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Research Article

Quantification of Quercetin 3-o-glucoside in Erandamoola Niruha Basti Prepared by Different Methods

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Abstract

Background: Basti Chikitsa is an important therapeutic modality in Ayurveda, where the efficacy of Niruha Basti depends largely on the proper emulsification of its multi-component formulation. Classical texts describe different methods of preparation, which may influence the physicochemical properties and therapeutic potential of the formulation. Objective: To compare the effect of different emulsification techniques on the pharmaceutical quality of Erandamoola Niruha Basti (EMNB) using chromatographic evaluation. Methods: EMNB was prepared using three distinct methods of emulsification. Trituration method– sequential homogenization using Khalva Yantra. Churning method – manual mixing followed by churning. Blending method – high-speed blending for uniform emulsification in a mixer. The prepared samples were subjected to extraction and analyzed using High Performance Liquid Chromatography (HPLC). Quantification of Quercetin-3-O-glucoside was performed as a marker compound to evaluate variation in phytoconstituent availability across methods. Results: Differences in chromatographic profiles and marker compound concentration were observed among the three methods, indicating variation in emulsification efficiency and phytochemical extraction. Conclusion: The method of emulsification significantly influences the pharmaceutical characteristics of Basti formulations. HPLC-based quantification of Quercetin-3-O-glucoside serves as a useful analytical approach for comparative evaluation; however, inclusion of additional analytical parameters is recommended for comprehensive standardization.

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Keywords: *Basti sammilana*, HPLC, Quercetin 3-o-glucoside, EMNB.

Introduction

Basti Chikitsa plays a role in managing systemic disorders, particularly those involving *Vata Dosha* (1). *Niruha Basti* is a complex polyherbal formulation consisting of aqueous (decoction) and lipid components, requiring proper emulsification for stability, absorption, and bioavailability. The therapeutic efficacy of Niruha *Basti* depends not only on its ingredients but also on the method of preparation, especially the process of emulsification Classical Ayurvedic texts describe different preparation techniques, notably (2):

1.Trituration method as per Acharya Charaka (3), in this method, initially Makshika is added with *lavana* in a *khalva yantra* and triturated till a homogenous mixture is formed, next the Sneha is added little by little to ensure proper mixing. Then kalka is added followed by Kashaya.

2. Churning method as per Acharya *Sushruta*(4), *Makshika* and *lavana* are mixed in a wide and deep plate with the help of hand till proper mixture is ensured. Then Sneha is added little by little ensuring proper emulsification, followed by adding of kalka. Then the mixture is transferred to a deep vessel and churned with a mechanical churner.

3. In contemporary practice (5), a blender or a hand mixer is used for convenience, and uniformity. However, the impact of these different emulsification techniques on the quality and phytochemical profile of Basti formulations remains inadequately explored.

Erandamoola Niruha Basti is widely used for its *Vatahara* properties. Among its phytoconstituents, Quercetin-3-O-glucoside, a flavonoid glycoside, is known for anti-inflammatory and antioxidant activities and serves as a suitable marker compound for analytical evaluation(6,7,8).

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High Performance Liquid Chromatography (HPLC) is a sensitive and reliable analytical tool for quantification of such phytoconstituents in complex herbal formulations. This study aims to evaluate how different emulsification techniques influence the availability of this marker compound. Objectives: To evaluate and compare the HPLC of EMNB prepared from different methodology.

Methodology

Materials and Methods

1. Pharmaceutical Study (Preparation of Basti)

Medicinal Source- Raw drugs for preparation was collected from GMP certified KLE ayurvedic Pharmacy, Belagavi. Authentication was ensured as per standard parameters.

Analytical source: HPLC was carried out in Ayush approved drug testing laboratory of Rajiv Gandhi Education society ayurvedic medical College & hospital & PG research centre, Rona, Gadag (dist.), Karnataka. Raw Materials

Ingredients (Uniform for all methods)
- *Makshika* (Honey) – 60 ml
- *Saindhava* (Rock salt) – 5 g
- *Morchita Tila Taila* (Sesame oil) – 60 ml
- *Shatapushpa Kalka* – 10 g
- *Erandamoola Kashaya* – 200 ml

Preparation of Kashaya

Prepared as per classical method (1:16 reduced to 1/8): 300 g Erandamoola + 4.8 L water → reduced to 600 ml[9].

