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Formulation Standardization And Quality Control Of Polyherbal Formulation For Treatment Of Type 2 Diabetes Mellitus

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Diabetes is one example of a multifactorial metabolic condition that can lead to multiple consequences, including immunosuppression, liver toxicity, and hyperlipidemia. Therefore, the combination of herbs is needed for disease treatment rather than single-drug therapy. The composition included herbs obtained from reputed suppliers situated in South India. The raw materials are standardized using shade-dried powdered plant components. All additional compounds utilized in this study were of analytical grade and procured only from approved vendors. The cholesterol profiles and blood glucose levels at rest were checked. When the mixture was compared to the normal range for people with pre-diabetes, it showed a big difference. Statistical analysis proved that giving the polyherbal mixture at a dose of 400 mg/kg lowered blood sugar levels by a large amount on the seventh day. Triglycerides, total cholesterol, and low-density lipoprotein cholesterol all went down, but high-density lipoprotein cholesterol went up. A phytochemical study proved that flavonoids were present. This could be where the strong anti-diabetic benefit comes from. Future clinical studies should include people as volunteers, and the stability of the polyherbal capsules that are being made should be tested. The anti-diabetic effect in

vitro was much stronger than that of standard Acarbose. It was seen that the formulation had a big impact on the measures of fasting blood glucose and lipid profile during an investigation into acute toxicity.

Keywords: Anti-diabetic, toxicity, blood glucose, lipid profile.

INTRODUCTION:

Existing pharmacotherapies are unable to fully correct hyperglycemia, have poor tolerance, and cause adverse effects. Consequently, there is a demand for alternative medicine, particularly from natural plant sources that have low adverse effects. The WHO expert committee on diabetes has advised investigating the effectiveness of these methods in managing the illness [1-3].

In most conventional systems, diabetes is more effectively controlled using a combination of herbs (polyherbal) rather than a single herb due to the synergistic effects and reduced occurrence of adverse effects. There are over two hundred polyherbal formulations available in the market for treating diabetes [3-5]. The majority of these formulations comprise eight or more pharmaceuticals in combination, making them difficult to standardize. As a result, the quality of these products cannot be guaranteed. According to FDA guidelines, it is recommended to have three or fewer ingredients in each combination. Therefore, in this study, the polyherbal formulation was adjusted using lyophilized hydroalcoholic extracts of only three medications [4-6].

The issue related to the utilization of herbal preparations lies in their limited bioavailability. Phytoconstituents such as flavonoids, tannins, and terpenoids have limited absorption due to their significant molecular size. Furthermore, its limited lipid solubility greatly hampers its capacity to traverse lipid-rich cellular membranes. Phospholipid-based vesicles can enhance the therapeutic efficacy, decrease toxicity, and improve the bioavailability of phytomedicines [5-7].

Herbal products like green tea, ginseng, hawthorn, ginkgo biloba, grape seed, and milk thistle have been put inside phytosomes, which are vesicle systems made of phospholipids. The flavonoid and terpenoid parts of these plant products bind strongly to phosphatidylcholine, which lets them stick to it directly [6–8]. Herbal extracts work better than regular herbal extracts because when their ingredients bind to phosphatidylcholine, they make a dosage form that is easier for the body to absorb. The study says that silybin from Silybin phytosomes is about seven times more likely to be absorbed than silybin from regular milk thistle extract. In this study, a new type of diabetes medicine is introduced. It is made from a mix of many herbs that are then put together into a system of phospholipid vesicles [7-14].

MATERIALS AND METHODS:

The composition included herbs obtained from reputed suppliers based in South India. The raw materials are standardized using powdered plant parts that have undergone shade-drying, including Emblica officinalis, Terminalalia belerica, and Cyperus rotundus. All additional compounds utilized in this investigation were of analytical quality and were bought only from approved vendors.

