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Research article

ANTI-DIABETIC ACTIVITY OF TRADITIONAL POLYHERBAL FORMULATION: DEVELOPMENT AND EVALUATION

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ABSTRACT

Introduction and Background: Herbal therapy has emerged as a prominent therapeutic approach for a wide range of ailments. In tandem with a nutritious diet and lifestyle, these interventions aim to address certain health objectives by supplying each cell with the most suitable and advantageous nourishment. There exists a botanical alternative for each synthetic medicine now available.

Material and Methods: The herbs utilized in the formulation were obtained from reputable vendors and subsequently verified by Department of Life Sciences, Garden City University, Bengaluru, Karnataka, India. For the purpose of standardizing raw materials, shade-dried powdered plant parts from the plants Berberis aristata (dried stem), Terminalia chebula (pericarp of matured fruit), Emblica officinalis (pericarp of dried mature fruit), Terminalalia belerica (pericarp of dried ripe fruit), and Cyperus rotundus (dried rhizome) are used.

Results: Blood glucose and lipid profiles were taken first thing in the morning. The formulation had a significant impact when compared to the typical range before diabetes was introduced. Total cholesterol, bad LDL cholesterol, and bad triglyceride levels were all reduced while HDL levels were increased. The phytochemical investigation backed up the claim that

flavonoids were present. This may explain why it has such a dramatic impact on treating diabetes. It is recommended that future clinical trials be conducted in Human Volunteers, and that stability studies be conducted on the manufactured polyherbal capsules.

Conclusion: The oldest type of therapy, herbal remedies are used to identify and treat illnesses. An animal model was used to assess the antidiabetic potency of five raw materials that were chosen for formulation into polyherbal capsules. The capsules significantly improved the lipid profile and fasting blood glucose indices, as well as their anti-diabetic efficacy. Future use is advised to pursue additional stability research and clinical trials.

Keywords: Anti-Diabetic, traditional, polyherbal formulation, evaluation

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INTRODUCTION

Herbal medicine has emerged as a popular treatment option for a wide range of ailments. In tandem with a nutritious diet and lifestyle, these interventions aim to address specific health objectives by supplying each cell with the most suitable and advantageous nourishment [1, 2]. Unlike synthetic substances, herbal supplements are devoid of any detrimental side effects that could potentially disrupt one's physical well-being. For each synthetic drug that exists, there is a corresponding herbal drug alternative. In the relentless pursuit of remedies for severe ailments, individuals have finally turned their attention to the field of indigenous medicine [3, 4].

Indigenous medicine, also known as traditional or folk medicine, is the body of medical knowledge that has been passed down from generation to generation across many cultures before the arrival of modern medicine. According to extant literature, the utilization of herbs can be traced back to around 5,000 years ago, during the reign of the ancient Sumerians [5, 6]. These historical records document the well-documented medical applications of several plant species. The natural world consistently serves as a prominent exemplar of the remarkable phenomenon of symbiosis. In contemporary times, individuals possess a comprehensive understanding of the efficacy and adverse effects associated with synthetic pharmaceutical substances [7, 8]. Consequently, there has been a growing fascination in natural product cures that adopt a fundamental approach rooted in nature. Natural products derived from botanical, zoological, and mineral sources have served as the fundamental basis for the therapeutic management of various human ailments. Approximately 80% of individuals residing in underdeveloped nations continue to rely on traditional medicine as their primary source of healthcare, predominantly derived from various plant and animal species. The incorporation of indigenous systems of medicine is vital in contemporary society [9, 10].

Ayurveda is an ancient school of medicine that employs a diverse array of modalities in order to promote and maintain optimal health and overall well-being. The primary objective of Ayurveda healthcare is to reinstate the equilibrium of physical, mental, and emotional well-being in individuals, so enhancing overall health, averting ailments, and addressing existing illnesses. The demand for alternative and herbal therapy among patients is experiencing significant exponential growth. Consequently, there is currently a significant demand for herbal medicines in developing nations as a means of primary healthcare [11, 12].

