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PREPARATION AND EVALUATION OF THE POLYHERBAL POWDER: THE NATURE'S PHARMACY FOR THE TREATMENT OF DIABETES MELLITUS AND ITS COMPLICATIONS

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ABSTRACT

Objective: Diabetes Mellitus (DM) is the major crippling disease, leading to huge economic loss in the developing country, India. Thus, the utilization of the nature's pharmacy is the pivotal forum of research in the treatment of DM and its complications. The main objective of the study was to prepare the Polyherbal Powder (PHP) extract for DM and its complications and evaluate it on the basis of organoleptic characteristics, and physico-phytochemical analysis. Materials & Methods: The PHP extracts were prepared by soxhlet extraction and evaluations were done for organoleptic properties, flow property of powder, and physico-phytochemical properties by standard procedures. Results: Organoleptic characters of the PHP are greenish brown in color, characteristic odor, astringent and bitter taste with moderately fine texture. Phytochemical qualitative analysis displayed presence of flavonoids, alkaloids, terpenoids, tannins, steroids, carbohydrates, and glycosides. The physicochemical analysis displayed longer stability results with acidic pH and excellent flow property of the PHP. Conclusion: PHP beholds felicitous and potent role in treatment of DM and its complications.

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Introduction

India is considered as the epicenter of the global diabetes epidemic and the diabetic capital in the world with the score of the second highest number of diabetic people in the world. It is estimated to have over 20 million diabetic cases which are estimated to increase to 57 million by 2025. Diabetes mellitus (DM) is the predominant public health concern disorder that causes substantial mortality, morbidity and long term health complications. It is basically the systematic metabolic disorder characterized by hyperglycemia, insulin resistance and relative insulin deficiency with disturbances of carbohydrate, fat and protein metabolism. It is increasing throughout the world at an alarming pace which is potential to cause grave complications in the body over time like neuropathy, nephropathy, retinopathy, cardiovascular diseases, retinopathy and dyslipidemia. In today's scenario, about 90% of the young population accounts for the major share in the development of incidences of Type II diabetes due to the shift from the diurnal standard of our ancestors living to the sedentary lifestyle i.e. of unhealthy diet habits and less physical activity. It accounts for 150 million people worldwide, which are estimated to increase to 300 million by 2025. Worldwide, it has become one of the major crippling diseases that leads to a huge economic loss. There is

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the availability of various synthetic drugs including insulin and oral hypoglycemic agents which controls the level of blood sugar, but their cost, complications and limited tolerability and various side effects cause the reduction in its wide acceptance. Thus, it is notably one of the refractory diseases identified by the Indian Council of Medical Research for which there is an alarming time for alternative medicine treatment. As per an estimate worldwide, about 12000 plants in the world are being used in medicinal purpose, but less than 10% out of them are investigated from the pharmacological point of view [1-5].

Considering the facts, the most commercially successful and widely used branch of alternative or complementary medicine is “phytotherapy” which acquires to be 'synergic' that is more effective than the sum of their parts. The emphasis is on the whole-plant material, rather than an individual chemical ingredient, that differentiates phytotherapy from the appropriation and synthetic manufacture by mainstream medicine of the active compounds of plants. India is considered as the “Emporium of Medicinal Plants”, because in different bioclimatic zones, there are varied and diverse availability of several thousands of medicinal plants and thus has a rich history of using herbal plants in medicinal purposes. Traditionally, herbal medicines and their preparations are used in various therapies owing to their natural origin and lesser side effects than synthetic drugs. These drugs comprise of raw materials that bring economic prosperity to the masses that grow these raw materials. Medicinal plants not only play a pivotal role in traditional systems of medicine but also in modern medicine. According to WHO reports, usage and practical application of herbal medicines in therapies constitute about 80% of the total world population. The traditional medicinal system is utilized by over 1.5 million practitioners in preventive, curative and promotional applications. There has been the revival of interest in the medicinal plants due to the recognition of the value of traditional claims regarding the natural products in health care. The plants are capable of retarding various pathological conditions of diseases [6-11].

Nature is the putative pharmacy for the prevention and treatment of DM as reported by numerous studies done on herbs and thus phytotherapy been practiced. Various anti-diabetic potential herbs include *Allium cepa, Ocimum sanctum, Trigonella foenum-graecum, Magnifera indica, Tinospora cordifolia, Acacia arabica, Ceylon cinnamon, Terminalia bellirica, Allium sativum, Azadirachta indica, Momordica charantia, Emblica officinalis, Aegle marmelos, Syzgium cumini*, and many others [12].