LOD Analysis:

The loss on drying test can be used to figure out how much water and volatile chemicals are

in the raw medicine. It is important to do the loss on drying test when plant ingredients are thought to be able to soak up water from the air. Excessive water addition to herbal products fosters microbial proliferation, fungal growth, insect infestation, and degradation. Recent developments in pharmaceutical technology have made it possible to learn a great deal about a drug's durability and production quality just by looking at its moisture content [8-10].

$$\text{LOD \%} = \frac{\text{Final weight of sample}}{\text{Initial weight of sample}} \text{ x } 100$$

Determination of ash values:

The byproduct generated from the combustion of medications often consists of granular ash. The medicine's composition may include intentionally added inorganic compounds with the purpose of adulterating, contaminating, or substituting it. In any case, the important parts are stable inorganic salts like silica and metallic salts that are already in the medicine and stay with it. It's important to look at home treatments. To find the ash level, you could measure the amount of total ash, acid-insoluble ash, or water-soluble ash. The ash value can also be utilized as a method to quantify the quantity of sulphated residue [10-12].

Phytochemical studies:

The herb has a biosynthetic factory that makes sugars, proteins, lipids, and other chemicals. Another group of substances that are present are flavonoids, alkaloids, glycosides, tannins, and others. To find the active ingredients in medicinal plants and learn about their possible drug effects, phytochemical studies of extracts and raw medicines are very important. These tests can measure and tell the difference between chemicals that have medicinal effects, which can help scientists make new drugs. At first, a phytochemical screening was done on the raw plant samples to find out what kinds of chemicals were in the plants [13-15].

Pre-formulation studies:

Before making the drug powder, it is very important to figure out its qualities by looking at the chemical and physical properties of the drug molecule. This knowledge is very important for figuring out the next steps and strategies in formulation development. Preformulation is the name for the first stage of learning. One of the main goals is to come up with a very efficient way to make medicines. As part of preformulation, the physiochemical properties of the drug option are looked at [16-20].

Formulation Development:

By changing the concentrations of the excipients to get the best flow qualities, four sets of capsules were made. It was studied how the mixed powder poured in all four test batches, looking at its bulk density, tapped density, compressibility index, Hausner's ratio, and angle of repose. The good qualities of the fourth trial batch were confirmed by the other trial batches, so it was chosen to be studied further (table 1) [21-25].

Table 1: Formulation Development Batches

Sr. No.	Components	Quantity
1	Berberis aristata	60

2	Cyperus rotundus	70
3	Terminalia chebula	60
4	Emblica officinalis	100
5	Terminalia belerica	110
6	Lactose	55
7	Magnesium Carbonate	20
8	Micro crystalline cellulose	25
9	Sodium methyl paraben	0.8
11	Starch paste	Aq.

Once the plants Cyperus rotundus, Berberis aristata, Terminalia chebula, Emblica officinalis, and Terminalia belerica were extracted with ethanol, they were freeze dried to keep their therapeutic properties. Each extract was given a certain amount of time to dry, which depended on how quickly it dried. The lyophilizer in the pharmacy lab at our university was used well. Wheat, lactose, magnesium stearate, and microcrystalline cellulose were used as diluents and were dried. The adsorbent that was used was magnesium carbonate, and the active ingredients were checked before sodium methyl paraben and bronopol, which are diluents and stabilizers, were added. Over the course of thirty minutes, the materials were carefully mixed in the best way possible. After that, the material was put into the polythene bags, which were then labeled and kept for further study [26-29].

In-vivo antidiabetic activity:

Due to its inherent properties, it is highly improbable that even the highest recommended dose will result in fatality. Consequently, we performed a single dose-limit test on all three animals, administering a dosage of 2000 milligrams per kilogram of body weight. The rats were deprived of food for one night. The next morning, they were injected with 50 mg/kg of newly made streptozotocin and 120 mg/kg of nicotinamide hydrochloride in a solution that also contained 0.1 mg of citrate buffer [30-35]. Researchers checked the fasting blood glucose levels of rats that were given STZ + NIC 48 hours after being given diabetes to see if they had diabetes. The four treatment groups for diabetic rats were chosen at random based on their fasting blood glucose levels, which were more than 200 mg/dl. For 28 days, both the plant mixture and the gold standard were given by mouth through a gavage. On a weekly basis, the researchers monitored the weight gain or loss of every animal involved in the experiment [36-42].

