Original Research Article

Pharmacopeial testing of Poly herbal formulation for management of Diabetes mellitus

Abstract

Diabetes mellitus is a global cause of morbidity and mortality. There is continuous rise in the patients of diabetes in Pakistan. Many conventional and natural origin medicines are available for maintaining optimum blood sugar level. There is a need to carry out standardization of the natural origin medicine to authenticate their efficacy and safety.

The objective of this research work was to carry out preliminary pharmacognostic, phytochemical, biological, pharmacological, toxicological studies on poly-herbal formulation.

The poly herbal preparation was prepared and evaluated for the standard Pharmacopeial tests. The results of the tests performed were found to be adequate to evaluate the poly-herbal formulation and may be used as reference standards in future for further studies.

Key Words: Organoleptic, Microscopic, Fourier transform Infra-red spectroscopy, Anti-microbial.

Introduction

Diabetes mellitus is a rising socio-economic burden globally. Pakistan is one of the 19 countries and territories of the International Diabetes Federation, Middle East and North Africa (IDF MENA) region. Worldwide, 425 million people have diabetes. In MENA region more than 39 million people suffer from diabetes and by 2045, the figure will rise to 67 million [1].

Syzygium cumini

Syzygium cumini (Linn.) Skeels (Myrtaceae) is commonly known as Indian blackberry; Jaman. The fruits are rich in raffinose, glucose, fructose, citric acid, mallic acid, gallic acid, anthocyanins; delphinidin-3-gentiobioside, malvidin-3-laminaribioside, petunidin-3-gentiobioside, cyaniding diglycoside, petunidin and malvidin. Black plum fruit and its leaves are good for diabetic patients. The black plum has anti-diabetic features. The fruit helps to convert starch into energy and keep your blood sugar levels in check [2].

Momordica charantia

M. charantia (bitter melon or bitter gourd) is a flowering vine in the family Cucurbitaceae. The main constituents of bitter melon

which are responsible for the antidiabetic effects are triterpene, protein, steroid, alkaloid, inorganic, lipid, and phenolic compounds. It is a popular plant used for the treating of diabetes. *M. charantia* has significant antidiabetic as well as hypolipidemic activity so that it can be used as an adjuvant along with allopathic treatment of medicine to treat diabetes as well as to delay the late complications of diabetes [3].

Wrightia tinctoria

Wrightia tinctoria (Indarjou, pale indigo plant) belongs to family Apocynaceae. The chemical constituents of *W. tinctoria* includes lipid, saponin, tannin, alkaloid, phenol, steroid, and flavonoid. W. tinctoria possess significant anti-diabetic activity [4].

Gymnema sylvestre

Gymnema sylvestre (Asclepiadaceae), popularly known as "gurmar" for its distinct property as sugar destroyer. The major constituents' saponins, alkaloids, anthraquinones, flavones, hentriacontane, pentatriacontane, phytin, resins, tartaric acid, formic acid, butyric acid, lupeol, β-amyrin related glycosides, stigmasterol, and calcium oxalate. The phyto-constituents responsible for sweet suppression activity includes triterpene saponins known as gymnemic acids, gymnemasaponins, and a polypeptide, gurmarin. G. sylvestre has prospective clinical data in support of treatment of diabetes as it displays favorable effects on blood sugar homeostasis, controls sugar cravings, and promotes regeneration of pancreas [5].

Material

All plants were procured from local pansare and authenticated. All the procured and authenticated individual drugs were dried in shade and cleaned by hand sorting. The individual drugs were then crushed using grinder and passed through mesh no. 40. The individual drugs were then weighed as per the quantity required. The drugs were mixed. The mixed formulation was unloaded, weighed, and packed in labeled glass bottles.

Methodology

Screening of anti-bacterial activity

The anti-bacterial activity of different medicinal plants five strains were explored in this study. Anti-bacterial activity of crude extract against the test organisms were determined by using agar-

well method. All plates were incubated at 28 + 2°C for 24-48 hours and after incubation diameter of zone of inhibition was measured [6].

Anti-inflammatory activity

The anti-inflammatory activity was evaluated by the Carrageenan induced paw edema method. [7]. The results were expressed as percentage reduction in edema volume, which can be calculated by using the formula:

% of inhibition = Control-Treated/ Control x 100

Fourier Transform Infrared Spectroscopy

Fourier Transform Infrared Spectrometry (FTIR) of DM was carried out on FT-IR model Nicolet Avator 330-FT-IR (USA) [8].

