Evaluation of a Polyherbal Powder for Treatment of Diabetes Mellitus

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Aziz et al.: Pharmacognostic and Physicochemical Evaluation of a Polyherbal Powder

Diabetes mellitus is a silent killer of mankind that leads to huge economic loss in a developing country like India. There is a need for better treatments with less adverse effects to minimise the burden on the health and economy of an individual and the society. The main aim of the study was to prepare a polyherbal powder for diabetes mellitus and evaluate the powder based on organoleptic, rheological and physico and phytochemical characteristics. Herbs used in the preparation of the polyherbal powder were Ocimum sanctum, Aegle marmelos, Emblica officinalis, Trigonella foenum-graecum, Terminalia bellirica, Terminalia chebula, Momordica charantia, Syzygium cumini, Cinnamomum zeylanicum and Curcuma longa. Evaluations were done using standard procedures. Organoleptic characters of the polyherbal powder were found to be dull brown in colour, characteristic odour and astringent taste with moderately fine texture. Phytochemical qualitative analysis indicated the presence of flavonoids, alkaloids, terpenoids, tannins, steroids, carbohydrates and glycosides. Physicochemical analysis revealed longer stability with good flow property of the polyherbal powder. Thus, the polyherbal powder was evaluated, which has a potential to treat diabetes mellitus.

Key words: Polyherbal powder, ash value, Carr’s index, phytoconstituents, moisture content

India is considered as the diabetic capital of the world. Diabetes mellitus (DM) is the systematic metabolic disorder characterized by hyperglycemia, insulin resistance and relative insulin deficiency with disturbances of carbohydrate, fat and protein metabolism. Its incidence is increasing throughout the world at an alarming pace, which is expected to cause grave secondary complications over time like neuropathy, nephropathy, retinopathy, cardiovascular disease, retinopathy and dyslipidemia. In today’s scenario, about 90 % of the young population accounts for a major share in the incidence of type II diabetes mainly due to a shift to the sedentary lifestyle comprising of unhealthy diet habits and less physical activity. Various synthetic drugs such as oral hypoglycemic drugs along with insulin are available to control the level of blood sugar, but their cost, complications, limited tolerability and various side effects hamper wider acceptance. Thus, it is notably one of the refractory diseases identified by the Indian Council of Medical Research for which there is a dire need for alternative medical treatment[1-5].

Considering the facts, the most commercially successful and widely used branch of alternate or complementary medicine is phyotherapy, which acquires to be ‘synergy’ that is more effective than the sum of their parts. India is considered as the emporium of medicinal plants because in different bioclimatic zones, there exists a diverse availability of several thousands of medicinal plants and thus has a rich history of using herbal plants for medicinal purposes. Traditionally, herbal medicines and their preparations are being used in various therapies owing to its natural origin and lesser side effects than synthetic drugs[6-11].

Phyotherapy flourishes having more than one herb in the formulation to achieve the extra therapeutic effectiveness known as polyherbalism. To acquire the synergistic effect, either pharmacodynamic or pharmacokinetic synergism is required i.e. either the herb will target the therapeutic activity to a receptor or will facilitate absorption, distribution, metabolism

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and elimination of the other herbs\cite{22}. The present study was conducted on a polyherbal powder (PHP) to study its pharmacognostic and physiological parameters. Some of the herbs of the PHP are discussed that hold a promise to treat DM.

*Ocimum sanctum* or *O. tenuiflorum* (family: Lamiaceae) also known as the holy basil or *tulsi* has pharmacological activities such as antioxidant, antidiabetic, antiinflammatory, antihypertensive, cardioprotective, hepatoprotective and renoprotective. It is highly recommended and one of the most potent traditional plant to manage diabetes. Extract of *tulsi* leaf has been shown to increase the insulin secretion from isolated islets, perfused pancreas and clonal pancreatic β-cells and thus possess antihyperglycaemic effect\cite{5,13-15}.