RESULTS AND DISCUSSION

LOD Analysis:

It was done to find out how much wetness was in the raw materials. The results and standard numbers that go with are all obtained within the accepted limits.

Total ash value analysis:

The amount of ash in the raw materials was measured. The data are shown in the table below, along with the ranges of what is acceptable. The whole quantity of ash was used up. It was measured how much acid-insoluble ash was in each raw material, and the results are shown

in a table. Table 2 shows the findings of the test that measured the amount of ash and water-soluble ash in each raw material.

Table 2: Total ash value

Sr. No	Plant Name	Total ash (% w/w)	Limits (w/w %)
1	Berberis aristata	5.93±0.25	Not More Than 14
2	Terminalia belerica	5.18±0.08	Not More Than 7
3	Cyperus rotundus	4.21±0.05	Not More Than 8
4	Emblica officinalis	5.17±4.66	Not More Than 7
5	Terminalia chebula	4.25±0.03	Not More Than 5

Analysis of heavy metal:

We documented and exhibited the findings of a quantitative test that assessed the presence of high amounts of metallic elements in the unprocessed substances in a tabular format. Heavy metal levels are within the allowed range, according to the sample analysis. This substance is considered harmless and does not cause any harm when consumed.

Table 3: Heavy Metal Analysis

Sr. No.		Obtained Values (PPM)			
		Mercury	**Arsenic**	**Cadmium**	**Lead**
1.	Cyperus rotundus	0.004	0.005	0.004	0.004
2.	Terminalia belerica	0.02	0.003	0.04	0.005
3.	Berberis aristata	0.004	0.002	0.015	0.003
4.	Terminalia chebula	0.002	0.003	0.005	0.002
5.	Emblica officinalis	0.003	0.004	0.003	0.004

In-vivo Antidiabetic activity:

There has been a revival in recent decades of the traditional use of herbs in medicine for diagnostics, treatment, and prevention. The antidiabetic effectiveness of a compound was evaluated using an animal model. After reviewing a great deal of relevant literature, the five basic components that made up the formulation were selected. Following this, the components were placed into polyherbal capsules.

The World Health Organization and the Indian Ayurvedic Pharmacopoeia gave us guidelines on how to find, evaluate, and improve the plant ingredients. To make sure that all of the drugs were the same, data on a number of physiochemical factors was collected. These

included extractive value, ash value, and drying loss. The plant chemicals were first looked at, and they were found to contain terpenoids, phenols, tannins, alkaloids, steroids, glycosides, and flavonoids.

The drugs were found to be safe, following the advice of the World Health Organization (WHO), after microbiological and heavy metal tests were done. Ethanol was used to get rid of the big particles on the plants that were picked. The ethanolic liquids that were freeze-dried were made. Transparent liquid chromatography (TLC) was used to separate the different plant products that make up the polyherbal extract. High-performance thin-layer chromatography was used to make a fingerprint of the polyherbal mix and test it. The resultant chromatogram clearly shows that every component has its own unique peak. Qualitative formulation studies can make use of the chromatogram as an index.

After four separate trial batches, the dried polyherbal extract's quality metrics and batch consistency were fine-tuned. Using preformulation standards, the testing was carried out consistently and to a high standard. The trial intravenous (IV) formulation of Polyherbal Capsules was fruitful since all metrics fell within acceptable ranges. Parameters related to physiochemistry, phytochemistry, disintegration time, weight consistency, moisture level, pH, and description were all standardized. Phytoconstituents such as tannin, flavonoid, and phenol levels were measured quantitatively. Heavy metal and microbiological load evaluations of the polyherbal formulation came back below safe limits, in accordance with WHO recommendations. Based on the results of the acute toxicity evaluation, the polyherbal capsules were shown to be safe for dosages up to 2000 mg/Kg, in compliance with OECD standards.