Phytotherapy flourishes more, 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 acquired i.e. either the herb will target the therapeutic activity to a receptor or will facilitate absorption, distribution, metabolism and elimination of the other herbs. Polyherbal acts on more targets at the same time to provide the assiduous and scrupulous relief. Thus polyherbal is expected to raise the effectiveness and potency of formulation, reduction of side effects, and the increase of life span. Thus, polyherbal formulations are gaining popularity when collating to single herb [13].

The recent scenario of phytotherapy assures the advantages of polyherbal, and thus holds the future prospects of a healthy population. The present study was conducted on the PHP to study its pharmacognostic and physiological parameters. Their constituent not only acts in maintaining the blood glucose level, but also provides with the protective effect thus preventing its long term complications.

Curcuma longa (Family Zingiberaceae) is the rhizomatous herb, commonly known as turmeric. It consists of curcumin as the principal curcuminoid that has been recently found as the anti-inflammatory, antioxidant, neuroprotective agent to reduce oxidative damage in neurodegenerative disorders. It is reported to accelerate the repair of sciatic nerve injury in rats through reducing Schwann cell apoptosis and promoting myelinisation and mainly accounts for the central mechanisms underlying neuropathic pain. It has no toxicity as an extract as observed in rats, guinea pig and monkey whereas isolated curcumin has a certain amount of toxicity in a dose-dependent manner, thus curcumin was not isolated and used as an extract [14-16].

Ocimum sanctum or *Ocimum tenuiflorum* (Family Lamiaceae) also known as Holy Basil or Tulsi has pharmacological activities as an antioxidant, antidiabetic, anti-inflammatory, antiallergic, immunomodulatory, antimicrobial, antistress, analgesic, antipyretic, antihypertensive, cardioprotective, gastroprotective, hepatoprotective, renoprotective, radioprotective, chemopreventive, and anticancer properties. 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 anti-hyperglycemic effect. Eugenol, rosmarinic acid and Apigenin (phenolic compounds) present in it have antinociceptive and anti-inflammatory activities that may either improve neuropathic pain or prevent nerve damage [5, 17-19].

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). 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 anti-diabetic and prevents its complications is that 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 [20, 21].

Cinnamomum verum (Family Lauraceae) commonly known as Dalchini has been shown to exhibit insulin potentiating effect in glucose metabolism. It is reported to enhance the glucose uptake by activating insulin receptor kinase activity, autophosphorylation of the insulin receptor and glycogen synthase activity [22].

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 it is used for delaying the onset of diabetes in prediabetes subjects by lowering blood glucose level in prediabetes and has an insulinotropic-effect. It exerts hypoglycemic 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 [23-25].
Momordica charantia (Family Cucurbetaceae) is commonly used as a vegetable in Asian cuisines and is commonly 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 hypoglycemic properties. The possible mechanism of action as suggested by the scientists includes the inhibition of glucose uptake and suppression of key glucogenic enzymes and enzymes of HMP Pathway. Thus, it is utilized as an anti-diabetic and also has pointed towards its preventive action in diabetic neuropathy as shown in numerous studies done by scientists [26].
Syzygium cumini (Family Myrtaceae) is effective in controlling high blood sugar levels and this has been already indicated in Ayurvedic pharmacopoeia. It is commonly called as Jamun. Several putative mechanisms have been reported by the scientists that make it an anti-diabetic potential. It reduces the free radicals and improves the functioning of β-cells of the pancreas causing low blood sugar level. It may also reduce the activity of α-amylase, which is upregulated in the DM [27, 28].

