

Pharmaceutico-analytical Study of *Dhananjayadi Vati* and Assessment of its Antihistaminic Activity: A Research Protocol

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ABSTRACT

Introduction: Histamine is the local hormone synthesised by mast cells in the tissue and basophils in the blood. Histamine and its Receptors (H1R-H4R) have a substantial impact on the onset of many allergic diseases. Allergic diseases, including asthma, pruritus, atopic dermatitis and allergic rhinitis, result from the complex interaction of inflammatory cells like basophils, mast cells, lymphocytes and dendritic cells responding to various environmental and allergic stimuli.

Need of the study: An antihistamine is a medication that works against histamines to relieve allergy symptoms. These medications help to address conditions triggered by histamine in the body. However, some non steroidal antihistaminic drugs are proven to be quick-acting but are associated with potential side-effects, such as organ damage. In Ayurveda, several medicinal preparations have been mentioned to combat the allergic condition and the *Dhananjayadi* tablet (*Vati*) might also be significantly effective in allergic conditions.

Aim: Pharmaceutico-analytical evaluation of *Dhananjayadi Vati* and assessment of its antihistaminic activity.

Materials and Methods: An in-vitro study will be conducted in the Department of *Dravyaguna* at Mahatma Gandhi Ayurved College Hospital Research Centre, Salod (H) Wardha, Maharashtra, India from August 2024 to March 2025. All herbal drugs will be collected, verified and primarily authenticated by the Department of *Dravyaguna* and will be prepared as per the references. Organoleptic parameters (touch, appearance, taste, odour) and physicochemical parameters (hardness, pH value, uniformity of weight, loss of drying at 105°C, total ash, acid-insoluble ash, water-soluble extractive values, alcohol-soluble extractive values, disintegration time, friability and High-Performance Thin-Layer Chromatography (HPTLC) of the drug) will be evaluated. The antihistaminic action of *Dhananjayadi* tablet (*Vati*) will be evaluated in-vitro while assessing the proportion of contraction of goat tracheal tissue. A paired t-test will be conducted for pre- and postintervention evaluation and a p-value <0.001 will be considered statistically significant.

Keywords: Antiallergic, Histamine, In-vitro study, Kasa, Physico-chemical

INTRODUCTION

Ayurveda is India's oldest and most well-known medical system. It is an ancient method with a history of more than 5,000 years and can successfully treat a wide range of illnesses [1]. Ayurveda, as a whole, represents the "Science of Life." It is a qualitative, all-encompassing approach to wellbeing and longevity [2].

Ayurvedic pharmaceutics (*Bhaishajya Kalpana*) is a science that involves applying various procedures (*Sanskars*) over medicine (*Bheshaja*). Medicine (*Bheshaja* or *Bhaishajya*) is one among the four pillars of treatment (*Chikitsa Chatushpada*) that eliminates the dread of disease or restores a person's health by stabilising the dosha and without which disease suppression is impossible. The term *Kalpana* refers to *Yojana* (Planning), which involves utilising various substances. Therefore, *Kalpana* is the process or modification through which a substance is converted into a medicinal form [3]. The medications require processing, which can be accomplished using the standard five pharmaceutical procedures (*Panchavidh Kashaya Kalpana*), namely Juice (*Swaras*), Paste (*Kalka*), Decoction (*Kwath*), Cold infusion (*Hima*) and Hot infusion (*Phant*) [4].

The pharmaceuticals must undergo processing before they can be used, which can be achieved using pharmaceutical techniques. Many other formulations have been developed to improve taste, extend shelf life, reduce dosage, ensure rapid effectiveness and make handling and dispensing easier [5]. Researchers have adapted the formulas to meet current requirements without compromising

their effectiveness or the aforementioned advantages, but it is crucial to demonstrate their usefulness in modern times [6].

'*Vati Kalpana*' is a significant secondary preparation (*Anukalpana*) in Ayurveda pharmaceutics. A tablet (*Vati*) is made from powdered medication, which is then mixed with liquid and shaped into a circular mass. This is the most widely produced and sold dosage form in the pharmaceutical world across all medication systems. It is prepared from herbal, herbs-mineral, or mineral drugs. The tablet (*Vati Kalpana*) is a commonly used form of medication due to its benefits, such as easy swallowing without irritation, ease of transportation and accurate dosing [7,8].