Methods of Emulsification

1. Acharya Charaka's Method (Trituration Method) (10,11): Sequential addition of ingredients in *Khalva Yantra*, Continuous trituration to achieve gradual emulsification which Emphasis on particle size reduction and uniform dispersion

2. Acharya Sushruta's Method (Churning Method) (12,13): Manual mixing of ingredients with palms, followed by churning to form emulsion, produces moderate shear and mixing efficiency

3. Blending Method (Mechanical Method) (14): All ingredients mixed using an electrical blender, High-speed mixing produces rapid emulsification, Ensures uniform dispersion but may alter thermolabile constituents

2. Analytical Study (HPLC Analysis)

Sample Preparation

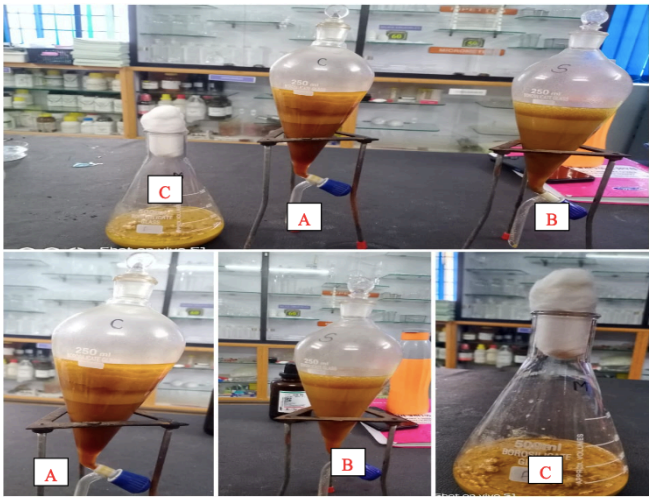
25 ml of fresh prepared different methods of Basti were taken washed with 100ml of petroleum ether in triplicates to separate fat contain, thus separated basti material was further extracted in 100ml Methanol in triplicates and filter with Whatman no. 43 filter paper and dried in rotary evaporator with temperature 35°C and stored in airtight container till it further used.

Thus obtained each extract was further 30 to 70mg was taken in 10m volumetric flask dissolved with methanol a studied on HPLC

Apparatus

The chromatography was performed on a Waters UPLC instrument equipped, auto sampler with PDA detector and empower software Symmetry C18 (4.6 x 250mm, 5p particle size) was used as stationary phase.

Figure 1: Preparation of the vasti dravya by different methods: A: Charaka's Method, B: Sushruta's Method, C: Modern Method



Reagents and Materials

The reference standard of Quercetin was purchased from Sigma Aldrich. HPLC grade Water, Methanol, Acetonitrile and Acetic acid made of Merck specialties Pvt. Ltd. Mumbai were used in study.

Table 1: Reference standard of Quercetin

Mobile Phase	1% Acetic acid: Methanol (40:60%v/v)
Stationary Phase	Symmetry C18 (4.6 × 250mm, 5µ particle size
Wavelength	334nm
Run time	6 Mins
Flow Rate	1ml/Min
Injection volume	100µL
Temperature	Ambient Isocratic elution
Mode of Operation	Isocratic

Preparation of Standard Stock Solution

An accurately weighed quantity of Quercetin (10mg) was transferred to a 100ml, volumetric flask, dissolved and diluted to the mark with Methanol to obtain standard stock solution of 1000µg/mL

Preparation of Calibration Curve

Aliquots of 0.8, 1, 1.5, 2 and 3mL. standard stock solution (1000µg/ml.) was transferred to 10ml. of volumetric flasks and made up to the mark with Methanol to get concentration of 80, 100, 150, 200 and 300µg/ml.

Quercetin quantification

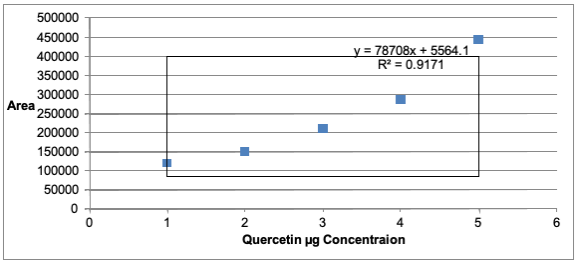
Table 2: Quercetin quantification

Conc µg	Area
80	119221
100	148955
150	209747
200	286548
300	443962

Table 3: Quercetin Quantification According to different methods

QTY	Method	Area	Conc	
10ml	Charaka	185	1.257648	Charaka method 40mg of 10 ml extract gave 12.83X10 = 128.3 Microgram of Quercetine
10ml	Sushruta	117133	79.62814	Sushruta method 71mg of 10 ml extract gaves 796.314X10 = 7963.14 microgram of Quercetine
10ml	Modern	8401	57.110814	Modern method 63mg in 10ml of extract gaves 571.36X10 = 5713.6 Microgram of Quercetine

Figure 2: Quercetin µg Concentraion



Sample / Method	Quantity of Study	Methanol extract in GM	Extract in mg	In 1mg of ext contains Quercetin in microgram	Quercetin in total ex microgram	Quercetin in mg
Charaka	25ML	6.18	6180	3.2075	19822.35	19.82235
Sushruta	25ML	4.68	4680	1121.569	524894.3	524.8943
Modern	25ML	5.68	5680	90.69206	515130.9	515.1309