Materials and Method

Selection and Collection of plant material: Leaves of tulsi, bael and fruits of amla were procured from the Botanical Garden of Pranveer Singh Institute of Technology (PSIT, Kanpur). Meethi, Harad, Baheda, Karela and Jamun seeds, barks of Dalchini and rhizomes of turmeric were procured from the local market. These marketed constituents were authenticated by Dr Naveen Kumar, the head of Botany Department of Christ Church College 012/2018/19-106.
Preparation of PHP extract: The plant materials were subjected to shade drying. Seeds of Jamun, Karela, Harad, Baheda and fruits of Amla were first crushed in a mortar pestle. Then each of the plant material was subjected separately to the electrical grinder for obtaining a fine powder. Seeds of Karela were passed through sieve no. 25 for fine powder; whereas seeds of Baheda and Harad were passed through sieve no. 44 and sieve no. 22 for obtaining fine powder after being ground in an electrical grinder. Fine powders of each of the plant material were thus obtained and mixed in a specific ratio.
With herbal processing, the type of extraction procedure can greatly affect the final natural products obtained. Thus, Soxhlet extraction technique was considered that involved the use of the Soxhlet apparatus for the extraction of herbs. The sample is placed in a thimble holder that is gradually filled with fresh solvent from a distillation flask. As the liquid reaches the overflow level, a siphon aspirates the solute from the thimble holder, moving the aliquot back into the distillation flask and carrying the extracted analytes into the bulk liquid. This process continues until the extraction process has been completed. The system operates in a continuous mode as the solvent is recirculated through the sample [29].
This technique was adopted for obtaining the extract of PHP. Aqueous and ethanolic extracts were obtained from soxhlet extraction by using solvents. For aqueous extract, soxhlet extraction was done with 500 ml water poured in gradation at temperature around 90ºC and was run upto 5 cycles. The ethanolic extract was obtained with 500 ml ethanol poured in gradation and was made 5 cycles to be run at the temperature of 45ºC.
Organoleptic Evaluation: Organoleptic evaluation refers to the evaluation of the formulation by the color, odor, taste, texture, etc. The method adopted for the organoleptic evaluation was described in Wallis [30].
Physicochemical and Phytochemical Evaluation: Various physicochemical parameters like moisture content, pH, and ash value were performed. PHP extracts were also analyzed for the preliminary phytochemical screening for the presence of organic constituents by standard methods [31-34].
Moisture content: Loss on drying is the parameter to keep the moisture content under check as the larger amount of moisture can promote hydrolytic reactions and can initiate the microbial growth. The moisture content was measured by the Gravimetric method and loss on drying was calculated. 2 g (W) sample was placed in a weighed preheated porcelain dish and then was kept in a hot air oven and dried at 105 °C till constant weight or two consecutive weights differing by 0.5mg was observed. Weight was taken after drying and was transferred to the desiccator to cool and then again porcelain dish was reweighed. The percentage of moisture content was calculated by following equation:

Moisture content (%) = {(W1-W2)/W}*100 where,
W = Weight of the sample (2g)
W1 = Weight (g) of sample before drying
W2 = Weight (g) of 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 3g (W2) of the air-dried sample was added in the previously weighed crucible. The sample was ignited gradually in an electrical muffle furnace, increasing the heat to 1000°C until it is white, indicating the absence of carbon. Then it was cooled in a desiccator and reweighed (W3).

W1= Weight of empty silica crucible
W2= Weight of sample including crucible weight for ignition
W3= Final weight of sample including crucible weight after ignition
Total ash content was calculated as:
Total ash (%) = {(W3-W1)/(W2-W1)}*100

Acid-insoluble Ash - 25ml of dilute HCl was added to the total ash containing crucible. It was then covered with watch-glass and boiled gently for 5 minutes. With 5ml of hot water, the watch glass was washed and the washings were added to the crucible. Then, the ashless filter paper was used to filter the insoluble matter and washed with hot water till 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 minutes and then reweighed.

W1= Weight of empty silica crucible
W2= Weight of sample including crucible weight for ignition
W3= Final weight of sample including crucible weight after ignition
W4= Constant weight after addition of HCl
Acid-insoluble ash content was calculated as:
Acid-insoluble ash (%) = {(W4 – W1)/(W2 – W1)}*100

Water-soluble Ash - In the total ash containing crucible, 25ml of water was added and boiled. Boiling was done for 5 minutes and then through the ashless filter-paper, filtration was done causing collection of insoluble matter on it. Further, the filter was washed with hot water and then ignited in a crucible for 15 minutes at a temperature not exceeding 500 °C. The residue was allowed to cool in a desiccator for 30 minutes, and then re-weighed (W5), calculations were done as:

W1= Weight of empty silica crucible
W2= Weight of sample including crucible weight for ignition
W3= Final weight of sample including crucible weight after ignition
W6= Weight of residue = W5 – W1
W7= Weight of ash = W3 – W1
Water-soluble ash (mg/g) = W7-W6
Water-soluble ash was calculated as:
Water-soluble ash (%) = (W7-W6)* 100

Flow Characteristics of powder (Rheological Parameters): The preformulation study is basically defined as the principal investigation technique in the development of the drug to obtain information on the previously known properties of the compound and propose the development schedule. Rheological characteristics of the formulated powder were studied and estimated like an angle of repose, bulk density, tapped density, and compressibility index.
Angle of Repose - An 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, powder 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.

h = Height of the cone
r = Radius of the cone base
$\tan \theta = h/r$
The angle of repose (θ) was calculated as:
Angle of repose $(\theta) = \tan^{-1} h/r$

Bulk Density - Some amount of powder (M) was added into a dry 100 ml cylinder, without compacting. The powder was carefully levelled without compacting and the unsettled apparent volume (V_o) was read and noted.

M = Weight of sample
V = Apparent volume of powder.