The word processing (*Sanskars*) is defined as "Samskaro hi Gunaantradhyanam," which means that the improvement in inherent characteristics of a drug is achieved by integrating specific qualities, resulting in a qualitative enhancement of its natural properties. One of the formulations listed in *Yogaratnakar* in the *Kasa Rogadhikara* is the *Dhananjayadi* tablet (*Vati*). This formulation contains *Terminalia arjuna* Roxb. (*Arjuna*), *Cinnamomum zeylanicum* Breyn. (*Twak*), *Elettaria cardamomum* Maton (*Ela*), *Cinnamomum tamala* Nees (*Patra*), *Piper longum* Linn. (*Pippalimoola*), *Zingiber officinalis* (Sunthi), *Piper nigrum* Linn. (*Maricha*) and *Piper longum* Linn. (*Pippali*) and is used to cure *Kasa* [9].

Cough (*Kasa*) is one of the pathological conditions covered in Ayurvedic literature. The *Kapha* and *Vata* doshas are the two main doshas that cause the illness known as cough (*Kasa*). Increased

Kapha forms a sort of mucus coating in the throat (*Upalepa*) in the respiratory system (*Pranavaha Srotas*), obstructing the normal pathway of air (*Vayu*) and leading to its vitiation. Cough (*Kasa*) may develop as an independent disease, with symptoms associated with other diseases and complications (*Upadra*) of the disease [10]. Early intervention is necessary to treat cough (*Kasa*); untreated cough (*Kasa*) can lead to dyspnoea (*Shwas*), emaciation (*Kshaya*) and sunken voice (*Swarasada*). Cough is one of the prevalent health issues, being the foremost commonly encountered symptom for which patients seek medical care [11]. It can happen due to secretions, irritants, foreign particles and microorganisms. Coughing can arise from a range of reasons, which encompass respiratory tract infections such as pneumonia, acute bronchitis and common cold, as well as lifestyle factors like smoking and health conditions like asthma and tuberculosis.

Antihistamines are commonly used to relieve allergy symptoms and are effective in managing conditions triggered by excess histamine, which is a compound produced by the body's immune system. However, some non-steroidal antihistamines have been shown to act quickly but may cause adverse effects, such as organ damage [12]. *Dhananjayadi Vati* is a formulation mentioned in the *Yogaratnakara* text under *Kasa Chikitsa*. It consists of eight ingredients in equal proportions, with *Ardraka Swarasa* used as the *Bhavana Dravya* and it is prepared using the *Niragni* method [13]. There is a continuous need to explore and expand therapeutic options beyond the current ones. The standard operating procedures for preparing *Dhananjayadi* tablets (*Vati*), along with their quality assurance parameters and pharmacodynamics, have not yet been established. Therefore, this study aims to conduct a pharmaceutical-analytical investigation of *Dhananjayadi Vati* and assess its antihistamine activity.

Primary objective:

1. To prepare *Dhananjayadi* tablet (*Vati*) according to classical reference and to develop a standard operating procedure for manufacturing *Dhananjayadi Vati*.
2. To analyse the *Dhananjayadi* tablet (*Vati*) for quality assurance.

Secondary objective:

1. In-vitro assessment of the antihistaminic activity of the *Dhananjayadi* tablet (*Vati*).

Null hypothesis: The *Dhananjayadi* tablet (*Vati*) will not demonstrate significant antihistaminic activity.

Alternate hypothesis: *Dhananjayadi* tablet (*Vati*) will demonstrate a significant antihistaminic activity.

REVIEW OF LITERATURE

Vati Kalpana plays a crucial role in Ayurvedic pharmaceutics due to its various advantages. *Dhananjayadi Vati* is one such formulation referenced in the *Kasa Chikitsa Adhyaya of Yogaratnakara* [7,8].