One primary rationale behind the utilization of herbal remedies is its integration into the cultural and belief systems of some individuals, serving as a means to uphold health and address certain medical conditions. One further factor contributing to the growing use of herbal remedies is the comparatively lower cost of herbal products, rendering them more

accessible and affordable for individuals with lower incomes. One further factor is the general perception held by the public towards herbal products, wherein the notion that these products are derived from natural sources leads to the belief that they are inherently safe. There exists a prevailing belief that herbal products are devoid of chemicals, unlike contemporary pharmaceuticals, which are associated with toxicity and hence pose greater risk [13, 14].

Materials and Methods

Materials

The herbs utilized in the formulation were obtained from reputable vendors and subsequently verified by Department of Life Sciences, Garden City University, Bengaluru, Karnataka, India.

Standardization of raw materials

For the purpose of standardizing raw materials, shade-dried powdered plant parts from the plants Berberis aristata (dried stem), Terminalia chebula (pericarp of matured fruit), Emblica officinalis (pericarp of dried mature fruit), Terminalalia belerica (pericarp of dried ripe fruit), and Cyperus rotundus (dried rhizome) are used [15].

Organoleptic evaluation

Color, smell, taste, and other sensory evaluations of pharmaceuticals are all part of the organoleptic profile, which defines the vast majority of information vital to establishing the material's quality. Physical appearance, flavor, and aroma were some of the organoleptic characteristics of plant materials tested and confirmed in this study [16].

Physico chemical evaluation

While Pharmacognosy has many real-world applications, the evaluation of crude pharmaceuticals is where it really shines for the pharmaceutical business. By analyzing these factors, one can get a good grasp on the unique qualities of illicit substances. Some naturally occurring inorganic and organic pollutants are almost impossible to eliminate during soil collection. Methods typically used to determine numerous criteria, such as the purity and standards of a crude medicine, which influence the quality of the product as a whole [17].

Loss on drying

The amount of water and volatile materials in the crude medication can be determined with the loss on drying test. When the herbal components are expected to be hygroscopic, the loss on drying test becomes crucial. Herbal substances that have had too much water added to them will foster the growth of microorganisms, as well as the presence of fungi, insects, and decay. The water content of a medicine can tell you a lot about how long it will keep and how well it was made, thanks to advances in pharmaceutical technology [18].

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

Determination of ash values

The byproduct of medication incineration is typically a coarse ash. Although it may include inorganic components added for adulteration, contamination, and substitution, it primarily reflects non-volatile inorganic salts like metallic salts and silica that occur naturally in and adhere to the drug. For evaluating home-made medicines, this is a must. Ash value can be indicated by total ash, acid insoluble ash, or water soluble ash. The ash value can also be used to quantify sulphated residue [19].

Microbial load analysis

Following these steps, we were able to estimate the number of aerobic bacteria contained in the herbal treatments and identify the species present.

Phytochemical studies

Food-useful chemical components like carbohydrates, proteins, and lipids are synthesized in the herb's biosynthetic laboratory. Secondary compounds such as glycosides, alkaloids, flavonoids, tannins, etc. are also present. In terms of their potential pharmacological effects, it is crucial that these active principles be identified in medicinal plants by phytochemical analysis of crude medications and extracts. Quantitative estimation and qualitative isolation of pharmacologically active chemical compounds can result from these tests, which may pave the way for future drug discovery and development. All plant raw materials underwent an initial phytochemical screening to identify the various plant components [20].

Development of formulation

Cyperus rotundus, Berberis aristata, Terminalia chebula, Emblica officinalis, and Terminalia belerica were all extracted using ethanol, and their medicinal qualities were preserved by freeze drying.Based on their individual drying rates, the extracts were dried for a predetermined amount of time. The lyophiliser was put to good use at the pharmaceutics lab at our institution. Microcrystalline cellulose, magnesium stearate, lactose, and flour are only some of the diluents that were dried [21].

Before adding the diluents and preservatives like sodium methyl paraben and bronopol, the active ingredients were measured out and magnesium carbonate was added as an adsorbent. For thirty minutes, everything was mixed together perfectly. The powder was then placed in the polythene bags, which were labeled and set aside for further analysis [22].

Table 1: Proposed formulation strength

S.no.	Excipients	Qty.
1	*Berberis aristata*	60
2	*Emblica officinalis*	110
3	*Terminalia chebula*	90
4	*Cyperus rotundus*	100
5	*Terminalia belerica*	110

Pre-formulation studies

It is crucial to first determine the drug powder's derived qualities from the drug molecule's physical and chemical properties before formulation. Many of the steps and strategies that follow in formulation development are determined by this data. Preformulation refers to this preliminary stage of education. The end goal is a drug production method that is as efficient as possible. The drug candidate's physiochemical characteristics are analyzed during preformulation [23].