CONCLUSION:

Herbal treatments, the first kind of medical treatment, are effective against many different diseases. Before being formulated into polyherbal capsules, the antidiabetic 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. The anti-diabetic activity in vitro was shown to be substantially higher than that of ordinary Acarbose.

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Conflict of Interest

None

REFERENCES:

1. Mahajan, S., Chauhan, P., Subramani, S. K., Anand, A., Borole, D., Goswamy, H., & Prasad, G. B. K. S. (2015). Evaluation of “GSPF kwath”: A Gymnema sylvestre-containing polyherbal formulation for the treatment of human type 2 diabetes mellitus. European Journal of Integrative Medicine, 7(3), 303-311.
2. John BIJU, Sulaiman CT, George S and Reddy VRK. (2014). Total phenolics and flavonoids in selected medicinal plants from Kerala. Int. J. Pharm. Pharm. Sci, 6(1), pp.406-408.
3. Patel, M., Thakkar, A., Bhatt, P., Shah, U., Patel, A., Solanki, N., Patel, S., Patel, S., Gandhi, K. and Patel, B. (2023). Prominent targets for cancer care: immunotherapy perspective. Current

Cancer Therapy Reviews, 19(4), 298-317.
4. Pandya, S., Savaliya, C., Thummar, K., Gothwad, A., Panchabhai, T., & Nagore, D. (2023). Validation of standardized polyherbal formulation in the management of type 2 diabetes mellitus: A randomized, double-blind, placebo-controlled trial. Journal of Diabetes & Metabolic Disorders, 22(1), 495-506.
5. Ahmad, Mohd F., Ahmad, S. M., Keservani, Raj K., Pradhan, A. "Anti-Ulcer Activity of Tuber Extracts of Solanum Tuberosum (Solanaceae) In Rats", Acta Fac. Pharm. Univ. Comen. LXII, 2015a (2): 32-37.
6. Patel, P., Shah, D., Bambharoliya, T., Patel, V., Patel, M., Patel, D., Bhavsar, V., Padhiyar, S., Patel, B., Mahavar, A. and Patel, R. (2024). A Review on the Development of Novel Heterocycles as α-Glucosidase Inhibitors for the Treatment of Type-2 Diabetes Mellitus. Medicinal Chemistry, 20(5), 503-536.
7. Ahmad, Mohd F., Keservani, Raj K., Babu, DJM., Interaction study between Ocimum sanctum and Glimepiride (sulfonylurea derivative) in diabetic rats, J. Chin. Pharm. Sci. 2015b, 24 (3), 156–163.
8. Patel, B.A. (2024). Permeation Enhancement and Advanced Strategies: A Comprehensive Review Of Improved Topical Drug Delivery. International Research Journal of Modernization in Engineering Technology and Science, 6(05), 6691-702.
9. Tiwari, G., Rao, DBS., Singh, G., Rao, NGR. (2024). Development and Assessment of Phytosomes for the Treatment of Polycystic Ovarian Syndrome. International Journal of Pharmaceutical Quality Assurance, 15(2), 845-848.