The bulk density (ρ_b) was calculated as:
$\rho_b = M / Vo$

Tapped Density - After following the procedure of apparent bulk density, the cylinder containing the sample was further undergone for the tapped density measurement. Initially, the sample was tapped 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 (V_f) was measured.

M = Weight of sample
V_f = Tapped volume of powder

The tapped density (ρ_{tap}) was calculated, in gm per ml, using the following formula.
$\rho_{tap} = M / V_f$

Carr's index - Carr's index is defined as the measure of the intensity of powder to be compressed. Its determination is done from the bulk and tapped density. In theory, it is believed that lower the compressibility of material, higher will be the flowability of the powder. As such, it is the measure of the relative importance of interparticulate interactions. In a free-flowing powder, such interactions are generally less significant, and the bulk and tapped densities will be closer in value. For poorer flowing materials, there are frequently greater inter-particle interactions, and a greater difference between the bulk and tapped densities will be observed. These differences are reflected in Carr's Index which is calculated using the following formula:
Carr's index = $[(\rho_{tap} - \rho_b) / \rho_{tap}] / \times 100$

Hausner's Ratio - It is defined as an indirect index of ease of flow of powder which is calculated using the formula:
Hausner's Ratio= ρ_{tap} / ρ_b

Table 1: Flow Characteristics of the Powder

S.No.	Angle of repose	Hausner's Ratio	Carr's Index	Relative flowability
1	25-30	1.00-1.11	<=10	Excellent
2	31-35	1.12-1.18	11-15	Good
3	36-40	1.19-1.25	16-20	Fair
4	41-45	1.26-1.34	21-25	Passable
5	46-55	1.35-1.45	26-31	Poor
6	56-65	1.46-1.59	32-37	Very Poor
7	>66	>1.60	>38	Extremely Poor

Angle of repose, Carr's index and Hausner's ratio values specifies the relative flowability of the powder within the specific range.

Phytochemical Screening: The phytochemical tests were carried out for the above mentioned aqueous and ethanolic plant extract separately using the standard procedures to identify the components.
Tests for alkaloids - Dragendorff's test- To 0.5 ml of plant extract, add Dragendorff's reagent (Potassium bismuth iodide solution). The appearance of reddish brown precipitate confirms that alkaloid is present i.e. test is positive.
Hager's test- To 0.5 ml of plant extract, add few drops of Hager's reagent. Formation of a yellow color precipitate confirmed the presence of alkaloids.
Wagner's test- To 0.5 ml of plant extract, add Wagner's reagent (solution of Iodine in Potassium Iodide). Observation of the reddish-brown precipitate confirms that the test is positive for alkaloids.
Mayer's test- To 0.5 ml of plant extract, Mayer's reagent (Potassium mercuric iodide solution) was added. A white creamy precipitate confirms that test as positive.
Tests for carbohydrates - General test: Molisch test- To 0.5 ml of plant extract, add few drops of alcoholic α-naphthol solution and then along the sides of test tubes, 0.2 ml of concentrated sulphuric acid was added. Formation of the reddish-violet ring at the junction of the two layers indicated the presence of carbohydrates, thus test being positive.
For reducing sugar: Benedict's test- 0.5 ml of plant extract was taken and was shaken with 2.5 ml of water. Then it was filtered and further the filtrate was heated to concentrate it. To the concentrated filtrate, add Benedict's solution (5 ml) and boil for 5 minutes. Brick red precipitate was formed. It indicated the presence of free reducing sugar.
Fehling's test- Equal volume of Fehling's A (copper sulphate in distilled water) and Fehling's B (potassium tartrate and sodium hydroxide in distilled water) reagents are mixed carefully. Then a few drops of plant extract were added and boiled. A brick red precipitate of cuprous oxide indicated the presence of free reducing sugar.
For monosaccharide: Barfoed test- About 0.5 ml of plant extract in distilled water was dissolved and filtered. About 1 ml of the filtrate was then mixed with 1 ml of Barfoed reagent and heated on a water bath for a period of two minutes. The brick red precipitate of cuprous oxide confirms that test as a positive for monosaccharide.