Formulation trial batch development

To get the best flow properties, four experimental batches of capsules were made by varying the excipient concentrations. All four trial batches' blended powder flow properties, including bulk density, tapped density, compressibility index, Hausner's ratio, and angle of repose, were examined. The fourth trial batch was picked for further examination after it was established from the aforementioned trial batches that it demonstrated the best qualities [24].

Table 3: Final Batch

Sr. No.	Components	Trail IV (Mg)
1	*Berberis aristata*	60
2	*Terminalia belerica*	110

3	*Emblica officinalis*	100
4	*Terminalia chebula*	80
5	*Cyperus rotundus*	90
6	Lactose	40
7	Micro crystalline cellulose	20
8	Magnesium Carbonate	10
9	Sodium methyl paraben	0.8
10	Bronopol	0.8
11	Starch paste	Aq

Preparation of capsules

The capsule is widely regarded as the most flexible medicinal dosage form. Capsules are a type of solid pharmaceutical formulation in which one or more active and inactive ingredients are enclosed in a small shell, typically made of gelatin. In the world of capsules, you can find the "hard" kind and the "soft" variety. The hard capsule is typically divided into two separate cylindrical halves, which is why it is often called a "two-piece" capsule.

Pharmacological evaluation

The Institutional Ethical Committee (IEC) granted approval for the procedure of the in vivo investigation conducted on female adult albino Wistar rats.

***In-vitro* anti – diabetic activity**

α-amylase inhibition assay

A concentration of 0.1 mg/mL of -amylase was present in phosphate buffer saline (PBS, 0.02 mol/L, pH 6.8). -amylase solution (0.010 mL) was added to sample solutions (0.25 mL) of different concentrations, and the mixture was incubated at 37 °C for 5 minutes. The reaction was initiated by adding 0.1 mL of a 1.0% (w/v) starch substrate solution to the incubation medium. After 3 minutes of incubation at 37 degrees Celsius, 1 milliliter of DNS reagent (1 percent Dinitrosalicylic acid, 0.05 percent sodium hydroxide, and 1 percent sodium hydroxide solution) was added, and the mixture was boiled for 5 minutes at 100 degrees Celsius, to stop the process. Once the sample had cooled to room temperature, the absorbance (Abs) at 540 nm was determined using a spectrophotometer. To calculate the level of suppression, we utilized the following formula:

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

Where, Abs1=sample and Abs2 = control.

***In vivo* antidiabetic activity**

Acute toxicity study

Since it is a natural remedy, even the highest recommended amount (2000 milligrams per kilogram of body weight) probably wouldn't kill you. As a result, we put all three animals through a single dose-limit test at 2000 milligrams per kilogram of body weight.

Table 4: Methods for Experiments on Animals

Sr. No.	Group	drug	Dose	Animals	Time
1	Group –1	Distilled Water	2ml p.o	6	30
2	Group –2	Polyherbal formulation	200mg /kgp.o	6	30

3	Group – 3	Polyherbal formulation	400mg/kgp.o	6	30
4	Group – 4	Standard (glibenclamide)	0.25mg/kgp.o	6	30

After an overnight fast, rats were injected intraperitoneally (i.p.) with 50 milligrams of freshly produced streptozotocin per kilogram of body weight, followed by 120 milligrams of nicotinamide hydrochloride (NIC) in 0.1 milliliters of citrate buffer (pH 4.5) per kilogram of body weight. After 48 hours of induction, diabetes was validated by monitoring fasting blood glucose levels in the STZ + NIC treated rats.
Rats with fasting blood glucose levels over 200 mg/dl were classified as diabetic and randomly assigned to one of four treatment groups. Both the gold standard (glibenclamide) and the herbal mixture were given orally by gavage once daily for 28 days. All of the experimental animals' weekly weight gains and losses were recorded.

RESULTS AND DISCUSSION

Loss on Drying

The process of determining the loss on drying for the raw materials was conducted. The obtained results and the corresponding standard values are presented in the table.

