Research Protocol

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Ayurveda Section

Exploring the Anti Diabetic Efficacy of *Trikatvadi Guggulu* through Pharmaceutical and Analytical Approaches: An In-vitro Research Study Protocol

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ABSTRACT

Introduction: Diabetes mellitus is a metabolic disorder characterised by high blood glucose levels resulting from inadequate insulin synthesis by the pancreas or the body's inability to respond to insulin. *Prameha* can be translated as *Prabhutmutrata* (polyuria) or *Avilmutrata* (turbid urine). It is a *Tridoshaja*, *Kapha*-dominant illness. *Acharya Charak*, *Sushrut*, and *Vagbhata* classify it under the *Ashtamahagada* category. There are 20 types of *Pramehas* in Ayurveda. Four *Vataja Pramehas*, six *Pittaja Pramehas*, and ten *Kaphaja Pramehas* are classified based on their *Dosha* dominance. *Madhumeha* is one of the varieties of *Vataja Prameha*. *Trikatvadi Guggulu* is an important herbal formulation mentioned in *Vaidya chintaamani*. It has a high therapeutic value, and its contents *Trikatu*, *Triphala*, *Musta*, and *Guggulu*. The liquid medium used for *Bhavana* is *Gokshura Kashaya*. It is traditionally indicated in *Prameha*, *Vata Vyadhi*, *Mutraghata*, *Mutradosha*, etc.

Need of the study: Diabetes mellitus is a global health burden with increasing prevalence and limited curative options. Current modern treatments primarily focus on glycaemic control and symptom management, but they often have adverse effects, emphasising the need for safer and more effective therapeutic alternatives. In Ayurvedic literature, *Prameha*, which closely resembles diabetes, is described as a chronic and difficult-to-treat disorder that requires holistic management. *Trikatvadi Guggulu*, an Ayurvedic polyherbal formulation, is traditionally indicated for *Prameha* and related urinary disorders. Although its ingredients-*Trikatu*, *Triphala*, *Musta*, *Guggulu*, and *Gokshura*-are known to possess antidiabetic properties, the compound formulation itself lacks adequate evaluation through standardised in-vitro assays. Thus, there is a pressing need to validate the antidiabetic potential of *Trikatvadi Guggulu* through modern scientific methods. The present study will focus on filling that gap by conducting a detailed pharmaceutical, analytical, and in-vitro assessment of *Trikatvadi Guggulu*, thereby contributing valuable evidence toward its therapeutic efficacy and supporting its standardisation for future clinical applications.

Aim: To evaluate the pharmaceutical and analytical characteristics of *Trikatvadi Guggulu* and to assess its in-vitro pharmacodynamic potential with respect to antidiabetic activity.

Materials and Methods: The present experimental analytical in-vitro study will be conducted at the Central Research Laboratory of Jawaharlal Nehru Medical College, Datta Meghe Institute of Higher Education and Research, Sawangi (Meghe), Wardha, Maharashtra, India, from July 2025 to November 2025. The raw materials required for the preparation of *Trikatvadi Guggulu* will be procured from *Dattatraya Ayurved Rasashala*, Wardha, and the formulation will be prepared in the Department of *Rasa Shastra* and *Bhaishajya Kalpana*, Mahatma Gandhi Ayurved College Hospital and Research Centre, Salod (H), Wardha, India. Organoleptic and physicochemical parameters will be evaluated. The antidiabetic effect of *Trikatvadi Guggulu* will be assessed using established in-vitro methodologies. One-way Analysis of Variance (ANOVA) will be employed for statistical analysis of the in-vitro results. A p-value of <0.05 will be considered statistically significant.

Keywords: Diabetes mellitus, Organoleptic, *Prameha, Vati,* Urinary tract disorders

INTRODUCTION

Ayurveda is India's oldest and best-known medical system. It is a more than 5000 years that may effectively treat a wide range of ailments. The term "Ayurveda" is comprised of two words: "Ayu" (life) and "ved" (knowledge or science), thus representing the complete Science of Life. Ayurveda provides a comprehensive and analytical approach to health and longevity [1].

Bheshajya, the Ayurvedic weapon, is a natural treatment provided by nature to prevent the spread of hazardous diseases. Pharmaceutical Science (*Bhaishajya Kalpana*) studies the *Samskara* (processing) applied to a drug (*Bheshajya*) [2]. The word medicine (*Bheshajya*) is originally defined as *Aushadh*. *Bhaishajya* is a *Chikitsa Chatushpada* that alleviates disease or restores health by stabilising the *Doshas*.