Tests for Flavonoids - To extract (1ml), diluted ammonia (5ml) was added followed by concentrated Sulphuric acid. The appearance of a yellow color indicates the presence of flavonoids.
Tests for glycosides - Anthraquinone Glycosides: Borntrager test- In plant extract, 0.5 ml of dilute ammonia solution and 1 ml of benzene were added. The appearance of reddish pink color indicated the presence of anthraquinone glycoside.
Cardiac Glycosides: Keller killiani's test- 0.5 ml of concentrated sulphuric acid, glacial acetic acid (0.4ml) containing traces of ferric chloride were added to the plant extract carefully. The appearance of the reddish-brown color at the junction of the two layers and bluish green of the upper layer indicated the presence of cardiac glycosides.
Legal's test: 1ml sodium nitroprusside and 1ml pyridine were added to the extract of the plant. Formation of pink to red color confirms the positive test for glycosides.
Test for Saponins - Froth test: A pinch of the dried powdered plant was added to 3 ml of distilled water. The mixture was shaken vigorously. Formation of foam indicated the presence of saponin.
Tests for Steroids and Triterpenoids - Liebermann-Burchard test: To 0.5 ml of plant extract, add few drops of acetic anhydride boil it and cool it. Along the sides of the test tube, concentrated sulphuric acid was added. Formation of a brown ring at the junction of two layers and the green color of the upper layer shows the presence of steroids whereas triterpenoids were indicated due to the formation of deep red color.
Salkowski Test: Chloroform was added to the plant extract (0.5 ml) with few drops of concentrated sulphuric acid. It was then shaken and allowed to stand for some time, the appearance of red color at the lower layer indicated the presence of steroids and formation of yellow color lower layer indicated the presence of Triterpenoids.
Tests for Tannins - Lead Acetate Test: Few drops of 10 % lead acetate was added to 0.5 ml of the plant extract. Formation of precipitate indicated the presence of tannins.
Ferric chloride Test: Few drops of 0.1% ferric chloride solution to 0.5 ml of plant extract was added. Tannins were indicated by either the formation of blue-black coloration or brownish green coloration.
Test for starch- To 0.5 ml of plant extract, iodine reagent was added. The appearance of a dark blue color which disappeared on heating and reappears on cooling indicated the presence of starch.
Tests for Phenolic compounds - Lead acetate Test: Few drops of 10% lead acetate solution was added to the plant extract and the presence of phenolic compounds were indicated by the formation of white precipitate.
Ferric chloride Test: Few drops of neutral 5% ferric chloride solution was added to the 0.5 ml of plant extract. Presence of phenolic compounds were indicated by the formation of dark green color.
Tests for Amino acids - Millon's Test: To extract of the plant, 2 ml of Millon's reagent (Mercuric nitrate in nitric acid containing traces of nitrous acid) was added. A white precipitate appears which turns red when gentle heating, indicated the presence of amino acids.
Ninhydrin Test: To 0.5 ml of plant extract, few drops of 5% ninhydrin was added and then was brought to boiling. The appearance of violet color indicated the presence of amino acids.
Tests for Protein- Biuret Test: To 0.5 ml of plant extract, 4% sodium hydroxide solution and few drops of 1% copper sulphate solution was added. Protein's presence was indicated by the appearance of violet color.
Test for oils and fats- A small quantity of the PHP was taken between the two filter papers and pressed. Presence of oil stain on the filter papers indicated the presence of oils and fats.
Test for Coumarins - To 0.5 ml of plant extract, the solution of 10% sodium hydroxide was added. The appearance of yellow color indicated the presence of coumarins.

Result and Discussion:

Organoleptic Characteristics: The organoleptic properties were evaluated and studied and it is represented in table no. 2

Table 2: Organoleptic Properties of PHP

S.No.	Organoleptic Property	Result
1	Color	Dull brown
2	Odor	Characteristic
3	Taste	Astringent and Bitter
4	Appearance	Moderately Fine, No clumping or aggregation

Evaluation of the organoleptic properties i.e colour, odour, taste and appearance of the PHP powder.

Physicochemical Parameters:
Moisture content - It is the major factor responsible for the deterioration of drugs and formulations. The presence of excessive amount of moisture in plant drugs causes the hydrolysis of constituents, bacteria and fungi growth and biochemical reactions. It is expected that the formulation with less moisture content is safe for the longer time. For the plant drug, it is mentioned that it should be less than 15%. Thus, in our study it is seen that the moisture content of the individual drug and the PHP were below 10% in the range of 5±0.01%w/w- 8±0.01%w/w and is depicted in figure no. 1.