Figure 3 : Analysis Reports

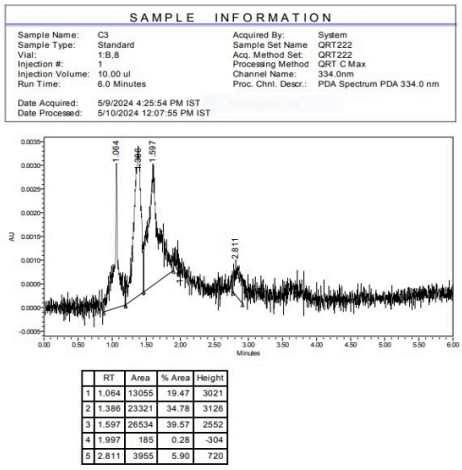


Figure 4: Analysis Reports

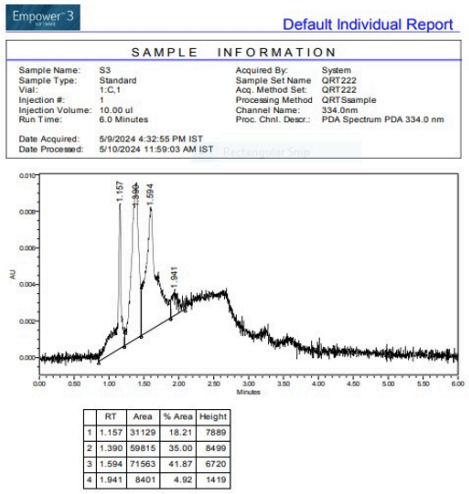
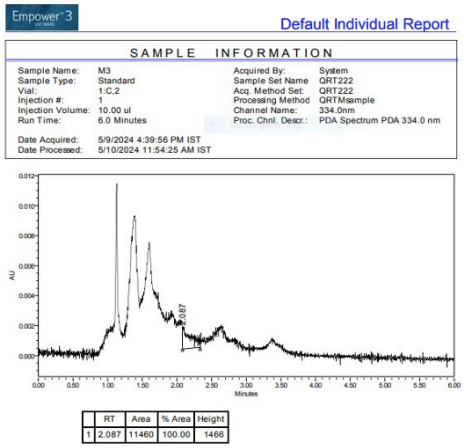


Figure 5 : Analysis Reports



Result

Table 4: Result of Quantification of Quercetin

Sl.No	Test	Result
1	Sushruta method	25ml EMNB contains 524.8943mg of Quercetin.
2	Modern method	25ml EMNB contains 515.1309mg of Quercetin.
3	Charaka method	25ml EMNB contains 19.822mg of Quercetin.

Discussion

Basti being a mixture of more than two immiscible ingredients, the method of preparation plays a very critical role in making it an emulsion. High shear mixing plays a pivotal role in the emulsification process by breaking down droplets and limiting the re-combining of droplets during processing, which improves the suspension characteristics of the basti (15). The highest quantification of Quercetin was found in Sushruta method.

In Sushruta Method - 25ml of EMNB contained 524.8943mg of Quercetin. For an emulsion to remain stable sufficient energy input is required and finer droplets are produced which improves the characteristics of the *basti* which was achieved with hand. In this method a large surface area of *basti dravya* came in contact with the hand, also a controlled and increased pressure was applied when compare to other two methods. Also, by *khaja* (churner) the mixing of uniform colloidal solution takes, that helps in friction and churning action on *basti dravya*.

In Modern method - 25ml EMNB contained 515.1309mg of Quercetin. Here basic Ingredients of the *basti* medicine are subjected to the blender. Blender method was found to have better chemical and physical properties. The base idea of modern method of *Basti* preparation in an electric motor which is set to run at a particular RPM using an electric circuit (16). By slicing mechanism and energy the droplets form a homogenous suspension which made *kwatha dravya* more variable.

In Charaka method - 25ml EMNB contained 19.822mg of Quercetin. This is probably due to less surface area contact while

preparing *basti Sammelana*, un-uniform pressure and no churning action on *basti dravya* comparatively other two methods.

Further on *basti Sammelana* Instrument with similar mechanism as that adopted by Acharya Sushruta can be developed for harvesting the maximum therapeutic benefit of Basti. Pharmacokinetics studies with respect to different methods of preparation can be undertaken.

Conclusion

Out of three method the Acharya Sushruta method was found to be more potent 25ml EMNB contained 524.894mg of Quercetin than compare to modern method 25ml EMNB contained 515.130mg of Quercetin and then Charaka method 25ml EMNB contained 21.822mg of Quercetin. This is attributed to more contact surface area, pressure and churning action adopted in this method.

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