*Emblica officinalis* or *Phyllanthus emblica* (family: Euphorbiaceae), commonly known as *amla* is the most extensively studied plant containing tannins, alkaloids, phenols and its fruit juice contains the highest concentration of vitamin C (ascorbic acid). The high concentration of vitamin C is effective in controlling diabetes. It is reported to be effective in reducing the fasting blood glucose level, post prandial blood glucose levels and HbA1c levels. Scientists have reported the possible mechanism by which it acts as an antidiabetic and prevents its complications. Ellagic acid in it is the potent α-amylase and α-glucosidase inhibitor. It is also reported to show the improvement in biomarkers of oxidative stress (nitric oxide, glutathione and malondialdehyde), HbA1c levels and high sensitivity C-reactive protein levels\cite{16,17}.

*Cinnamomum zeylanicum* (family: Lauraceae) commonly known as *dalchini* has been shown to exhibit insulin potentiation effect in glucose metabolism. It is reported to enhance the glucose uptake by activating the insulin receptor kinase, autophosphorylation of the insulin receptor and glycogen synthase activity\cite{18}.

*Trigonella foenum-graecum* (family: Fabaceae) is commonly known as fenugreek (*meethi*). Fenugreek seed is used as a source of the antidiabetic compound in various model systems. It lowers fasting serum glucose level. A study revealed its use in delaying the onset of diabetes in prediabetes subjects by lowering blood glucose level in prediabetes and has an insulinotropic effect. It exerts hypoglycaemic effects by stimulating glucose-dependent insulin secretion from pancreatic beta cells, as well as by inhibiting the activities of α-amylase involved in carbohydrate metabolism\cite{19-22}.

*Momordica charantia* (family: Cucurbitaceae) is a commonly used vegetable in Asian cuisines and is known as *karela*. Its chief chemical constituents include momorcharin (glycoprotein), momordicin (alkaloid), momordin and charantin (glycosides), polypeptide-p (insulin-like peptides) which have been found to possess hypoglycaemic properties. The possible mechanism of action as suggested by the scientists includes inhibition of glucose uptake and suppression of key glucogenic enzymes and enzymes of hexose monophosphate pathway. Thus, it is utilized as an antidiabetic\cite{23}.

*Syzygium cumini* (family: Myrtaceae) is effective in controlling high blood sugar levels and this has been indicated in the Ayurvedic Pharmacopoeia. It is popularly known as *jamun*. Several putative mechanisms have been reported to explain the antidiabetic potential. It reduces the free radicals and improves functioning of β-cells of the pancreas leading to lowering of blood sugar levels. It may also reduce the activity of α-amylase, which is upregulated in DM\cite{24-26}.

**MATERIALS AND METHODS**

**Selection and collection of plant material:**

Leaves of *O. sanctum*, *A. marmelos* and fruits of *E. officinalis* were procured from the Botanical Garden of Pranveer Singh Institute of Technology. *T. foenum-graecum*, *T. bellirica*, *T. chebula*, *M. charantia* and *S. cumini* seeds, barks of *C. zeylanicum* and rhizomes of *C. longa* were freshly procured from the local market. These raw materials were shade dried and ground properly in an electrical grinder. They were mixed in a specific ratio with maximum of *O. sanctum*, *A. marmelos*, *C. zeylanicum*, *T. bellirica* and *T. chebula* and remaining herbs were added half of the quantity of above mentioned herbs.

**Organoleptic, Physicochemical and phytochemical evaluation**\cite{27-31}:

Organoleptic evaluation refers to the evaluation of the formulation by the colour, odour, taste and texture. The method adopted for the organoleptic evaluation was as described in Wallis\cite{26}. Various physicochemical parameters like moisture content, pH, ash value were determined. PHP was also subjected to preliminary phytochemical screening to detect the presence of organic constituents using standard methods.

**Moisture content:**

Loss on drying is a parameter to keep the moisture
content under check as the large amounts of moisture can promote hydrolytic reactions and microbial growth. The moisture content was measured using the gravimetric method and loss on drying was calculated. Two grams of PHP was placed in a weighed preheated porcelain dish and then was kept in a hot air oven and dried at 105\(^\circ\)C till constant weight or two consecutive weights differing by 0.5 mg was observed. Weight was taken after drying and was transferred to the desiccator to cool and then again porcelain dish was reweighed. Percent moisture content was calculated using the Eqn., 

\[
\% \text{ moisture content} = \frac{(W_1-W_2)/W}{100}
\]

where, W is the weight of the sample (2 g), W1 is the weight of the sample before drying and W2 is the weight of the sample after drying.