In a study by Mudukannanavara S et al., the traditional *Dhananjayadi Vati* formulation was modified into *Dhananjayadi Vati* lozenges for added convenience. The analysis of *Dhananjayadi Vati* and its lozenges showed that both met standards, each having less than 1.0% w/w of certain components, indicating they can handle stress well. However, the weight consistency was poor. *Dhananjayadi Vati* contained 8.39% w/w total ash, compared to 0.39% w/w in the lozenges, meaning it has more inorganic compounds. *Dhananjayadi Vati* is made by mixing powdered ingredients with ginger juice and drying them, while lozenges combine caramel syrup with decoction and powders. The acid-insoluble ash was low (0.07% for *Vati* and 0% for lozenges), indicating few indigestible substances. Both had a pH of 6.0, showing mild acidity. The water-soluble extract was 64.5% for *Vati* and 92.5% for lozenges, suggesting more active ingredients in the lozenges. The alcohol-soluble extracts were 5.46% in *Vati* and 2.28% in lozenges, indicating more phytochemicals in the *Vati*. HPTLC showed eight spots for *Vati* and three for lozenges at 254 nm, with potential ellagic acid present. Microbial contamination was "too numerous to count" for *Vati* and 50 for lozenges, which suggests better safety for the lozenges due to their preparation process. Phytochemical screening found alkaloids, resin, carbohydrates and tannins in both, with steroids and coumarins in *Vati* and flavonoids and terpenoids in lozenges. This analysis highlights the differences and potential benefits of each formulation. Since there are no established standards for *Dhananjayadi Vati* and its lozenges, the findings from this study serve as a reference for future evaluation of the product [13].

MATERIALS AND METHODS

An in-vitro study will be conducted in the Department of *Dravyaguna* at Mahatma Gandhi Ayurved College Hospital Research Centre, Salod (H), Wardha, Maharashtra, India from August 2024 to March 2025. IEC no. MGACHRC/IEC/July-2022/559. The drug will be procured from Dattatraya Ayurveda Rasashala and Herbal Garden at MGACH and RC Salod (H), Wardha. Standardisation, authentication and verification of drugs will be done by taxonomists.

Drug Review

Dhananjayadi Vati contains *Arjuna tvaka*, *Tvak*, *Ela*, *Patra*, *Pippali*, *Sunthi*, *Maricha* and *Pippalimoola*, as shown in [Table/Fig-1] [14-19].

S. No.	Dravya (material)	Rasa (taste)	Guna (quality)	Veerya (potency)	Vipaka (bio-transfor medrasa)	Karma (action)	Activity	Proportion and part used
1.	<i>Terminalia arjuna</i> Roxb. (<i>Arjuna</i>) [14]	Kashaya (astringent),	<i>Laghu</i> (light), <i>Ruksha</i> (dry)	Sheeta (cold)	Katu (pungent)	<i>Kaphaghna</i> <i>Pittaghna</i> <i>Hridya</i> <i>Udardprashaman</i>	Antiallergic	1 part (bark)
2.	<i>Cinnamomum Zeylanicum</i> Breyn. (<i>Tvaka</i>) [15]	Katu (pungent), <i>Madhura</i> (Sweet)	<i>Laghu</i> (light), <i>Ruksha</i> (dry), <i>Tikshna</i> (sharp)	Ushna (hot)	Madhura (sweet)	<i>Kaphavata shamak</i>	Anti-inflammatory Antioxidant	1 part (bark)
3.	<i>Elletaria cardamomum</i> . Maton (<i>Ela</i>) (<i>Sukshma ela</i>) [15]	Katu (pungent), <i>Madhura</i> (Sweet)	<i>Laghu</i> (light), <i>Snigdha</i> (unctuousness), <i>Sukshma</i> (minute)	Sheeta (cold)	Madhura (sweet)	<i>Kaphavata shamak</i> <i>Pitta shamak</i>	Antimicrobia Antioxidant	1 part (seed)
4.	<i>Cinnamomum tamala</i> . Nees (<i>Tejapatra</i>) [16]	Katu, (pungent), <i>Tikta</i> , (bitter), <i>Kashaya</i> (astringent)	<i>Laghu</i> (light) <i>Tikshna</i> (sharp)	Ushna (hot)	Madhur (sweet)	<i>Kaphavata shamak</i>	Anti-inflammatory, Antimicrobia	1 part (patra)
5.	<i>Zingiber officinalis</i> (<i>Sunthi</i>) [17]	Katu (pungent)	<i>Laghu</i> (light), <i>Snigdha</i> (unctuousness)	Ushna (hot)	Madhura (sweet)	<i>Kaphaghna</i> <i>Vataghna</i>	Anti-inflammatory, Antimicrobial, Anticancer, Antioxidant	1 part (Rhizome)
6	<i>Piper longum</i> Linn. (<i>Pippali</i>) [18]	Katu (pungent)	<i>Laghu</i> , (light), <i>Snigdha</i> (unctuousness), <i>Tikshna</i> (sharp)	Anushna (lukewarm)	Madhura (sweet)	<i>Kaphavata shamak</i> , <i>pittashamak</i>	Anti-inflammatory, immunostimulatory, antilulcer, antiamoebic, Antioxidant, hepatoprotective	1 part (fruit)

