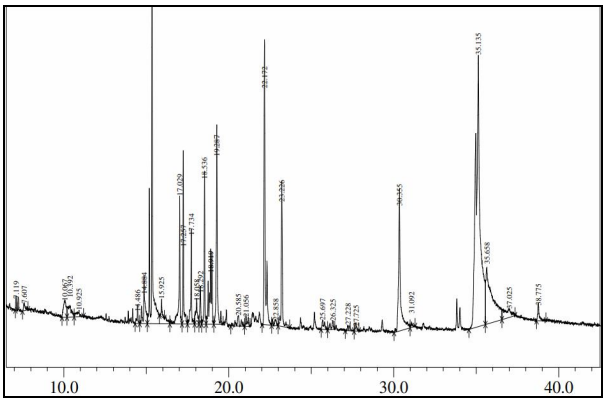

Figure 2. **GC-MS profile of antidiabetic polyherbal formulation**

Table 4. **Phytochemicals detected in polyherbal formulation using GC-MS technique.**

S.No	Retention time (min)	Peak area %	Molecular Formula	Names of phytochemical compound
1	7.119	0.21	CH_5N_3O	Hydrazinecarboxamide
2	7.607	0.31	CH_5N_3O	Carbohydrazide
3	10.067	1.18	$C_3H_8N_2O_2$	Propanoic acid
4	10.392	1.10	$C_{11}H_{24}$	Nonane, 3, 7-dimethyldecane
5	10.925	0.49	$C_{10}H_{14}$	Menthatriene
6	14.486	0.42	C_9H_8O	Cinnamaldehyde
7	14.884	1.99	C_9H_8O	Cinnamaldehyde
8	15.360	12.01	C_9H_8O	Cinnamaldehyde
9	15.925	0.38	$C_9H_{10}O_2$	2-methoxy-4-vinylphenol
10	16 .029	3.05	$C_{15}H_{24}$	Copaene
11	17.257	2.78	$C_{11}H_{14}O_2$	9-Methoxybicyclo
12	17.734	2.04	$C_{15}H_{24}$	Caryophylene
13	18.058	1.01	$C_{10}H_{10}O_2$	Propenal
14	18.292	0.89	$C_{15}H_{24}$	Cycloundecatriene
15	18.536	2.48	$C_{15}H_{22}$	α -Curcumene, Naphthalene
16	18.919	3.92	$C_{15}H_{24}$	α-Muurolene
17	19.287	4.92	$C_{15}H_{24}$	Caryophyllene, Cyclohexene
18	20.585	0.73	$C_{15}H_{24}O$	2H-Pyran,
19	20.056	0.48	$C_{15}H_{24}$	Cedrene, Thujopsene
20	22.172	8.22	$C_{15}H_{20}O$	Ar-tumerone, Cinnamylangelate
21	22.858	0.36	$C_{10}H_{16}O$	cis-Chrysanthenol
22	23.226	3.75	$C_{15}H_{22}O$	Curcumin, Curlone, β-Turmerone
23	25.697	0.46	$C_{10}H_{14}NO_2$	α curcumene, Pentene
24	26.325	0.46	$C_{26}H_{38}O_8$	Tumerone
25	27.228	0.29	$C_{15}H_{26}O$	1-Pentene
26	27.725	0.25	$C_{10}H_{18}O_2$	Mentha
27	30.355	6.56	$C_{15}H_{30}O_2$	Palmitic acid
28	31.092	0.19	$C_{11}H_{18}O_2$	1H-Pyrrole
29	35.135	27.96	$C_{16}H_{30}O$	9-Octadecenal, Olealdehyde
30	35.658	8.86	$C_{15}H_{30}O_2$	Tetradecanoic acid
31	37.025	1.60	$C_{11}H_{18}O$	1,5-Naphthalenedione
32	38.775	0.65	$C_{19}H_{26}O_4$	Gingerol, Shogaol acetate

In a similar study, the GCMS analysis of PHF prepared from spices revealed the presence of phytochemicals having antioxidants and antidiabetic agents that play a role in controlling hyperglycemia [30]. Moreover, PHF decreases oxidative stress which in turn has a synergistic antioxidant effect [31].