**Ash content:**

The ash values usually represent the inorganic residues such as phosphates, carbonates and silicates present in herbal drugs. These are important indices to illustrate the quality as well as purity of herbal medicine. The objective to evaluate is to remove all traces of organic matter, which may otherwise interfere in an analytical determination.

**Total ash:**

Empty silica crucible was weighed (W1). About 3 g (W2) of the air-dried PHP was added to the previously weighed crucible. The sample was ignited gradually in an electrical muffle furnace, increasing the heat to 500-600\(^\circ\)C until it is white, indicating the absence of carbon. Then it was cooled in a desiccator and reweighed (W3). Total ash content was calculated as % total ash = ((W3-\(W_1\))/(W2-\(W_1\)))\(\times\)100.

**Acid-insoluble ash:**

Twenty five millilters of dilute HCl was added to the total ash containing crucible. It was then covered with watch-glass and boiled gently for 5 min. With 5 ml of hot water, the watch glass was washed and the washings were added to the crucible. Then, ashless filter paper was used to filter the insoluble matter and washed with hot water until the neutral filtrate was obtained. The filter paper containing the insoluble matter was transferred to the original crucible, dried on a hotplate and ignited to constant weight (W4). The residue was allowed to cool in a desiccator for 30 min and then reweighed. W1 is weight of empty silica crucible, W2 is the weight of sample including crucible for ignition, W3 is the final weight of sample including crucible weight after ignition and W4 is the constant weight after addition of HCl. Acid-insoluble ash content was calculated as, % acid-insoluble ash = \((W_4-W_1)/(W_2-W_1))\times100.

**Water-soluble ash:**

In the total ash containing crucible, 25 ml of water was added, boiled for 5 min and filtered through an ashless filter-paper. The insoluble matter collected on the filter paper was washed with hot water and then the filter paper ignited in a crucible for 15 min at a temperature not exceeding 500\(^\circ\)C. The residue was allowed to cool in a desiccator for 30 min and re-weighed (W5). % water-soluble ash was calculated as, \((W_7-W_6)\times100\), where, W1 is the weight of empty silica crucible, W2 is the weight of sample including crucible weight for ignition, W3 is the final weight of sample including crucible weight after ignition, W6 is the weight of residue, which is W5–W1, W7 is the weight of ash, which is W3–W1 and water-soluble ash is W7–W6 mg/g.

**Flow characteristics of powder (rheological parameters):**

A preformulation study is defined as the principal investigation technique in the development of a drug product to obtain information on the previously known properties of the compound to propose a development schedule. Rheological characteristics of the formulated powder were studied and estimated like an angle of repose, bulk density, tapped density, compressibility index. The angle of repose was measured by the fixed funnel method, where a funnel was placed above the graph paper on a flat horizontal surface secured with its tip at a given height (h). Through the funnel, PHP was poured until the tip of the funnel was just touched by the apex of the conical pile. The radius (r) formed on the base by the heap of the conical pile was measured. Angle of repose \((\theta) = \tan^{-1}h/r\), where, h is the height of the cone, r, the radius of the cone base and \(\tan \theta = h/r\).

Carr’s Index defines the measure of the intensity by which the powder can be compressed and Hausner’s ratio is defined as the indirect ease of the flow of the powder. Thus, their determination requires the determination of true density and tapped density. Carr’s index is calculated using the following formula, Carr’s index = \((\rho_{\text{tap}}-\rho_{\text{b}})/\rho_{\text{tap}})\times100\). Hausner’s ratio is calculated using the formula, Hausner’s ratio = \(\rho_{\text{tap}}/\rho_{\text{b}}\).