10. Shah, U., Shah, A., Patel, S., Patel, A., Patel, M., Solanki, N., Patel, S., Patel, A., Patel, V. and Patel, B. (2023). Atorvastatin's Reduction of Alzheimer's Disease and Possible Alteration of Cognitive Function in Midlife as well as its Treatment. CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders), 22(10), 1462-1471.
11. Tiwari, G., Singh, S., Ahammed, SYE., Raju, R., Jaiswal, A.A. (2024). Hydrocortisone-loaded Lipid-Polymer Hybrid Nanoparticles for Macrophage Targeted Delivery in Chronic Obstructive Pulmonary Disease. International Journal of Drug Delivery Technology, 14(1), 160-164.
12. Tiwari, R., Tiwari, G., Mishra, S. and Ramachandran, V. (2023). Preventive and Therapeutic Aspects of Migraine for Patient Care: An Insight. Current Molecular Pharmacology, 16(2), 147-160.
13. Kumar T and Chandrashekar KS. (2011). Bauhinia purpurea Linn: A review of its ethnobotany, phytochemical and pharmacological profile. J. Med. Plant Res. 5(4), pp.420-431.
14. Shweta M, Shivshankar R and Vaghela DB. (2020). Shelf life evaluation of Laghu Sutashekhara Rasa–a preliminary assessment. J Ayurveda Integr Med. 11(3), pp.213-216.
15. Raut NA and Gaikwad NJ. (2006). Antidiabetic activity of hydro-ethanolic extract of Cyperus rotundus in alloxan induced diabetes in rats. Fitoterapia, 77(7-8), pp.585-588.
16. Reddy KR, Reddy VJ and Palagati S. (2022). An Overview of Lipid Nanoparticles for Drug Targeting. J.adv.med.med.res. Vol. 7, pp.1-8.
17. Rane, Bhushan R., Bharath, Mounika S., Patil, Rutuja R., Keservani, Raj K., Jain, Ashish S. (2020b). Novel Approaches in Nutraceuticals, In: Enhancing the Therapeutic Efficacy of Herbal Formulations through Novel Drug Delivery Systems Edited by Rajesh Kumar Kesharwani, Raj K. Keservani, Anil K. Sharma, IGI Global International Publisher, Pennsylvania, USA, Chapter 11, 241-266. ISBN13: 9781799844532.
18. Tiwari, R., Tiwari, G., Sharma, S. and Ramachandran, V. (2023). An Exploration of herbal extracts loaded phyto-phospholipid complexes (Phytosomes) against polycystic ovarian syndrome: Formulation considerations. Pharmaceutical Nanotechnology, 11(1), 44-55.
19. Vyas, N., Keservani, R. K., Nayak, A., Jain, S., &Singhal, M. (2010). Effect of Tamarindus Indica and Curcuma Longa on stress induced Alopecia. Pharmacologyonline, 1, 377-384.
20. Tiwari, G., Singh, G., Shekhar, R. and Tiwari, R. (2022). Development and qualitative evaluation