FTIR Evaluation for Fingerprint Spectra

In this study, the qualitative analysis of PHF was carried out by means of FTIR spectroscopy which identified 21 peaks as shown in Fig. 3. In the single bond range (2500–4000 cm^{-1}), a wide-ranging absorption band in the frequency range at 3414.12 cm^{-1} peak was found, indicating hydrogen-bonded hydroxyl groups (O-H stretch) showing the presence of alcohol and amine groups. The highest antioxidant activity compounds present in excessive quantity were aromatic amines (Table 5). The frequencies peaks between 2960.83 cm^{-1} and 2926.11 cm^{-1} represents the C-H stretching which is usually found in lipids. Moreover, the frequency range at 2854.74 peaks indicating the C-H stretch depicting the existence of Alkanes.

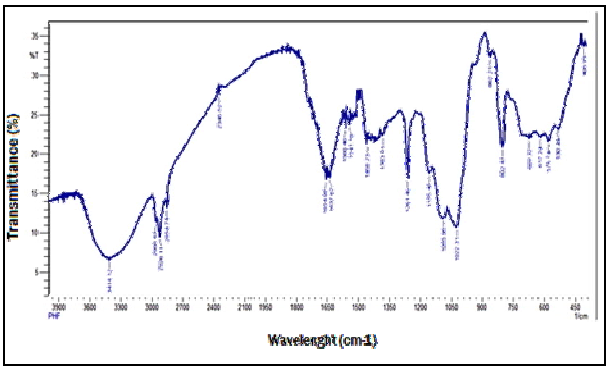

Figure 3. **FTIR Spectra showing different peaks of polyherbal formulation**

In the double bond region (1500–2345 cm^{-1}), five peaks were detected. The highest peak was found at 2345.52 cm^{-1} which indicates the presence of carboxylic acid. A medium peak was found band at 1654.98 cm^{-1} which showed the presence of amides. In addition, peak 1637.62 cm^{-1} represents alkenes compounds. On the basis of FTIR results, high antioxidant potential can be attributed to excessive quantity of alkenes. However, these results indicate a strong possibility that these alkenes also play a role in the antioxidant activity of the herbs. Moreover, the peak 1560.46 cm^{-1} shows the existence of alkene and nitro compounds. The last double bond peak 1541.18 cm^{-1} indicates aromatic & nitro compounds. Aromatic compounds have anti-diabetic and antioxidant activities.

Table 5. **FTIR interpretation of polyherbal formulation.**

Wave number cm^{-1} (Test Sample)	Wavenumber cm^{-1} (Reference) Coates, 2000	Functional groups	Phyto compounds
3414.12	3570-3200	O-H Stretch, Hydroxy group, H-bonded	Poly Hydroxy
2960.83	2970-2950	C-H Stretch	Alkene, alkyl compounds
2926.11	2935–2915	Asymmetric stretching –CH (CH2) vibration	Alkene, alkyl Lipids, proteins
2854.74	2865-2845	Symmetric stretching of –CH (CH2) vibration	Proteins, lipids
1636.62	1680-1620	C=C Stretch	Olefinic alkene compounds
1560.46	1650-1550	>N-H bend	Secondary Amine
1541.18	1555-1485	NO_2 stretch	Aromatic nitro compounds
1458.01	1510-1450	C=C-C, Aromatic ring stretch	Aromatic compounds
1384.49	1410-1310	O-H bend, Alcoholic group	Phenol or tertiary alcohol
1155.40	1190-1130	CN stretch	Secondary Amine Lipids, proteins
1085.96	1090-1020	CN stretch	Primary Amine
1022.31	1090-1020	CN stretch	Primary Amine
862.21	890-820	C-O-O stretch	Peroxides
669.32	705-570	C-S stretch	Di sulphides Thiols and thio-substituted compounds

In the fingerprint region (400–1500 cm^{-1}), several peaks were found. The first peak was found at 1458.01 cm^{-1}, indicating the presence of nitro compounds. Peaks found at 1384.49 cm^{-1} shows the existence of alkyl, aryl halides, and nitro compounds and peaks 1155.40 cm^{-1}, 1085.96 cm^{-1}, 1022.31 cm^{-1} show the presence of alkyl & aryl halides. Peaks found at 862.21 cm^{-1} reveal the aromatic compounds, while peak at 802.41 cm^{-1} shows the availability of alkyl & aryl halides. Peak found at 669.32 cm^{-1}, 617.24 cm^{-1}, 576.74 cm^{-1}, 530.44 cm^{-1} and 406.99 cm^{-1} shows the presence of alkyl & aryl halides.

