VOLUME 10 ISSUE 2 2024

ISSN 2454 – 3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 10, No. 2, 50-57 (2024)

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Polyherbal Formulation Development and Quality Control for Type 2 Diabetic Mellitus Management

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Received: 6th February, 2024; **Accepted:** 22nd May, 2024; **Published online:** 6th July, 2024

https://doi.org/10.33745/ijzi.2024.v10i02.006

Abstract: Nowadays, herbal remedies are used to treat a wide range of illnesses. Along with a balanced diet and regular exercise, they work to improve health by supplying each cell with the nutrients it needs to thrive. The ancient Indian medical practice of Ayurveda employs a broad variety of techniques to promote health and wellness. The formulation's herbs were sourced from reliable sources in India. Standardization of raw materials is achieved by using powdered, shade-dried plant parts from the following plants: *Cyperus rotundus*, *Terminalia belerica*, *Emblica officinalis*, *Berberis aristata*, and *Terminalia chebula*. The phyto-chemical investigation provided evidence supporting the assertion that flavonoids were detected. This may elucidate the reason behind its profound efficacy in the treatment of diabetes. Future clinical trials should be conducted using Human Volunteers, and stability studies should be performed on the manufactured polyherbal capsules. Herbal treatments, the oldest therapy, diagnose and treat illnesses. Polyherbal capsule raw materials were tested for anti-diabetic efficacy in an animal model. The capsules improved anti-diabetic effectiveness.

Keywords: Traditional, Polyherbal, Anti-diabetic, Assessment, Diabetes mellitus, Phyto-chemicals, *Cyperus rotundus*, *Terminalia belerica*, *Emblica officinalis*, *Berberis aristata*, *Terminalia chebula*

Citation: Upadhye Shashikant Sudarshan, Janet Beula S., Srinivasa Rao Desu Brahma, Dev Dhruv, Pradhan Pankaj, Rihana Syed and Karajgi Santosh: Polyherbal formulation development and quality control for type 2 diabetic mellitus management. Intern. J. Zool. Invest. 10(2): 50-57, 2024.

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Introduction

A growing number of people are turning to herbal remedies as a means of alleviating a variety of health problems. These therapies, in conjunction with a healthy diet and way of life, try to accomplish particular health goals by providing the best possible sustenance to each cell. When compared to synthetic medications, herbal supplements do not have any negative side effects that could harm one's physical health (Kumar *et al.*, 2011; Shweta *et al.*, 2020). A natural medicine is available as a substitute for every synthetic medicine. People have recently begun to focus on traditional medicine as a possible solution to their chronic health problems (Raut and Gaikwad, 2006; Reddy *et al.*, 2022; Ahire *et al.*, 2023).

The medical knowledge that has been handed down from one culture to another prior to the advent of modern medicine is referred to as indigenous medicine, traditional medicine, or folk medicine. The use of plants was first documented in writings from the time of the ancient Sumerians, around 5,000 years ago. The historical records provide evidence of the well-established medicinal uses of various plant species. Symbiosis is an unique occurrence that is remarkably illustrated by the natural world (John *et al.*, 2014; Talele *et al.*, 2021; Pathan *et al.*, 2024).

All the benefits and risks of synthetic pharmaceuticals are well known to modern people. As a result, treatments based on natural products, which take a more minimalist approach, have become increasingly popular. The medicinal therapy of numerous human illnesses has historically relied on natural goods sourced from botanical, zoological, and mineral sources. Around 80% of people in developing countries still use traditional medicine, which is mostly made from different kinds of plants and animals, for their health care needs. It is critical for modern culture to incorporate traditional medical practices (Kumar *et al.*, 2010; Ishtiaq *et al.*, 2014).

Ayurveda is a traditional medical system with a long history of use that combines many different approaches to health and wellness. Restoring harmony between an individual's mental, emotional, and physical health is central to Ayurvedic medicine, which aims to improve general health, prevent disease, and treat existing conditions. Among patients, the demand for herbal and alternative therapies is growing at an exponential rate. Hence, herbal medications are currently in high demand in underdeveloped nations for primary healthcare (Kaul and Dwivedi 2010; Rizwan *et al.,* 2017).

The cultural and religious integration of herbal remedies as a tool to promote health and treat specific medical issues is a major motivation for their use. The considerably reduced cost of herbal products is another reason for their increasing popularity as a remedy. This makes them more accessible and affordable for persons with lower incomes (Choudhary and Sekhon, 2011). Additionally, there is the public's overall view of herbal products, which holds that they are naturally safe since they are made from natural ingredients. A common misconception is that herbal remedies are safer than modern medications because they do not contain any chemicals. In this study polyherbal capsule raw materials were tested for anti-diabetic efficacy in an animal model.