Bulk density is determined as follows, 5 g of PHP (M) was added into a dry 100 ml cylinder, without compacting the powder was carefully levelled and the unsettled apparent volume (V0) was read and noted. The bulk density \((\rho_{b})\) was calculated as \(\rho_{b} = M/V0\),
where, M is the weight of sample and V is the apparent volume of powder. Tapped density is determined by tapping the PHP 500 times, followed by additional taps of 750 times, then 1250 until the difference between succeeding measurement is less than 2 % and then tapped volume (Vf) was measured. The tapped density (\(\rho_{\text{tap}}\)) was calculated, in g/ml, using the following formula, \(\rho_{\text{tap}} = \frac{M}{V_f}\), where M is the weight of the sample and Vf is the tapped volume of powder. Summary of the relative flowability of the powder concerning the angle of repose, Carr’s index and Hausner’s ratio is depicted in Table 1.

**Phytochemical screening, test for alkaloids:**

The phytochemical tests were carried out on the PHP using standard procedures to identify the components. Dragendorff’s test was used to detect alkaloids. To 0.5 ml of aqueous PHP solution, Dragendorff’s reagent (potassium bismuth iodide solution) was added. The appearance of reddish-brown precipitate confirms the presence of alkaloids. Hager’s test was carried out by adding to 0.5 ml of aqueous PHP solution a few drops of Hager’s reagent. Formation of a yellow colour precipitate confirmed the presence of alkaloids. Wagner’s test was carried out by adding Wagner’s reagent (solution of iodine in potassium iodide) to 0.5 ml of aqueous PHP solution, formation of a reddish-brown precipitate confirmed presence of alkaloids. Mayer’s test was done by adding to 0.5 ml of aqueous PHP solution, Mayer’s reagent (potassium mercuric iodide solution) and the formation of a white creamy precipitate indicated that the test is positive.

**Tests for carbohydrates:**

Molisch test was conducted by adding to 0.5 ml of aqueous PHP solution a few drops of alcoholic \(\alpha\)-naphthol solution followed by the addition along the sides of test tubes 0.2 ml of concentrated sulphuric acid. Formation of a reddish-violet ring at the junction of the two layers indicated the presence of carbohydrates. For reducing sugars Benedict’s test was performed by taking 0.5 ml of aqueous PHP solution, shaking with 2.5 ml of water, filtered and the filtrate was heated to concentrate. To the concentrated filtrate, 5 ml of Benedict’s solution was added and boiled for 5 min. Formation of a brick red precipitate indicated the presence of free reducing sugar. Fehling’s test was conducted by mixing equal volumes of Fehling’s A (copper sulphate in distilled water) and Fehling’s B (potassium tartrate and sodium hydroxide in distilled water) reagents and to the mixture a few drops of aqueous PHP solution were added and boiled. A brick-red precipitate of cuprous oxide indicated the presence of free reducing sugar.

Monosaccharides were detected using the Barfoed test. About 0.5 ml of aqueous PHP solution was diluted with distilled water, filtered and 1 ml of the filtrate was mixed with 1 ml of Barfoed reagent and heated on a water bath for 2 min. The brick-red precipitate of cuprous oxide confirmed the presence of monosaccharides. Starch was detected by the appearance of a dark blue colour when 0.5 ml of aqueous PHP solution was mixed with iodine reagent and the colour disappears on heating and reappears on cooling.

**Tests for flavonoids and glycosides:**

Flavonoids were detected by adding 5 ml of dilute ammonia to 1 ml of aqueous PHP solution, followed by the addition of concentrated sulphuric acid. The appearance of a yellow colour indicated the presence of flavonoids. Anthraquinone glycosides were detected using Born Trager test. To 0.5 ml of aqueous PHP solution, 0.5 ml of dilute ammonia solution and 1 ml of benzene were added. The appearance of reddish-pink colour indicated the presence of anthraquinone glycosides. Keller Killiani’s test was performed to detect cardiac glycosides. A mixture of 0.5 ml of concentrated sulphuric acid, 0.4 ml of glacial acetic acid containing traces of ferric chloride was added to the aqueous PHP solution carefully. The appearance of a reddish-brown colour at the junction of the two layers and bluish green of the upper layer indicated the presence of cardiac glycosides. Legal’s test, in which, 1 ml sodium nitroprusside and 1 ml of pyridine were added to the aqueous PHP solution and the formation of pink to red colour confirmed presence of glycosides.