The term *Prameha* consists of two parts: "*Pra*" meaning abundant, and "*Meha*" meaning the passage of large quantities of urine. *Prameha* refers to either polyuria or turbid urine. It is a *Tridoshaja* and *Kapha*-dominant disease [3]. *Acharya Charak*, *Sushrut*, and *Vagbhata* classify it under the *Ashtamahagada* category [4]. There are 20 types of *Prameha*: four *Vataja*, six *Pittaja*, and ten *Kaphaja* types, classified according to *Dosha* dominance. *Madhumeha* is considered one of *Vataja Pramehas* [5,6].

Diabetes Mellitus Type 2 is characterised by elevated blood sugar due to insulin deficiency or resistance [7]. Lifestyle changes, lack of exercise, poor diet, and sedentary behaviour are major risk factors [8]. Diabetes mellitus is a major health issue in the 21st century. India ranks among the top five countries with the highest diabetic population, earning the title "Diabetes Capital of the World." Diabetes, is known as the silent killer, poses significant risk to communities and healthcare resources worldwide, and if left untreated, it can result in a variety of health complications, including death. Currently, more than 62 million people in India suffer from diabetes [9]. According to the International Diabetes Federation (IDF), 415 million adults worldwide suffer diabetes, and this number is expected to rise to 642 million by 2040 [10]. Globally, an estimated 8.8% of adults, or 415 million people, have diabetes [11].

REVIEW OF LITERATURE

In Ayurveda, *Prameha* is classified as a *Mahagada* (incurable or poor prognosis disorder) and is characterised by increased frequency and altered turbidity of urine. Based on its symptoms, diabetes mellitus can be correlated with *Prameha*. Major contributing factors include a sedentary lifestyle, excessive sleep, a diet high in curds, meat soups from aquatic, marsh, or domesticated animals, milk and its derivatives, newly harvested meals, jaggery preparations, and other *Kapha*-aggravating influences [12].

The study by Shekhar C et al., concluded that the therapeutic effi cacy as well as inbuilt potential of a commonly used preparation *Triphala* demonstrates therapeutic efficacy in various ailments, in a very effectively way but its antidiabetic potential [13]. Chhabra V et al., concluded that the multi-target and multi-pathway approach of *Trikatu*, suggesting further pharmacokinetics/Pharmacodynamics studies [14]. Singh P et al., demonstrated the use of *Cyperus rotundus* in traditional Indian medicine for diabetes management [15].

Islam WU et al., reported that Myrrhanol-B and Myrrhanone-B from *Commiphora mukul* exhibit potent in-vivo antidiabetic and antihyperlipidemic effects by normalising cholesterol and triacylglycerol levels. In-silico docking studies predicted strong inhibition of α-glucosidase via binding to active site residues, and in-vitro assays confirmed excellent α-glucosidase inhibition (<20 μM). Kinetic experiments revealed mixed inhibition for Myrrhanol-B and competitive inhibition for Myrrhanone-B [16].

The aim of the present study is a pharmaceutical and analytical study of *Trikatvadi Guggulu* and assessment of its in-vitro pharmacodynamic potential with respect to antidiabetic activity.

Primary objectives:

1. To evaluate glucose uptake in 3T3-L1 cell lines treated with *Trikatvadi Guggulu*.
2. To assess insulin secretion in 3T3-L1 cell lines treated with *Trikatvadi Guggulu*.
3. To evaluate the enzyme inhibition assay (Alpha-Amylase) by *Trikatvadi Guggulu* in 3T3-L1 cell line.
4. To evaluate the enzyme inhibition assay (Alpha-Glucosidase) by *Trikatvadi Guggulu* in 3T3-L1 cell line.

Secondary objectives:

1. To develop standard operating procedure for *Trikatvadi Guggulu*.
2. To analyse *Trikatvadi Guggulu* on different analytical parameters.

Null hypothesis (H0): *Trikatvadi Guggulu* has no significant effect on antidiabetic parameters in the 3T3-L1 cell line.

Alternate hypothesis (H1): *Trikatvadi Guggulu* exhibits a significant effect on antidiabetic parameters in the 3T3-L1 cell line.

MATERIALS AND METHODS

The present experimental analytical in-vitro study will be conducted at the Central Research Laboratory of Jawaharlal Nehru Medical College, Datta Meghe Institute of Higher Education and Research, Sawangi (Meghe), Wardha, Maharashtra, India, from July 2025 to November 2025. The ethical approval was obtained from the Institutional Ethics Committee (IEC) (MGACHRC/IEC/Jun-2024/852).