Gas Chromatography-Mass Spectroscopy

Gas Chromatography-Mass Spectroscopy (GC-MS) was carried out using GC-MS 1. In the GC/MS process, a sample is first injected into a gas chromatograph, where components are separated according to size and/or polarity. Then, the components pass into a device known as a mass selective detector. It's at this stage that a mass spectrum is obtained and compared against standard reference libraries in order to identify unknown components in the sample. [9].

Microscopic evaluation

Microscopic evaluation was carried out using scanning electron microscope (SEM) [10].

Results

Microscopic Evaluation

Prominent microscopic features found were parenchymatous cells, starch grains, epicarp, mesocarp, vascular bundles, epidermis, hypodermis, endosperm, testa, schizogenous cavities, trichome, cortex and vascular bundle (See figure 1 – 7).

Fourier Transform Infra-red Spectroscopy

The results can be seen in figure 8 – 9.

GC-MS Evaluation

GC-MS spectra can be seen in figure 10.

Anti-bacterial Activity

The results of anti-bacterial activity are exhibited in Table 1 below.

Table 1: Anti-bacterial activity

Name of Bacteria	% Inhibition of Compound	% Inhibition of Drug
Escherichia coli	No Inhibition	91.69%
Bacillus subtilis	No Inhibition	89.56%
Staphylococcus aureus	No Inhibition	94%
Pseudomonas aeruginosa	No Inhibition	88.2%
Salmonella typhi	No Inhibition	93%

Anti-inflammatory Activity

The results of anti-inflammatory activity may be observed in table 2 below.