Table 5: LOD of plant

Sr. No	Plant Name	LOD (% W/W)	Acceptable Limits (W/W %)
1	*Berberis aristata*	2.63±0.28	Not More Than 8
2	*Emblica officinalis*	4.45±0.34	Not More Than 6
3	*Terminalia chebula*	3.79±0.24	Not More Than 8
4	*Cyperus rotundus*	4.38±0.03	Not More Than 5
5	*Terminalia belerica*	4.47±0.08	Not More Than 5

Total ash Content

The raw materials' total ash content was calculated, and the results, along with their allowable ranges, are listed in the table below.

Table 6: Total ash value

Sr. No	Plant Name	Total ash (% w/w)	Limits (w/w %)
1	*Berberis aristata*	4.78±0.37	Not More Than 14
2	*Cyperus rotundus*	6.27±0.03	Not More Than 8
3	*Terminalia chebula*	2.42±0.01	Not More Than 5
4	*Terminalia belerica*	5.08±0.05	Not More Than 7
5	*Emblica officinalis*	5.41±2.64	Not More Than 7

Acid insoluble ash

The entirety of the ash was utilized. The acid insoluble ash concentration of each individual raw material was measured and the findings are presented in a tabular format.

Table 7: Acid insoluble ash

Sr. No	Plant Name	Acid Insoluble Ash (% W/W)	Limits (W/W %)
1	*Terminalia chebula*	0.38±0.01	Not More Than 5
2	*Berberis aristata*	0.37±0.02	Not More Than 5
3	*Cyperus rotundus*	2.16±0.03	Not More Than 4
4	*Terminalia belerica*	0.56±0.02	Not More Than 1
5	*Emblica officinalis*	1.26±0.21	Not More Than 2

Water Soluble Ash

Each individual raw materials ash content and water soluble ash content were assessed, and the results are shown in the table.

Table 8: Water soluble ash

Sr. No	Plant Name	Water Soluble Ash	Limits
1	*Cyperus rotundus*	6.47±0.32	NMT 7
2	*Terminalia belerica*	1.98±0.61	NMT 6
3	*Terminalia chebula*	3.14±0.03	NMT 5
4	*Berberis aristata*	0.81±0.02	NMT 3
5	*Emblica officinalis*	2.14±0.48	NMT 3

Analysis of heavy metal

An analysis was conducted to estimate the presence of heavy metals in the raw materials, and the findings were documented and presented in a tabular format.

Table 9: Heavy metals test

Sr. No.	Plant Name	Results (ppm)			
		Arsenic (NMT5)	Lead (NMT10)	Cadmium (NMT0.3)	Mercury (NMT0.5)
1	*Emblica officinalis*	0.003	0.003	0.002	0.002
2	*Berberis aristata*	0.001	0.002	0.014	0.003
3	*Terminalia chebula*	0.002	0.001	0.004	0.001
4	*Cyperus rotundus*	0.004	0.003	0.003	0.005
5	*Terminalia belerica*	0.002	0.004	0.03	0.03

The analysis of heavy metals in the sample indicated that the concentrations of heavy metals fall within the specified thresholds. The consumption of this substance is considered harmless and does not pose any adverse effects.

Microbial load analysis

Table 10: Microbial load analysis

Sr. No.	Parameters	*Berberis aristata*	Terminalia chebula	Emblica officinalis	Terminalia belerica	Cyperus rotundus
1	Total aerobic count	-	-	100	100	-

2	Yeast and mould count	-	-	-	-	-
3	*E.coli*	Ab	Ab	Ab	Ab	Ab
4	*Salmonella*	Ab	Ab	Ab	Ab	Ab
5	*Pseudomonas*	Ab	Ab	Ab	Ab	Ab

The findings indicate that the powdered raw materials meet the microbial load analysis guidelines set by the World Health Organization (WHO), suggesting that they are suitable for internal consumption due to their safety.

Phytochemical testing

The chemical analyses were conducted to determine the presence of different phytoconstituents in the raw materials. The obtained data were documented and presented in a tabular format.