In a similar study, FTIR analysis showed different characteristic band values with functional groups in the PHF such as amines, alcohol, phenols, alkanes and alkenes having bioactive phytochemicals with therapeutic values [32]. In another study, a polyherbal formulation was developed and evaluated for its antidiabetic effect [33]. Similarly, a polyherbal formulation was developed and standardized containing seven herbs and FTIR spectroscopy revealed the absence of interaction among herbs [34]. The chemical composition and fingerprints obtained by FTIR help to maintain the quality and specifications of PHF in order to obtain approval from regulatory authorities. The current study on potential herbs for effective management of diabetes has facilitated in identifying the major bioactive compounds and their mechanism of action [35].

Effect of Polyherbal Formulation on Glycemic Control in Type 2 Diabetic (T2D) Patients

The supplementation of polyherbal formulation in two doses PHF 1.5 g, and PHF 3.0 g and metformin resulted in a significant decrease ($P<0.05$) in the fasting blood sugar while in the placebo group, the decrease in fasting blood sugar was not significant ($P>0.36$) as shown in Figure 4A. The fenugreek intervention in patients with T2D showed a significant decrease in FBS [36]. Moreover, treatment with polyherbal formulation along with prescribed diabetes medication resulted in a significant reduction in FBS levels [12].

The treatment of T2D patients with polyherbal formulation and metformin resulted in a significant ($P<0.05$) reduction in RBS levels. The two groups PHF 1.5 and PHF 3 and the metformin group showed significant reduction in random blood sugar. However, no significant ($P>0.05$) change in the placebo group, as shown in Figure 4B. These results in agreement with the administration of ginger powder supplementation significantly reduced RBS in newly diagnosed T2D patients compared with metformin [37]. In a similar study, the polyherbal formulation made from spices had significant reduced RBS in patients with T2D [38].

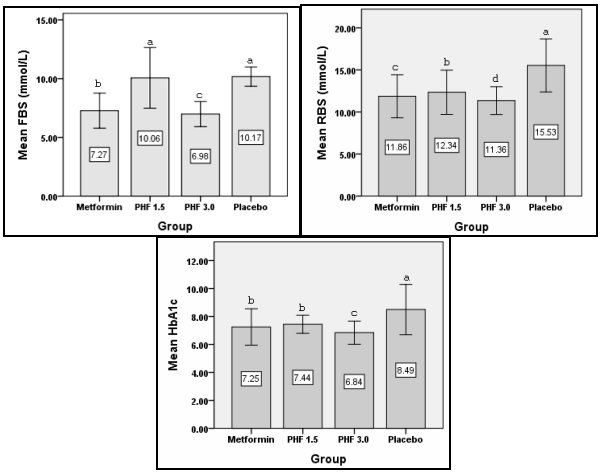

Figure 4 ***Effect of PHF on glycemic control parameters (HbA1c, FBS and RBS) in various groups of T2D patients.***

The group PHF 3.0 gm showed relative greater reduction in HbA1c than PHF 1.5. On the contrary, the placebo group revealed significant decrease ($P>0.05$) in HbA1c value. In a study turmeric

supplementation (2100 mg) showed a significant effect on HbA1c [39]. Moreover, treatment with polyherbal formulation along with medication caused more than a 1% decrease in HbA1c within each group [12]. The PHF was effective in achieving glycemic control in type 2 diabetic patients with diabetic diet plans.

Conclusion

The unique GCMS and FTIR spectrums and composition of PHF provide standard chemical patterns for reproducibility and identification that are required for assuring quality. Moreover, the formulation is a rich source of bio-active phytochemical compounds having therapeutic potential for diabetes prevention and management. Future studies may be conducted on various ratios of composition and different doses of product in the management of pre-diabetic, type 2 diabetic and type 1 diabetes patients.

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Conflict of Interest

The authors have not declared any conflict of interest.

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