Drug preparation: Ingredients required for drug preparation will be procured from *Dattatraya Ayurved Rasashala* and authenticated by the Department of *Dravyaguna*, MGACH&RC, Wardha.

The purification of *Guggulu* (*Shodhana* in Ayurveda) is a meticulous process aimed at detoxifying and enhancing therapeutic properties. Initially, raw *Guggulu* is examined to remove physical impurities such as sand, stones, or glass fragments. The cleaned *Guggulu* is then broken into smaller pieces and placed into a cloth bundle (*pottali*). The *pottali* is suspended in a vessel containing *Triphala Kashaya* (*Triphala* decoction) and boiled using the *Dola Yantra* apparatus until the *Guggulu* softens and dissolves. The mixture is filtered to separate the dissolved *Guggulu* from the residual impurities, then boiled further to evaporate the liquid, leaving behind purified *Guggulu*. The purified *Guggulu* is then dried and ground into a fine powder using a morter and pestle. This traditional method ensures the removal of toxins and enhances the efficacy of *Guggulu* in various Ayurvedic formulations [17].

To prepare *Trikatvadi Guggulu Vati*, equal quantities of fine *churna* of *Triphala*, *Trikatu*, *Musta*, and purified *Guggulu* are mixed thoroughly in a *Khalva Yantra*. *Gokshura Kashaya* is gradually added, and the entire blend is triturated (*Bhavana*) for approximately three hours to ensure uniform absorption. The sticky mixture is then rolled into *Vati* of the desired size, dried, and stored in airtight containers to maintain their efficacy and prevent moisture absorption [18].

Ingredients of *Trikatvadi Guggulu* are listed in [Table/Fig-1].

S. No.	Ingredients	Proportion	Part used
1	*Trikatu (Pippali, Marich, Sunthi)*	1 part each	Fruit
2	*Triphala(Amalaki, Haritaki, Bibhitaki)*	1 part each	Fruit
3	*Musta*	1 part each	Rhizome
4	*Guggulu*	1 part each	Exudate
5	*Goksurabhavana Dravya*	Q.S	Root

[Table/Fig-1]: Ingredients of *Trikatvadi Guggulu*.

Properties of drugs: A thorough understanding of drug properties is fundamental for optimising therapeutic outcomes while minimising risks to patient health. The properties of *Trikatvadi Guggulu* drugs are explained in [Table/Fig-2], and properties of *Bhavana Dravya* (*Gokshura*) are presented in [Table/Fig-3] [19].

Method of Anti-diabetic Cell Line Preparation

1. **Cell line selection:** Choose an appropriate cell line representative of the disease process. For example, in the case of diabetes, pancreatic beta cells (which produce insulin) or hepatocytes (which take up glucose from the blood) may be used.
2. **Preparation of test compounds:** Prepare the test compounds to be evaluated for antidiabetic activity. This may involve dissolving the compounds in a suitable solvent or preparing extracts from natural products.
3. **Cell culture:** Culture the cells under appropriate conditions, typically in a humidified incubator at 37°C with 5% CO_2.
4. **Treatment of cells:** Treat the cells with the test compounds at different concentrations. Include a control group that is not treated with any compound.
5. **Assessment of antidiabetic activity:** After treatment, evaluate the antidiabetic activity by measuring parameters including glucose uptake (indicating the efficiency of cellular glucose absorption), insulin secretion (reflecting the capacity of cells to produce insulin), and cell viability (demonstrating overall cellular health and functionality).

Primary outcome: *Trikatvadi Guggulu* exhibited significant antidiabetic activity in 3T3-L1 adipocyte cells, demonstrated by enhanced glucose uptake, stimulated insulin secretion, and notable inhibition of α-amylase and α-glucosidase enzymes.

Secondary outcome: *Trikatvadi Guggulu* will be prepared and standard operating procedure will be determined from the present study. Quality assessment parameters can be derived, which will aid further research studies.