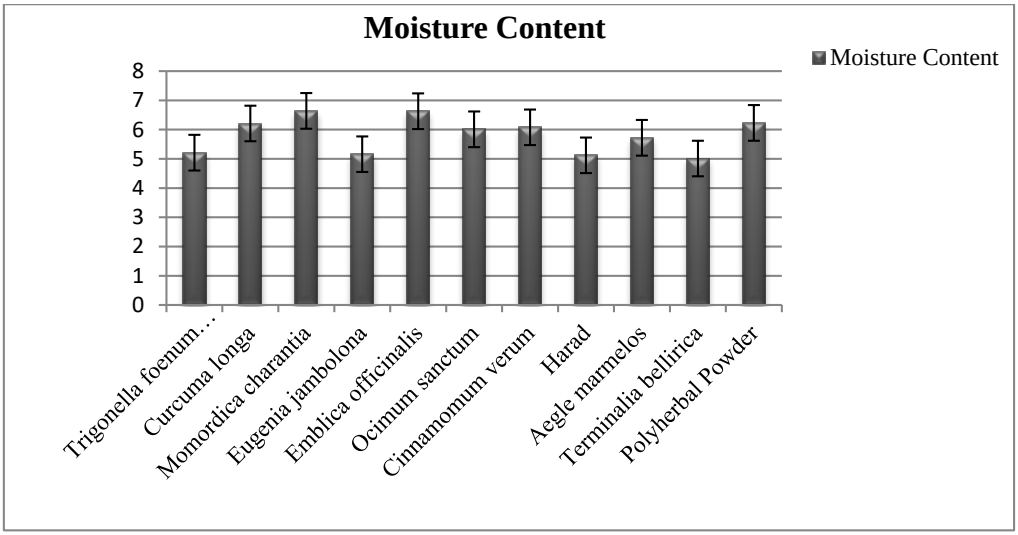

Fig. 1: Moisture content of the individual herb and PHP (%w/w)

Ash Content - The most important parameter for the quality control of herbal drugs is the ash value. High ash value indicates the adulteration, contamination, substitution or carelessness in preparing the drug. In our study, the total ash value was in the range of 6±0.01%w/w-9±0.01%w/w indicating low contamination**.** The part of the total ash content, which has solubility in water is called the water-soluble ash, which is primarily utilized as the indicator for the incorrect preparation or presence of previous extraction of water soluble salts in the drug. Thus, it is basically the difference in weight between the total ash and the residue obtained after treatment of total ash with water [35]. The water-soluble ash values of the individual drugs and PHP were in the range of 2±0.01 %w/w-3±0.01% w/w. This range of water soluble ash shows the normal quality of the drugs. The acid-insoluble ash values of the individual drugs and PHP ranges from 1±0.01%w/w-2.5±0.01%w/w. Total ash value, water-soluble ash, and acid-insoluble ash of the individual drug and PHP is depicted in figure 2.

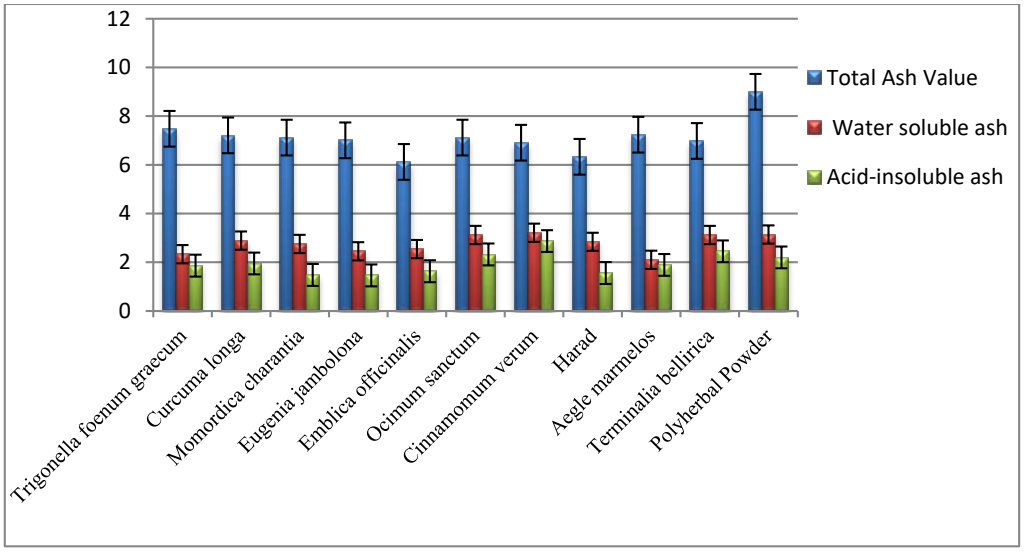

Fig. 2: Percentage ash values of individual herb and PHP (%w/w)

pH - pH of aqueous extract of PHP was found to be 4±0.02 and of ethanolic extract of PHP was 2±0.01. Range was in acidic range. Aqueous extract was slightly acidic. The correlation between the pH and microbial contamination was studied by Abba et al. and suggested that a neutral or alkaline pH favors high microbial contamination levels of the herbal preparations. Thus, our formulation falling in acidic range favors to have more stability [36].