Materials and Methods

The formulation incorporated herbs procured from reputable purveyors located in India. Powdered plant parts that have been shade-dried (*Cyperus rotundus*, *Terminalia belerica*, *Emblica officinalis*, *Berberis aristata*, and *Terminalia chebula*) are utilized in order to establish standards for the raw materials.

Organoleptic evaluation:

The organoleptic profile encompasses the assessment of colour, smell, taste, and other sensory attributes of pharmaceuticals. This profile plays a crucial role in determining the quality of the substance by providing essential information. This study confirmed and examined the organoleptic features of plant materials, including

their physical appearance, flavour, and scent (Bhutani, 2003; Sable *et al*., 2023).

Physicochemical evaluation:

Pharmacognosy demonstrates its true value in the pharmaceutical industry by its significant contributions in the assessment of raw pharmaceutical materials. Through the examination of these aspects, individuals can gain a comprehensive understanding of the distinctive characteristics of illegal substances. Certain naturally occurring inorganic and organic contaminants are extremely challenging to remove during the process of soil collecting. Various methodologies are commonly employed to assess multiple factors, including the purity and compliance of a raw medication, which significantly impact the overall quality of the product (Groop and Pociot, 2014; Sable *et al*., 2023).

Loss on drying:

The loss on drying test can be used to measure the quantity of water and volatile substances present in the crude medicine. When the herbal components are anticipated to have the property of absorbing moisture from the air, the loss on drying test becomes essential. The excessive addition of water to herbal compounds promotes the proliferation of germs, as well as the occurrence of fungi, insects, and deterioration. The moisture content of a medication can provide valuable insights into its stability and manufacturing quality, owing to advancements in pharmaceutical technology (Kumudhaveni and Radha, 2017; Sable *et al.*, 2023).

$$\text{Loss on Drying \%} = \frac{\text{Final weight of sample}}{\text{Initial weight of sample}} \text{ x } 100$$

Determination of ash values:

The residue produced from the burning of pharmaceuticals is usually a granular ash. The composition of the medicine may contain inorganic substances that are deliberately introduced to adulterate, contaminate, or substitute it. However, the main components are non-volatile inorganic salts, such as metallic salts and silica, which naturally occur in the drug and stick to it. This is essential for assessing homemade remedies. Ash content can be determined by measuring total ash, acid insoluble ash, or water soluble ash. The ash value can also serve as a means to measure the amount of sulphated residue (Rao and Chandira, 2020; Sable *et al*., 2023).

Phytochemical studies:

The herb's biosynthetic laboratory synthesizes food-useful chemical components like as carbs, and proteins. Secondary chemicals, including glycosides, alkaloids, flavonoids, tannins, and others, are also found. Identifying the active components in medicinal plants by phytochemical analysis of crude drugs and extracts is essential for understanding their possible pharmacological effects. These assays can provide a quantitative assessment and qualitative separation of pharmacologically active chemical compounds, thereby facilitating future drug discovery and development. The plant raw materials were subjected to a first phytochemical screening in order to determine the different components present in the plants.

Development of formulation:

The plants *Cyperus rotundus*, *Berberis aristata*, *Terminalia chebula*, *Emblica officinalis* and *Terminalia belerica* were subjected to ethanol extraction, and their therapeutic properties were maintained after freeze drying.The extracts were dried for a specific duration based on their respective drying rates. The lyophilizer was utilised effectively at the pharmaceutical laboratory at our university. Microcrystalline cellulose, magnesium stearate, lactose, and wheat are among the diluents that underwent a drying process. Prior to including diluents and preservatives such as sodium methyl paraben and bronopol, the active components were quantified and magnesium carbonate was introduced as an adsorbent. During a duration of thirty minutes, all components were thoroughly blended together in

Table 1: Final Batch

S. No.	Components	Batch (in mg)
1	*Terminalia chebula*	50
2	*Cyperus rotundus*	80
3	*Berberis aristata*	50
4	*Terminalia belerica*	100
5	*Emblica officinalis*	110
6	Magnesium Carbonate	15
7	Sodium methylparaben	0.9
8	Lactose	50
9	Microcrystalline cellulose	30
11	Starch paste	Aq.

an ideal manner. Subsequently, the powder was deposited into the polythene bags, which were subsequently marked and reserved for subsequent examination.