Table 2: Anti-inflammatory activity

Sample Code	Conc. (mg/ml)	% Inhibition
DM	25 µg/ml	0.7

Fig 1- 7 Electron Powder Microscopy

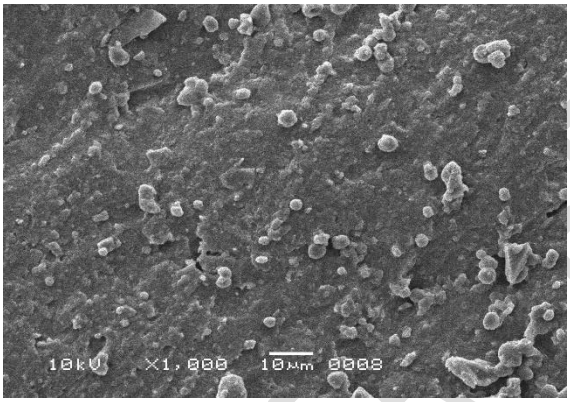

Figure 1

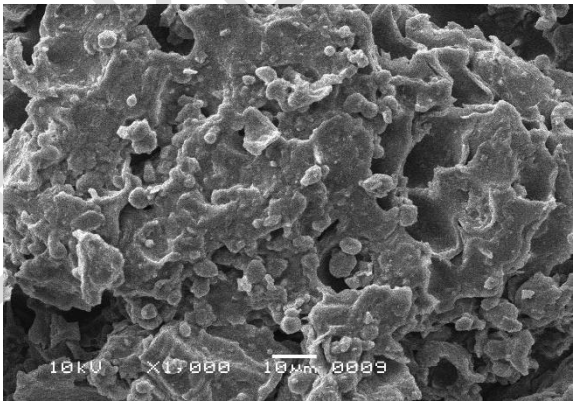

Figure 2

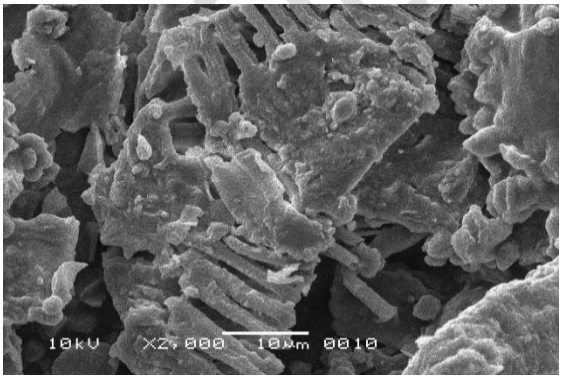

Figure 3

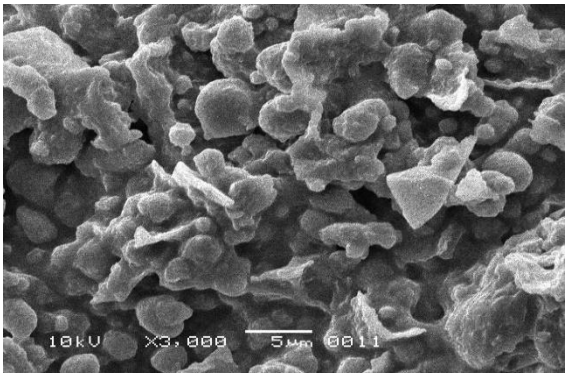

Figure 4

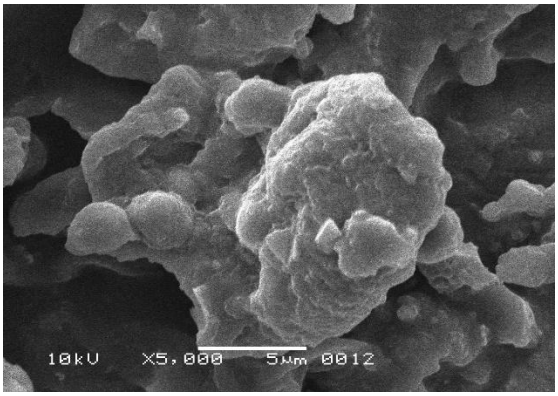

Figure 5