A. Organoleptic characteristics:

1. Touch

S. No.	Drug (Common name)	*Rasa* (Taste)	*Guna* (Qualities)	*Virya* (potency)	*Vipaka* (Post-digestive effect)	*Karma* (Therapeutic action)
1	*Haritaki* (*Terminalia Chebula* Retz.)	*Pancharasa*(all tastes except *Lavana*)	*Laghu* (light), *Ruksha* (dry)	*Ushna* (hot)	*Madhura* (sweet)	*Tridoshahara*(balances all threedoshas)
2	*Bibhitaki* (*Terminalia bellirica* Roxb.)	*Kashaya* (astringent)	*Laghu* (light), *Ruksha* (dry)	*Ushna* (hot)	*Madhura* (sweet)	*Kapha-Pittahara* (balances *Kapha* and *Pitta*)
3	*Amalaki* (*Emblica officinalis* Linn.)	*Pancharasa* (excluding *Lavana*) *Amla* dominant	*Guru* (heavy), *Ruksha* (dry), *Sheeta* (cold)	*Sheeta* (cold)	*Madhura* (sweet)	*Tridoshahara* (balances all three *doshas*)
4	*Marich* (*Piper nigrum* Linn.)	*Katu* (pungent)	*Laghu* (light), *Tikshna* (sharp)	*Ushna* (hot)	*Katu* (pungent)	*Dipana* (appetiser), *Pachana* (digestive)
5	*Pippali* (*Piper longum* Linn.)	*Katu* (pungent)	*Laghu* (light), *Snigdha* (unctuous), *Tikshna* (sharp)	*AnushnaSheeta* (moderately cold)	*Madhura* (sweet)	*Tridoshahara* (balances all three doshas)
6	*Shunthi* (*Zingiber officinale*)	*Katu* (pungent)	*Laghu* (light), *Snigdha* (unctuous)	*Ushna* (hot)	*Madhura* (sweet)	*Amapachana* (digests toxins), *Vata-Kaphahara* (balances *Vata* and *Kapha)*
7	*Musta* (*Cyperus rotundus* Linn.)	*Tikta* (bitter), *Katu* (pungent), *Kashaya* (astringent)	*Laghu* (light), *Ruksha* (dry)	*Sheeta* (cold)	*Katu* (pungent)	*Dipana* (appetiser), *Pachana* (digestive)
8	*Guggulu* (*Commiphora wightii* (Arn.)Bhandari)	*Tikta* (bitter), *Katu* (pungent)	*Laghu* (light), *Ruksha* (dry)	*Ushna* (hot)	*Katu* (pungent)	*Medohara* (reduces fat), *Vatabalasaji* (strengthens Vata)

[Table/Fig-2]: Properties of *Trikatvadi Guggulu* [19].

S. No.	*Dravya* (Common name)	*Rasa* (Taste)	*Guna* (Qualities)	*Virya* (Potency)	*Vipaka* (Post-digestive effect)	*Karma* (Therapeutic actions)
1.	Gokshura (*Tribulus terrestris*)	*Madhura* (Sweet)	*Guru* (Heavy), *Snigdha* (Unctuous)	*Sheeta* (Cold)	*Madhura* (Sweet)	*Vatahara* (Alleviates Vata), *Kaphahara* (Alleviates *Kapha*), *Mutrala* (Diuretic), *Vrishya* (Aphrodisiac), *Rasayana* (Rejuvenative), *Hridya* (Cardiotonic), *Deepana* (Digestive Stimulant)

[Table/Fig-3]: Properties of *Bhavana Dravya* [19].

2. Appearance
3. Taste
4. Colour

B. Physicochemical parameters:

1. High-Performance Thin-Layer Chromatography (HPTLC)
2. Potential of Hydrogen (pH) Value
3. Loss of drying
4. Total ash
5. Acid-insoluble ash
6. Water-soluble extractive
7. Alcohol-soluble extractive
8. Disintegration time
9. Friability
10. Hardness

STATISTICAL ANALYSIS

All experimental results will be performed using Statistical Package for the Social Sciences (SPSS) software version 17. One-way ANOVA will be applied to the data. The p-value of <0.05 will be considered statistically significant.

Protocol amendments: There are no plans to notify significant protocol changes (e.g., changes in drugs, outcomes, or analysis) to relevant parties (e.g., investigators, journals, and regulators).

Declaration of interests: Principal investigators overseeing the entire trial and at each study site will transparently disclose any financial or other competing interests that could potentially influence the present study's integrity or interpretation of results.

Data availability: Access to the final trial dataset will be restricted to authorised personnel directly involved in the present study. Any existing contractual agreements limiting access for investigators will be openly disclosed.

Dissemination: The present protocol will be published as a thesis to share the research on anti-diabetic effect of *Trikatvadi Guggulu*. The thesis will provide a thorough overview of the present study's design, methodology, data gathering methods, data analysis plan, and ethical considerations. By making this protocol public, the authors aim to expand knowledge in the field and promote future research.

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