Pre-formulation studies:

Prior to formation, it is imperative to ascertain the derived characteristics of the drug powder based on the physical and chemical properties of the drug molecule. This data plays a crucial role in determining many of the subsequent phases and tactics in formulation development. The ultimate objective is to develop a medicine manufacturing process that maximizes efficiency. Pre-formulation involves the analysis of the drug candidate's physiochemical features.

Formulation trial batch development:

Four experimental batches of capsules were produced by altering the concentrations of the excipient in order to achieve optimal flow characteristics. The flow parameters of the blended powder in all four trial batches, including bulk density, tapped density, compressibility index, Hausner's ratio, and angle of repose, were analyzed. The fourth trial batch (Table 1) was selected for additional scrutiny based on the previous trial batches, which confirmed that it exhibited the most favourable characteristics.

Preparation of capsules:

In terms of pharmaceutical dose forms, the capsule is generally considered to be the most versatile. One solid pharmaceutical formulation is the capsule, which consists of a tiny shell, usually composed of gelatin, enclosing one or more active and inactive components. There is a "hard" kind and a "soft" kind of capsule in the capsule universe. One common name for the hard capsule is the "two-piece" capsule because of the common practice of dividing them into two cylindrical halves.

Pharmacological evaluation:

The *in vivo* study on adult female albino Wistar rats was approved by the Institutional Ethical Committee.

In-vitro anti- diabetic activity:

α-amylase inhibition assay:

There was -amylase in the phosphate buffer saline at a concentration of 0.1 mg/ml. Mixtures of sample solutions with varying concentrations were treated with amylase solutions and then incubated at 37 °C for 5 min. To start the reaction, 0.1 ml of a starch substrate solution with a concentration of 1.0% was added to the incubation medium. It was necessary to boil the mixture for 5 min at 100 °C. The process was terminated after adding 1 ml of DNS reagent after 3 min of incubation at 37 °C. The absorbance at 540 nm was measured using a spectrophotometer after the sample had cooled to room temperature. Following is the formula that we used to determine the level of suppression:

$$\text{Inhibition (\%)} = [(Abs1 - Abs2)/Abs1] \times 100$$

Where, Abs1=sample and Abs2=control.

In vivo anti-diabetic activity:

Being a natural medication, it is quite unlikely that even the maximum dosage would be fatal. Therefore, we conducted a single dose-limit test on all three animals at a dosage of 2000 mg/kg body weight. Rats were administered 50 mg of freshly made streptozotocin per kg of body weight, 120 mg of nicotinamide hydrochloride in 0.1 mg of citrate buffer per kg of body weight intraperitoneally after an overnight fast. Fasting blood glucose levels in rats treated with STZ + NIC were monitored after 48 h of induction to confirm the presence of diabetes. Diabetic rats were randomly assigned to one of four treatment groups based on their fasting blood glucose levels, which were more than 200 mg/dl. For a duration of 28 days, both the gold standard and the herbal mixture were administered orally through gavage. Every week, the weight growth or loss of each animal in the experiment was observed.

Results and Discussion

Loss on Drying:

The assessment of the moisture content in the raw materials was carried out. The results obtained and their matching standard values are displayed in the table.

Total ash Content:

The total ash content of the raw materials was determined and the results, together with their permissible ranges, are presented in Table 2.

Acid insoluble Ash:

All of the ash was fully utilized. The acid insoluble ash concentration of each individual raw material was quantified.

Water Soluble Ash:

The ash content and water soluble ash content of each individual raw material were evaluated.

Analysis of heavy Metal:

A quantitative examination was performed to assess the occurrence of high levels of metallic elements in the unprocessed substances, and the results are depicted in table 3. The sample analysis revealed that the levels of heavy metals are within the prescribed limits. The ingestion of this chemical is deemed innocuous and does not provide any detrimental consequences.

Phyto-chemical testing:

Chemical tests were performed to ascertain the existence of several phytoconstituents in the raw materials. The presence/absence of phyto-chemicals are shown in Table 4.