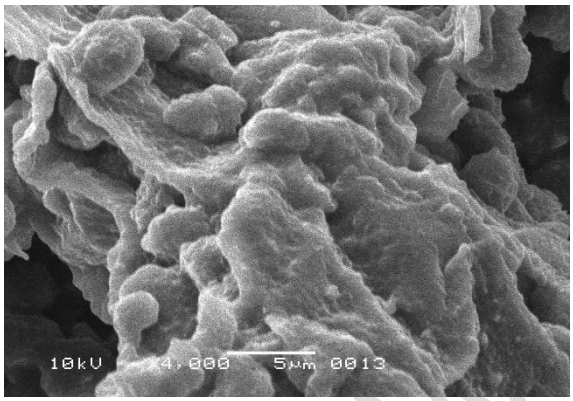

Figure 6

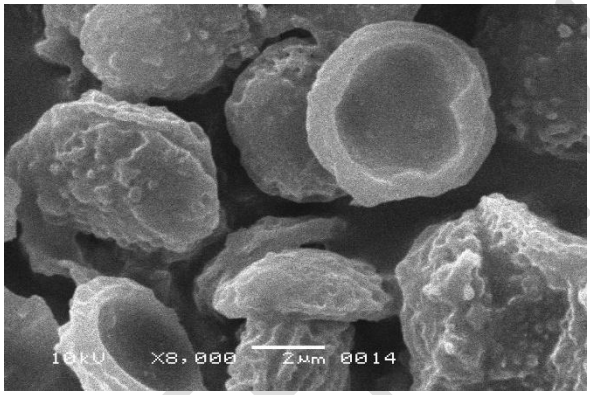

Figure 7

Fourier Transform Infra-red Spectroscopy

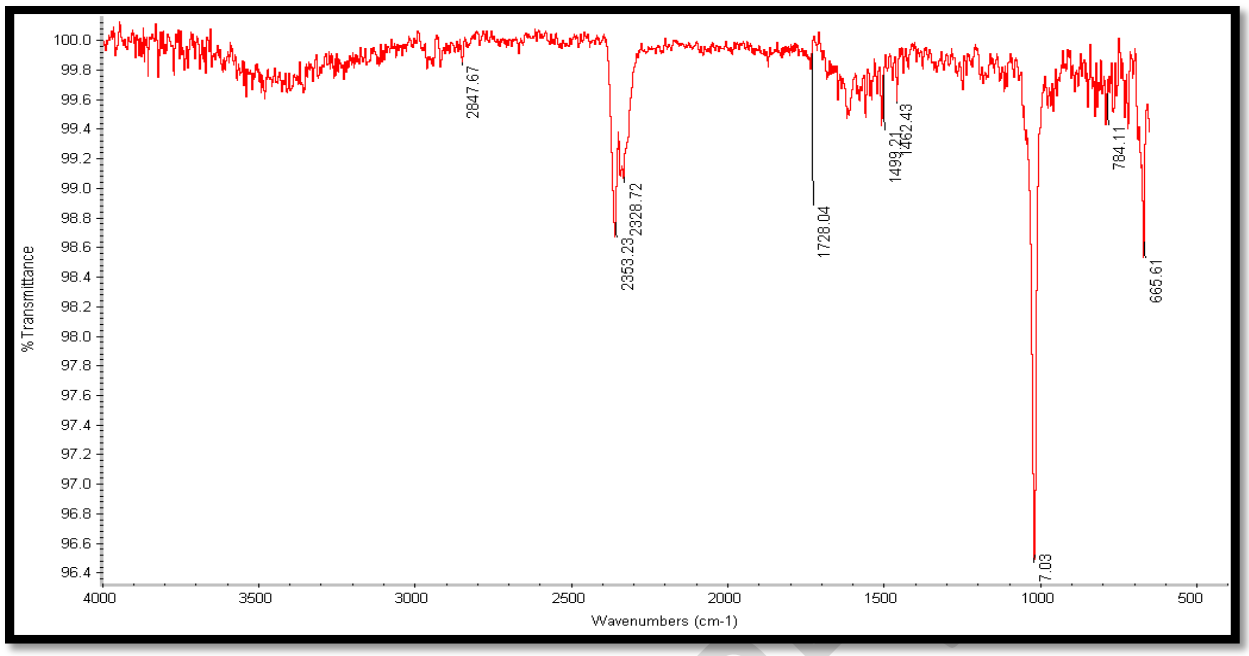

Figure 8: Significant peaks - C-H (stretch); O=C=O (stretch); C=O (stretch); C-H (bending)

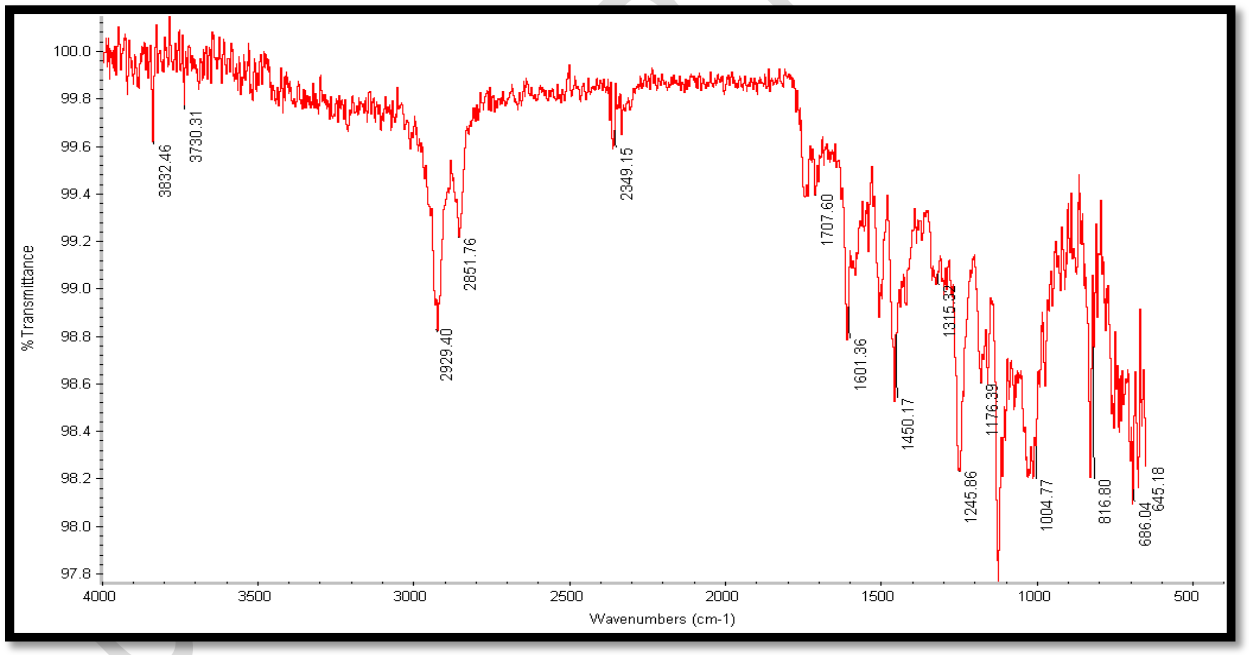

Figure 9:Significant peaks - C-H (stretch); O=C=O (stretch); C=O (stretch); C=C (stretch); C-O (stretch)