**Test for saponins, steroids and triterpenoids:**

A pinch of the dried PHP was added to 3 ml of distilled water and the mixture was shaken vigorously.

<table>
<thead>
<tr>
<th>Angle of Repose</th>
<th>Hauser’s Ratio</th>
<th>Carr’s Index</th>
<th>Relative Flowability</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-30</td>
<td>1.00-1.11</td>
<td>&lt;=10</td>
<td>Excellent</td>
</tr>
<tr>
<td>31-35</td>
<td>1.12-1.18</td>
<td>11-15</td>
<td>Good</td>
</tr>
<tr>
<td>36-40</td>
<td>1.19-1.25</td>
<td>16-20</td>
<td>Fair</td>
</tr>
<tr>
<td>41-45</td>
<td>1.26-1.34</td>
<td>21-25</td>
<td>Passable</td>
</tr>
<tr>
<td>46-55</td>
<td>1.35-1.45</td>
<td>26-31</td>
<td>Poor</td>
</tr>
<tr>
<td>56-65</td>
<td>1.46-1.59</td>
<td>32-37</td>
<td>Very Poor</td>
</tr>
<tr>
<td>&gt;66</td>
<td>&gt;1.60</td>
<td>&gt;38</td>
<td>Extremely Poor</td>
</tr>
</tbody>
</table>

The angle of repose, Carr’s index and Hauser’s ratio values specifies the relative flowability of the powder within the specific range.
Formation of foam indicated the presence of saponin. Positive Liebermann-Burchard test confirmed presence of steroids and terpenoids. To 0.5 ml of aqueous PHP solution, few drops of acetic anhydride was added, boiled and cooled. Along the sides of the test tube, concentrated sulphuric acid was added. Formation of a brown ring at the junction of two layers with green coloured upper layer indicated the presence of steroids whereas triterpenoids were indicated by the formation of deep red colour. Salkowski test was performed by adding chloroform to 0.5 ml of aqueous PHP solution with a few drops of concentrated sulphuric acid. It was then shaken and allowed to stand. The appearance of red colour at the lower layer indicated the presence of steroids and the formation of yellow coloured lower layer indicated the presence of triterpenoids.

**Tests for tannins:**

Tannins were detected by the lead acetate test, in which few drops of 10 % lead acetate were added to 0.5 ml of the aqueous PHP solution. Formation of a precipitate indicated the presence of tannins. Ferric chloride test in which a few drops of 0.1 % ferric chloride solution was added to 0.5 ml of aqueous PHP solution and presence of tannins were indicated by either the formation of a blue-black or brownish-green colouration.

**Tests for phenolic compounds:**

Few drops of 10 % lead acetate solution were added to the aqueous PHP solution and the presence of phenolic compounds was indicated by the formation of white precipitate. Few drops of neutral 5 % ferric chloride solution was added to the 0.5 ml of aqueous PHP solution. Presence of phenolic compounds were indicated by the formation of dark green colour.

**Tests for amino acids:**

Millon’s test in which to 0.5 ml of aqueous PHP solution, 2 ml of Millon’s reagent (mercuric nitrate in nitric acid containing traces of nitrous acid) was added. A white precipitate appeared which turned red when gentle heating, indicated the presence of amino acids. Ninhydrin test was performed by adding to 0.5 ml of aqueous PHP solution a few drops of 5 % ninhydrin followed by boiling. The appearance of violet colour indicated the presence of amino acids.

Proteins were detected with Biuret test in which to 0.5 ml of aqueous PHP solution, 4 % sodium hydroxide solution and few drops of 1 % copper sulphate solution were added. Protein’s presence was indicated by the appearance of violet colour. Oils and fats were detected when a small quantity of the PHP was taken between two filter papers and pressed. Presence of oil stain on the filter papers indicated the presence of oils and fats. Presence of coumarins was tested by adding to 0.5 ml of aqueous PHP solution 10 % sodium hydroxide. The appearance of an yellow colour indicated the presence of coumarins.