In vivo anti-diabetic activity:

We used the following method to divide the Albino Wistar rats into four separate groups of six. Herbal treatments have a long history that precedes modern medicine. Traditional uses of herbs for medical purposes, including prevention, diagnosis, and therapy, have seen a renaissance in recent decades. An animal model was used to assess the anti-diabetic efficacy of a formulation. The formulation consisted of five raw materials that were chosen following extensive literature research (Shelke *et al.*, 2017; Ahire *et al.*, 2020). The ingredients were then enclosed in polyherbal capsules. We used procedures suggested by the WHO and the Ayurvedic Pharmacopoeia of India to identify, evaluate, and confirm the purity of the herbal raw materials. Data on a variety of physiochemical parameters, including drying loss, ash value, and extractive value, were computed; this information will be utilized to guarantee pharmaceutical uniformity. Phyto-chemical examination revealed that the raw materials contained alkaloids, steroids, glycosides, flavonoids, phenols, tannins, and terpenoids (Khemuka *et al*., 2014; Ahirrao *et al*., 2022).

The quality metrics and batch consistency of the dried polyherbal extract were fine-tuned by making four independent trial batches. The testing was conducted consistently and at a high standard using preformulation standards. Polyherbal Capsule's trial IV formulation was successful since all metrics were within acceptable values. Physiochemical parameters, phyto-chemical

Table 2: Total ash value

S. No.	Plant Name	Total ash (%w/w)	Limits (w/w%)
1	*Cyperus rotundus*	5.30±0.04	Not More Than 8
2	*Emblica officinalis*	4.22±3.55	Not More Than 7
3	*Berberis aristata*	5.82±0.41	Not More Than 14
4	*Terminalia belerica*	4.07±0.06	Not More Than 7
5	*Terminalia chebula*	3.51±0.02	Not More Than 5

Table 3: Metallurgical analysis

S. No.		Values (PPM)			
		Mercury	Arsenic	Cadmium	Lead
1.	*Cyperus rotundus*	0.005	0.004	0.003	0.003
2.	*Terminalia belerica*	0.03	0.002	0.03	0.004
3.	*Berberis aristata*	0.003	0.001	0.014	0.002
4.	*Terminalia chebula*	0.001	0.002	0.004	0.001
5.	*Emblica officinalis*	0.002	0.003	0.002	0.003

Table 4: Phytochemical analysis

S. No.	Phyto-constituents	*Terminalia chebula*	*Cyperus rotundus*	*Berberis aristata*	*Terminaliab elerica*	*Emblica officinalis*
1	Flavanoids	-	+	+	+	-
2	Tannins	-	-	+	+	+
3	Alkaloids	+	+	+	+	-
4	Steroids	-	-	+	-	+
5	Glycosides	+	+	+	+	-
6	Saponins	+	+	+	-	-
7	Proteins	+	+	+	+	+
8	Carbohydrates	+	+	+	+	+
9	Terpenoids	-	-	+	+	+

research, disintegration time, weight uniformity, moisture content, pH, and description were all standardized. Quantitative assessments of phytoconstituents, including flavonoid, phenol, and tannin levels, were conducted. The results of the polyherbal formulation's heavy metal and microbial load assessments were within safe limits, as per the recommendations of the World Health Organisation (Ahire *et al*., 2020; Reddy *et al*., 2019).

To test the anti-diabetic activity *in vitro*, the beta-amylase inhibition assay was utilized. It has a substantially stronger anti-diabetic effect than conventional Acarbose. The polyherbal capsules were evaluated for acute toxicity and were determined to be safe up to a dosage of 2000 mg/Kg, in accordance with OECD guidelines 423. To evaluate the therapeutic effectiveness of the synthetic polyherbal pill, streptozotocin-induced diabetes was utilized as an *in vivo* model (Kumar *et al*., 2006; Sabu and Kuttan, 2009).

The results of the phytochemical investigation proved that flavonoids were present. This might be the source of the potent anti-diabetic impact. Future clinical trials involving human volunteers are recommended, as are stability tests of the

manufactured polyherbal capsules (Arora *et al*., 2012; Reddy *et al*., 2019; Sable *et al*., 2023).

Conclusion

Herbal treatments, the first kind of medical treatment, are effective against many different diseases. Before being formulated into polyherbal capsules, the anti-diabetic potency of five selected raw ingredients was evaluated using an animal model. Raw materials were tested for authenticity, quality, and purity before physiochemical characteristics were determined. A number of phytoconstituents were discovered through preliminary phytochemical investigation by means of heavy metals and microbiological screening. The polyherbal extract was fine-tuned for batch consistency and quality with the greatest results coming from trial IV. The produced pills were standardized with regard to their botanical testing, physiochemical characteristics, weight, disintegration time, water content, pH, and description. The anti-diabetic activity *in vitro* was shown to be substantially higher than that of ordinary Acarbose. It is recommended to conduct further stability studies and clinical trials for future use.

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