7.	<i>Piper nigrum</i> . Linn (Maricha) [19]	Katu (pungent)	Tikshna (sharp)	Ushna (hot)	Katu (pungent)	Vataghna Kaphaghna	Antiallergic, anti-inflammatory, Antioxidant	1 part (fruit)
8.	<i>Piper longum</i> . Linn (Pippalimool) [19]	Katu (pungent)	Laghu (light) Snigdha (unctuousness), Tikshna (sharp)	Anushna (lukewarm)	Madhura (sweet)	Kaphavatashamak Pittashamak	anti-inflammatory, analgesic, antibacterial	1 part (root)

[Table/Fig-1]: Illustrating the ingredients and properties of *Dhananjayadi Vati*.

Preparation of *Dhananjayadi Tablet (Vati)*: Raw materials will be procured and authenticated according to Active Pharmaceutical Ingredient (API) standards. Each ingredient will then be pulverised individually. The drugs are sieved through a mesh of size number 80, individually. The fine powders of drugs are taken in mortar and pestle (*Khalva yantra*). Bhavana will be given with ginger juice (*Ardraka Swarasa*) for seven days for three hours. Then, tablets of a specific size (*Vati*) are prepared [Table/Fig-2] [3].



[Table/Fig-2]: *Dhananjayadi Vati*.

Primary Outcomes

Standardisation Parameters:

i) Analytical parameters of the *Dhananjayadi Tablet (Vati)*:

A. Organoleptic characters [20]:

1. Touch
2. Appearance
3. Taste
4. Odour

B. Physicochemical parameters:

1. pH value: A digital pH meter with a combined electrode will be used to measure pH. Before starting the experiment, the instrument will be calibrated using buffer solutions with pH values of 4.0, 7.0 and 9.20 to ensure its accuracy [21].

2. Hardness: Hardness plays an important role in the quality control of tablets, as the compression process influences all aspects of tablet performance, including disintegration, dissolution and stability. It also reflects the bonding strength, internal integrity and brittleness of the tablets. Ensuring that tablets remain intact during transportation and handling until they reach the consumer is essential. The hardness of the drug will be measured using a Monsanto tablet hardness tester [22].

3. Uniformity of weight: To assess drug content uniformity, half-tablets will be compared to one-half of the mean drug content found in the entire sample. To evaluate weight uniformity, the weights of half-tablets will be compared to one-half of the sample's mean weight for whole tablets using a Mettler balance [23].

4. Loss of drying at 105°C: A 10-gram sample of the compound will be evenly distributed in a shallow petri dish. The dish will then be subjected to controlled heating at 105°C, followed by cooling in a desiccator before weighing. This process will be repeated several times until two consecutive weight measurements are consistent.

The percentage of weight loss will then be calculated based on the initial weight [24].

5. Total ash: Two grams of each ash sample will be accurately weighed and placed into silica crucibles. The samples will be evenly distributed across the bottoms of the crucibles, then incinerated, allowed to cool and weighed. The total ash value is determined by subtracting the weight of the empty crucible from the weight of the crucible with the incinerated sample [23,24].

6. Acid insoluble ash: The residues from the total ash determination will be boiled in hydrochloric acid. After boiling, the insoluble parts will be rinsed with hot water, placed in a crucible, dried and then weighed. The difference in weight between the crucible containing the incinerated sample and the empty crucible provides the measurement for acid-insoluble ash [24,25].