Powder Characterization- 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 the resistant to flow. From our study, it was found that the tapped density was in the 0.65±0.01% w/w- 0.92±0.01%w/w. Thus, having low value of tapped density. Tapped density, bulk density and Hausner's ratio of individual drug and PHP is depicted in figure no. 3.

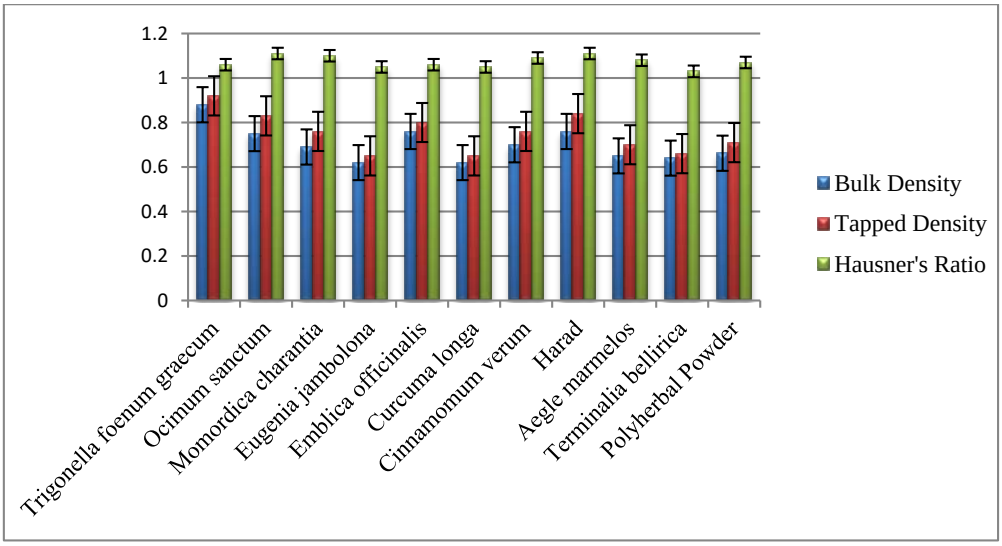

Fig. 3: Bulk density, Tapped density and Hausner's Ratio of the individual herb and PHP (%w/w)

Carr's Index and Angle of repose indicate the compressibility and free flowing property of the powder. Their range falls in the excellent flow property of the powder which is illustrated in figure no. 4 and 5.

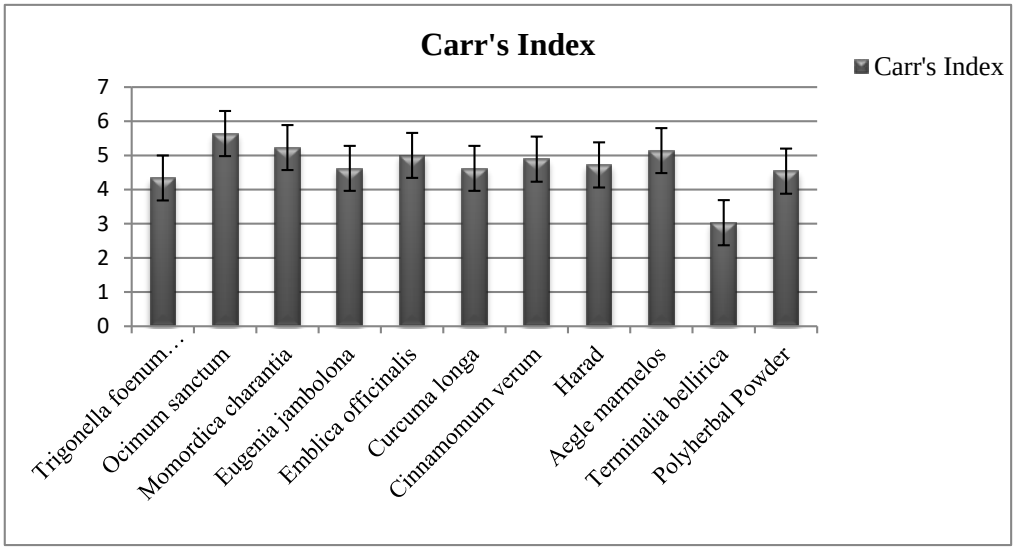

Fig. 4: Carr's Index of the individual herb and PHP (%w/w)