Table 11: phytochemical testing

Phytoc-constituents	*Berberis aristata*	*Emblica officinalis*	*Cyperus rotundus*	*Terminalia chebula*	*Terminalia belerica*
Phenolic compounds	ab	p	p	p	p
Flavanoids	p	p	p	p	p
Tannins	ab	p	p	p	p
Alkaloids	p	p	p	p	ab
Steroids	p	p	_	p	_
Glycosides	p	p	p	p	p
Saponins	p	_	p	p	p
Proteins	p	p	p	p	p

***In-vivo* Antidiabetic activity**

The Albino Wistar rats were partitioned into four distinct groups, each consisting of six individuals, in the following manner.

Traditional herbal remedies predate all other medical practices. Several age-old methods of using herbs for the diagnosis, prevention, and treatment of illness have gained popularity in recent years. The antidiabetic efficacy of a formulation comprising five raw materials selected after a thorough literature research and then encapsulated in polyherbal capsules was tested in an animal model. The identification, quality, and purity of the herbal raw ingredients were determined using methods recommended by the World Health Organization and the Ayurvedic Pharmacopeia of India. Loss on drying, ash values, and extractive values, among other physiochemical characteristics, were calculated; these data will be used to ensure that pharmaceutical consistency. Alkaloids, steroids, glycosides, flavonoids, phenols, tannins, and terpenoids were found in the raw materials by preliminary phytochemical analysis [24, 25].

Heavy metal analysis and microbiological testing confirmed that the raw ingredients were within the World Health Organization's (WHO) permissible limits. Ethanol was used to extract the coarse powders of the chosen plants. The freeze-dried ethanolic extracts were included into the final product. TLC was used to separate the components of the polyherbal extract from the individual plant extracts. The polyherbal formulation was also subjected to HPTLC fingerprinting, with the resulting chromatogram revealing the presence of peaks corresponding to the various ingredients. In qualitative study of the formulation, the

chromatogram might serve as an index [26].

By creating four separate trial batches (Trial I, II, III, and IV), the dried polyherbal extract was fine-tuned for quality metrics and batch consistency. Preformulation parameters were used to ensure consistency and quality in the testing. All parameters were found to be within acceptable ranges, indicating that the trial IV utilized to create Polyherbal Capsule was effective. Standardization was achieved in the following areas: description, weight uniformity, disintegration time, moisture content, pH, physiochemical parameters, and phytochemical research. Flavonoid, phenol, and tannin levels were all calculated quantitatively as phytoconstituents. According to the World Health Organization's recommendations, the microbiological load and heavy metal analyses of the polyherbal formulation were both within safe parameters [27].

The beta-amylase inhibition assay was used to measure anti-diabetic efficacy in vitro. When compared to regular Acarbose, it has much higher antidiabetic action. According to OECD guidelines 423, the polyherbal capsules were tested for acute toxicity and found to be non-hazardous at doses of up to 2000 mg/Kg. Streptozotocin-induced diabetes was used as an in vivo model to assess the therapeutic efficacy of the designed and standardized polyherbal capsule.

The lipid profile and fasting blood glucose levels were measured. When compared to the normal range before the introduction of diabetes, the formulation exhibited a substantial effect. On day 7, there was a statistically significant drop in blood sugar after taking the polyherbal formulation at a dose of 400 mg/kg. HDL levels were raised while total cholesterol, LDL cholesterol, and triglyceride levels were lowered. The presence of flavonoids was confirmed by the phytochemical analysis. The powerful anti-diabetic effect may stem from this. Stability investigations of the prepared polyherbal capsules and future clinical trials in Human Volunteers are advised [28].

CONCLUSION

The oldest kind of treatment, herbal remedies are used to identify and cure a wide range of illnesses. An animal model was used to assess the antidiabetic potency of five raw materials that were chosen for formulation into polyherbal capsules. The identity, quality, and purity of the raw materials were examined, and physiochemical parameters were calculated. Using heavy metals and microbiological screening, preliminary phytochemical analysis identified a variety of phytoconstituents. The quality and batch consistency of the polyherbal extract were optimized, with trial IV being the best. For description, weight, disintegration time, moisture content, pH, physiochemical parameters, and phytochemical tests, the created capsules underwent standardization. When compared to regular Acarbose, in-vitro anti-diabetic activity was discovered to be significantly higher. An acute toxicity study was conducted, and the formulation significantly affected the parameters of fasting blood glucose and lipid profile. Future use is advised to pursue additional stability research and clinical trials.

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Conflict of Interest

None

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