7. Water soluble extractive values: A conical flask with a glass stopper will be used to mix 5 g of the drug with 100 mL of distilled water and alcohol. After six hours of gentle shaking at regular intervals, the mixture will be left to stand undisturbed for 18 hours. After filtration, 25 mL of the solution will be evaporated using a water bath. The residual residue will be dried at 105°C for six hours, cooled in desiccator for 30 minutes and promptly weighed. The percentage of water-soluble materials will be estimated using the weight of the air-dried medication [22].

8. Alcohol soluble extractive values: The proportion of alcohol-soluble matter will be calculated using the same approach as for water extractive values, but using alcohol instead of water [22].

9. Disintegration time: Disintegration time is a crucial parameter that evaluates the ability of dosage forms, such as tablets, capsules, boluses, pessaries and suppositories, to break down within a specified timeframe when immersed in a liquid medium under controlled experimental conditions. This metric serves as an indicator of the drug's quality and the characteristics of the binding agent will be used [25].

10. Friability: To analyse tablet friability, standard pharmacopeial procedures require a collection of identical tablets from the same batch. For a single examination, at least 6.5 grams of tablets are required. The test involves dropping the tablets 100 times from an established height while the friability apparatus is in motion. After the process, the tablets are collected, washed and weighed to determine the overall weight reduction. A weight loss of less than 1% is considered acceptable for proven compressed and uncoated tablet formulations [25].

11. HPTLC: HPTLC plays a crucial role in detecting adulterants and evaluating drug quality. This method enables the separation of different chemical components, allowing for the determination of Rf values after spot detection, which is essential for verifying the drug's identity, purity and potency [22].

Secondary Outcome

The trachea of a newly slaughtered goat will be obtained from the slaughterhouse and promptly placed in a thermostat flask that holds a chilled Krebs-Hansleit solution at 4°C until it is needed the following day.

Isolated goat tracheal chain preparation: Isolated adult goat tracheal tissue will be collected immediately after the goats are slaughtered. The trachea will be segmented into separate rings and then linked together sequentially to create a chain. The trachea will be placed in a bath containing Krebs' solution with the following

components: CaCl_2 (0.28), KCl (0.35), NaCl (6.9), NaHCO_3 (2.1), MgSO_4 (0.28), KH_2PO_4 (0.16) and glucose (2.0) g/L at a temperature of $37 \pm 0.5^\circ\text{C}$. One extremity of the tracheal chain will be connected to an S-shaped aerator tube, while the other end will be linked to an isotonic frontal writing lever connected to a magnified smoked drum (with a magnification of 10-12 times). The tissue will be given 45 minutes under a 400 mg load of *Dhananjayadi* tablet (*Vati*) before proceeding. By maintaining a 15-minute time cycle, a dosage-response curve for histamine will be measured. The *Dhananjayadi* tablet (*Vati*) will be placed in the appropriate reservoir after getting a dosage-response curve of histamine on the trachea and repeated doses of histamine will be supplied.

In-vitro/Tissue Model: The tracheal chain from the goat trachea is suitable for screening the activity of a drug on respiratory smooth muscles. To examine the contractile and dilator responses to both agonists and antagonists, the contraction of tracheal or bronchial smooth muscle in-vitro will be employed [26]. The tracheal muscle in goats contains H1, M3 and B2 receptors. The bronchial smooth muscle contracts in response to stimulation of H1 receptors [27]. The dose-response curve for histamine, both in the absence and presence of the *Dhananjayadi* tablet (*Vati*), will be recorded using a graph that shows the percentage of maximum contractile response on the ordinate and the negative logarithm of histamine's molar concentration on the abscissa [28,29].

STATISTICAL ANALYSIS

The IBM Statistical Package for the Social Sciences (SPSS) statistics software (version 29.0) for the statistical analysis will be used. A paired t-test will be applied for pre- and postintervention analysis and a p-value <0.05 will be considered statistically significant.

Reporting guidelines: Checklist for Reporting in-vitro Studies (CRIS) Guidelines checklist for pharmaceutico-analytical study of *Dhananjayadi Vati* and assessment for its antihistaminic activity-study protocol checklist.

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