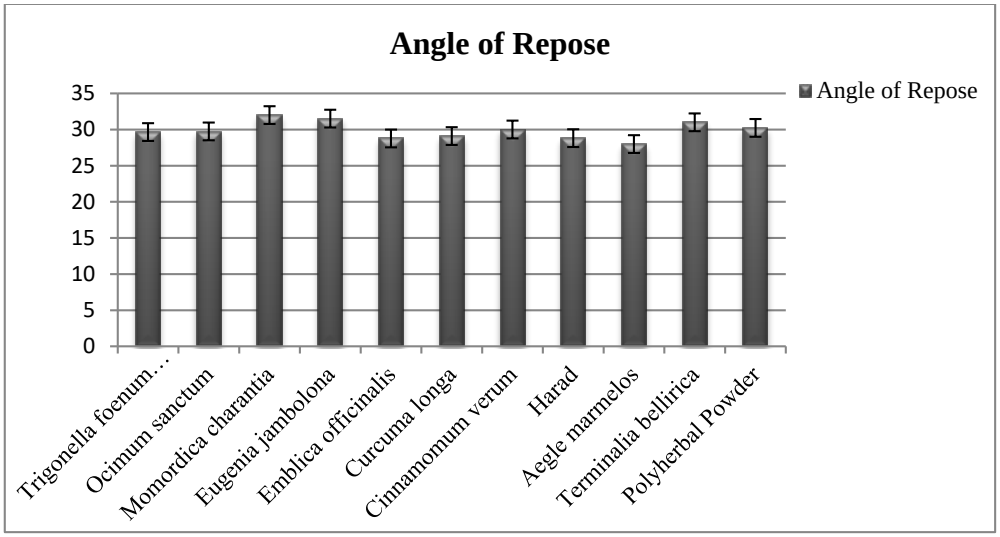

Fig. 5: Angle of Repose of the individual herb and PHP (%w/w)

Phytochemical Screening - It has been suggested after extensive studies by researchers that multiple mechanisms in the treatment of diabetes will effectively control and modify the deteriorating conditions of the patients [37]. PHPs targets at different pathological events associated in the development and progression of diabetes from different mechanistic approaches and abolish its further complications. The ongoing researches for the potential herbs in effective control of diabetes and its complications putatively met with Cinnamon that has been found to exhibit inhibitory actions on α-glucosidase, [38] and also α-amylase associated with anti-hyperglycaemic actions associated with Tulsi. Greater inhibition of enzyme maltase that delays the release of glucose into 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, tannins etc. [39, 40]. As in our study, the phytochemical analysis of the PHP confirmed the presence of active constituents such as glycosides, flavonoids, tannins that ultimately is effective in controlling blood glucose level and preventing its complications. As the pathophysiological mechanism of various diabetic complications like diabetic cardiomyopathy, diabetic neuropathy and others are initiated and promoted by oxidative stress that ultimately is prevented by our prepared PHP, such that not only lowers blood glucose level but also prevents its complications.

It has also been investigated that several herbs have multiple mechanisms in lowering the blood glucose level. As in this PHP, Karela seeds attribute in multiple mechanism thus not only can contribute in lowering the blood glucose level but may also prevent its further complications [41]. Furthermore, seeds of fenugreek also assist in multiple ways in diabetes treatment by its alkaloidal content which is believed to prevent the catabolism processes such as glycogenolysis, lipolysis, modulates insulin secretion and also contribute in the antioxidant action, thus it will also synergistically account in lowering the blood glucose level as other herbs mentioned earlier [42]. These herbs are majorly employed in our formulation of PHP. The phytochemical screening of both extracts of PHP is depicted in table no. 3.

Table 3: Phytochemical Screening of the PHP Extract

S. No	Phytoconstituents	Water Extract	Ethanolic Extract
1.	Test for Alkaloids		
	A. Dragendorff's test	+	+
	B. Hager's test	+	+
	C. Wagner's test	+	+
	D. Mayer's test	+	+
2.	Test for Carbohydrates		
	A. Molisch test	+	+
	B. Benedict's test	+	+
	C. Fehling's test	+	-
	D. Barfoed test	-	-
3.	Test for Flavonoids	+	+
4.	Test for Glycosides		
	A. Borntrager test	+	+
	B. Killer Killiani's test	+	-
5.	Test for Steroids & Triterpenoids		
	A. Liebermann-Burchard test	+	+
	B. Salkowski test	+	+
6.	Test for Tannins		
	A. Lead acetate test	+	+
	B. Ferric chloride test	+	+
7.	Test for Phenolic compounds		
	A. Lead acetate test	+	+
	B. Ferric chloride test	+	+
8.	Test for Coumarins	+	+
9.	Test for Saponins	+	+
10.	Test for Starch	-	-
11.	Test for Amino Acids	+	+
12.	Test for Proteins	+	+
13.	Test for oils and fats	-	-

Evaluation of the phytochemical constituents of the both aqueous and ethanolic PHP extracts
(+) = Present, (-) = Absent.

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