**RESULT AND DISCUSSION**

Organoleptic properties of PHP were evaluated and represented in Table 2. Previous studies reporting organoleptic evaluations of polyherbal formulations for DM indicated the presence of bitter taste decreased patients’ acceptance [32-35]. However with the PHP developed in this investigation patient’s acceptability would increase due to its acceptable taste with specific combination of herbs.

Moisture content is the major factor responsible for the deterioration of drugs and formulations. The presence of excessive moisture in plant drugs causes hydrolysis of constituents, bacterial and fungal growth and biochemical reactions. It is expected that the formulation with less moisture content would be stable for a longer time. For a plant drug, it is mentioned that the moisture content should be less than 14 % [29]. Thus, in this study it is seen that the moisture content of individual drugs and the PHP were below 10 % in the range of 5±0.01-8±0.01 % w/w as depicted in fig. 1.

The most important parameter for the quality control of herbal drugs is the ash value. High ash value indicated adulteration, contamination, substitution or carelessness in preparing the drug. In this study, the total ash value was in the range of 6±0.01-9±0.01 % w/w indicating low contamination. A part of the total ash content, which is soluble in water is called the water-soluble ash, which is primarily utilised as an indicator for the incorrect preparation or presence of previously extracted water-soluble salts in the drug. Thus, it is the difference in weight between the total ash and the residue obtained after treatment of total ash with water [26]. The water-

<table>
<thead>
<tr>
<th>TABLE 2: ORGANOLEPTIC PROPERTIES OF POLYHERBAL POWDER</th>
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<tbody>
<tr>
<td><strong>Organoleptic Property</strong></td>
</tr>
<tr>
<td>Colour</td>
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<tr>
<td>Odour</td>
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<tr>
<td>Taste</td>
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<tr>
<td>Appearance</td>
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</tbody>
</table>

Evaluation of the organoleptic properties i.e. colour, odour, taste and appearance of the PHP powder.
soluble ash values of individual drug and PHP were in the range of 2±0.01-3±0.01 % w/w. This range of water-soluble ash indicated normal quality of the drugs. The acid-insoluble ash values of individual drugs and PHP ranged from 1±0.01-2.5±0.01 % w/w. Total ash value, water-soluble ash, and acid-insoluble ash of the individual drug and PHP are depicted in fig. 2.

The pH of the aqueous solution of PHP was found to be 6±0.02. Study of bulk density and tapped density are required as it determines the packaging of the powder. Tapped density of the powder gives the information on the consolidation of powder. More the powder is consolidated; more will be resistant to flow. From our study, it was found that the tapped density was in the range of 0.65±0.01-0.92±0.01 % w/w. Thus, having a low value of tapped density. Tapped density, bulk density and Hausner’s ratio of individual drugs of the PHP is depicted in fig. 3. Carr’s index and angle of repose indicate the compressibility and free-flowing property of the powder. Their range indicated excellent flow property of the powder which is illustrated in figs. 4A and B.

The ongoing research on potential herbs for effective control of diabetes and its complications indicated that *C. zeylanicum* was found to exhibit inhibitory actions on α-glucosidase[18] and also α-amylase associated with antihyperglycemic actions associated with *O. sanctum*. Greater inhibition of maltase delays release of glucose into the blood stream is attributed to the high total polyphenols and flavonoids contents. Phytochemical analysis has also shown that aqueous extract of *tulsi* leaves contains cardiac glycosides, flavonoids, glycosides and tannins[36,37]. As found in this study, the phytochemical analysis of the PHP confirmed the presence of active constituents such as glycosides,
In the present study, a PHP was evaluated using organoleptic, physico-chemical and phytochemical parameters to set its physicochemical standards (moisture content, flow property, loss of weight on drying, total ash, water-soluble, acid-insoluble ash). The research findings of the standardization could be used for evaluating the quality and purity of the formulations. This formulation could be used in the treatment of DM.

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### Conflicts of interest:

No conflict of interest.

### REFERENCES