of periodontal gel containing an antibacterial agent for periodontal disease. Research Journal of Pharmacy and Technology, 15(11), 5225-5231.
21. Talele SG, Ahire ED, Talele GS and Derle DV. (2021). An Innovative Approach as Self-Emulsifying Drug Delivery System for Phytoconstituents. In Enhancing the Therapeutic Efficacy of Herbal Formulations (pp. 69-84). IGI Global.
22. Ahire ED, Sonawane VN, Surana KR, Talele SG, Talele GS, Kshirsagar SJ, Khairnar SJ, Thombre NA and Mahajan SK. (2023). Preventive Measures of Type 2 Diabetes via Nutrition. In The Metabolic Syndrome (pp. 71-99). Apple Academic Press.
23. Kaul S and Dwivedi S. (2010). Indigeneous ayurvedic knowledge of some species in the treatment of human disease and disorders. Int. j. pharm. life sci, 1(1), pp.44-49.
24. Rizwan M, Khan A, Nasir H, Javed A and Shah SZ. (2017). Phytochemical and biological screening of Berberis aristata. Adv. Life Sci. 5(1), pp.01-07.
25. Choudhary N and Sekhon BS. (2011). An overview of advances in the standardization of herbal drugs. J. Pharma. Edu. Res. 2(2), p.55.
26. Khemuka N. (2014). Preliminary Pharmacognostical and Phytochemical Evaluation of Prashasta and Aprashasta Haritaki (Terminalia Chebula Retz.) Research Article Nidhi Khemuka, Harisha CR 2, Shukla VJ 3, Galib R 4, Patgiri BJ 5, Prajapati PK 6. Int. j. Ayurvedic med.5(3), pp.257-268.
27. Reddy KR, Satyanarayana SV and Reddy VJ. (2019). Factorial design based optimization and in-vitro ex-vivo evaluation of clobetasol loaded nano structured lipid carriers. Int. J Pharm. Sci. Res, 10(9), pp.4374-83.
28. Ramesh Reddy K, Jayashankar Reddy V. (2019). Clobetasol-loaded dermal Nanostructured lipid carriers for the treatment of imiquimod induced psoriasis in mice. Asian J. Pharm. 13(4): 71- 82.
29. Sabu MC, Kuttan R. (2009). Antidiabetic and antioxidant activity of Terminalia belerica.Roxb. Indian J. Exp. Biol. 47(4): 270-275
30. Kumar GPS, Arulselvan P, Kumar DS and Subramanian SP. (2006). Anti-diabetic activity of fruits of Terminalia chebula on streptozotocin induced diabetic rats. J. health sci. 52(3), pp.283-291.
31. Bhutani KK. (2003). Herbal medicines an enigma and challenge to science and directions for new initiatives. Indian J Nat Prod, 19(1), pp.3-8.
32. Groop L and Pociot F. (2014). Genetics of diabetes–are we missing the genes or the disease? Mol. Cell. Endocrinol. 382(1), pp.726-739.
33. Kumudhaveni B and Radha R. (2017). Anti-diabetic potential of a traditional Polyherbal formulation-A review. Res. J. Pharm. Techno. 10(6), pp.1865-1869.
34. Rao TM and Chandira M. (2020). A Study on Antioxidant Activity of Three Ethanolic Extract Polyherbal Formulation. Int. J. Pharma. Res. Health Sci. 8(3), pp.3155-60.
35. Shelke DP, Shendarkar GR, Daswad AK, Pohare JG, Wangawar SN and Roge AB. (2017). Design and development of polyherbal formulation with antioxidant potential. J. Med. Plants Stud. 5(2), pp.207-213.
36. Sukalingam, K., Ganesan, K., & Ponnusamy, K. (2015). Evaluation of antidiabetic activity of polyherbal formulations on type 2 diabetic patients: A single blinded randomized study. Int. J. Intg. Med. Sci, 2, 90-98.
37. Tiwari G, Gupta M, Devhare LD, Tiwari R. Therapeutic and Phytochemical Properties of Thymoquinone Derived from Nigella Sativa. Current Drug Research Reviews Formerly: Current Drug Abuse Reviews. 2024 Jul 1;16(2):145-56.
38. Tiwari R, Khatri C, Tyagi LK, Tiwari G. Expanded Therapeutic Applications of Holarrhena Antidysenterica: A Review. Combinatorial Chemistry & High Throughput Screening. 2024 Jun 1;27(9):1257-75.
39. Tiwari G, Tiwari R, Kaur A. Pharmaceutical Considerations of Translabial Formulations for Treatment of Parkinson's Disease: A Concept of Drug Delivery for Unconscious Patients.

Current Drug Delivery. 2023 Oct 1;20(8):1163-75.
40. Tiwari R, Tiwari G, Parashar P. Theranostics Applications of Functionalized Magnetic Nanoparticles. In Multifunctional and Targeted Theranostic Nanomedicines: Formulation, Design And Applications 2023 Aug 24 (pp. 361-382). Singapore: Springer Nature Singapore.
41. Tiwari R, Mishra J, Devhare LD, Tiwari G. An updated review on recent developments and applications of fish collagen. Pharma Times. 2023 Jun;55(06):28.
42. Bisht, A., Dwivedi, H., & Rawat, A. K. S. (2021). Development and Evaluation of Polyherbal Formulation for Diabetes. Asian Pac. J. Health Sci, 8(4), 48-54.