GC-MS Spectrum

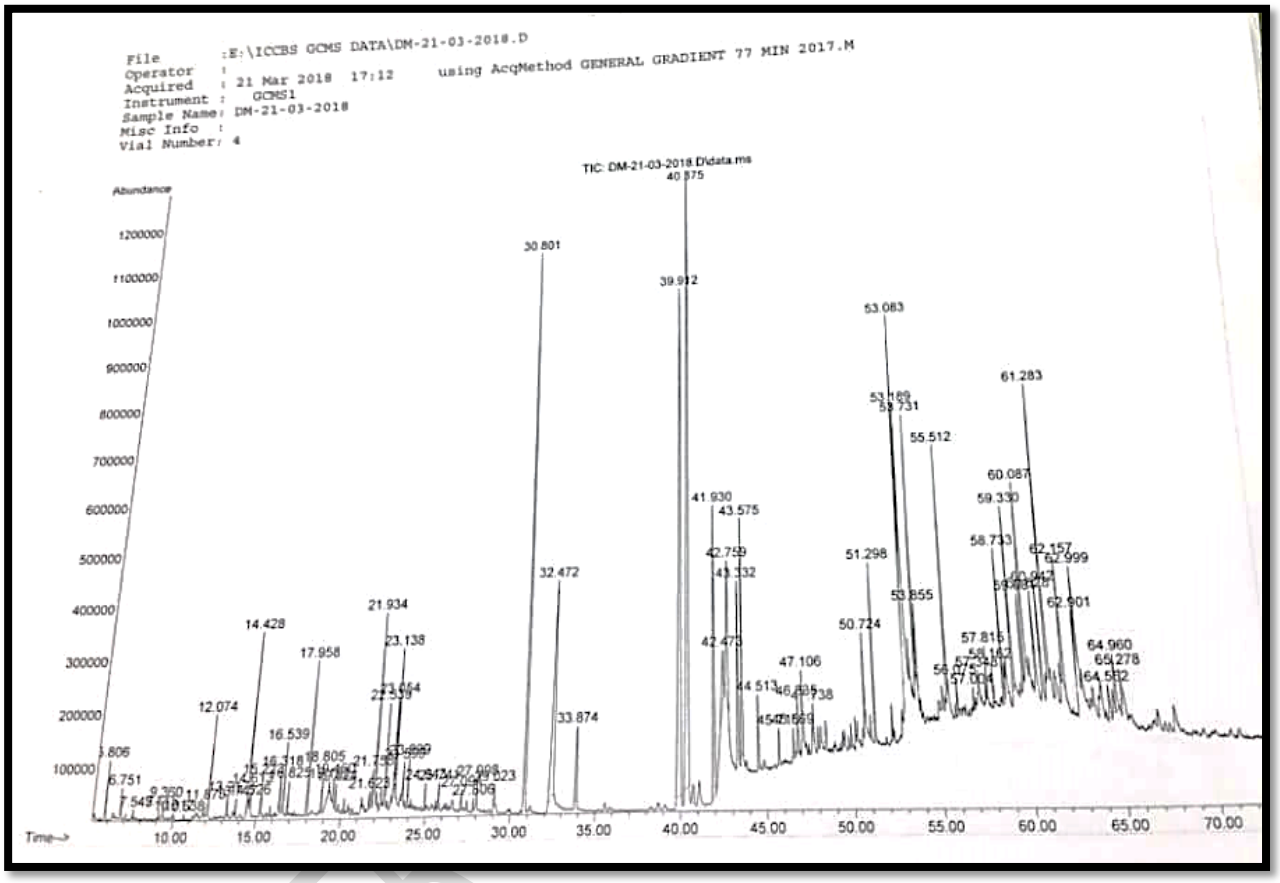

Figure 10: Significant peaks: Octadecanonic acid, n- Hexadecanoic acid; 2- Heptenal; Piperiene; Stigmasterol; Lupeol; Betulin; Ethyl iso-allocholate

Discussion

Natural source especially the herbals are in the lime light of discovery of novel molecules with better efficacy and lesser adverse events. The researches are being carried out to explore probable mechanism of action of the pharmacologically active ingredients present in any herb or other natural source. Standardization of the herbal and other natural origin medicines is essential and plays a pivotal role in maintaining the quality control of the formulations prepared by them. In the current study the formulation was prepared with all those herbals that have already

reported data of their anti-diabetic activity (Chandel et al. 2011; Ekka et al. 2008; Paramanick & Sharma 2017). *Syzygium cumini* contains mycaminose, which possess anti-diabetic activity (Kumar et al. 2008). *Momordica charantia* contains triterpene, proteid, steroid, alkaloid, inorganic, lipid, and phenolic compounds that possess anti-diabetic activity. Ursolic acid and chlorogenic acid, Aldose reductase inhibitors present in *Wrightia tinctoria* may be responsible for its antidiabetic potential (Oviya et al. 2015). *Gymnema sylvestre* contains triterpene saponins known as gymnemic acids, gymnemasaponins, and a polypeptide, gurmarin that may be responsible for anti-diabetic activity (Tiwari et al. 2014). The results of our study revealed that the poly-herbal preparation for the treatment of diabetes mellitus contains carbohydrates, triterpenes, steroids, glycosides, polypeptides, inorganic and phenolic compounds. Due to the presence of the above mentioned constituent the formulation has potent anti-bacterial and anti-inflammatory activity.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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