



PHARMACEUTICO ANALYTICAL STANDARDISATION OF GUGGULVASAVA

An Experimental Study

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Abstract: Sandhāna Kalpanā is an important pharmaceutical process described in Ayurveda in which liquid preparations undergo fermentation under suitable conditions. Asava and Ariṣṭa are important dosage forms prepared through this process and are characterized by the generation of alcohol during fermentation, which facilitates extraction of alcohol- and water-soluble constituents and contributes to preservation of the formulation. Guggulvāsava is one such classical fermented formulation described in *Gada Nigraha*, containing Guggulu along with various herbal ingredients, sweetening substances and fermentation-supporting drugs. Despite the therapeutic importance of classical fermented preparations, maintaining uniformity in their pharmaceutical processing remains challenging because natural fermentation is influenced by several intrinsic and extrinsic factors. Hence, systematic pharmaceutical documentation and analytical evaluation are necessary for establishing reproducibility and quality-control parameters.

Keywords: Guggulvāsava, Guggulu, Sandhāna Kalpanā, Asava, fermentation, pharmaceutical standardisation, analytical standardisation, HPTLC.

GA- Guggulvasava

I. INTRODUCTION

Ayurveda encompasses a wide range of pharmaceutical preparations designed to improve the therapeutic utility, stability and acceptability of medicinal substances. Among these, Sandhāna Kalpanā represents an important fermentation-based pharmaceutical process and forms the basis for the preparation of Asava and Ariṣṭa.

Asava and Ariṣṭa are fermented liquid preparations in which medicinal substances are allowed to undergo natural fermentation in the presence of suitable fermentable substrates. During this process, microorganisms convert available sugars into alcohol and other fermentation products. The self-generated alcohol acts as a solvent and preservative and assists in extracting both hydrophilic and relatively lipophilic constituents from the incorporated drugs.

Guggulvāsava is a classical Ayurvedic formulation described in *Gada Nigraha*. Guggulu, obtained from *Commiphora mukul*, constitutes an important component of the formulation. Classical indications of Guggulvāsava include Bhagandara, Udara Roga, Plīha, Urustambha, Kāmalā, Śvāsa, Kāsa, Śopha, Krimi, Kuṣṭha and Prameha, and the formulation is also attributed with Agnidīpana activity.

Several Guggulu-containing formulations are available in dosage forms such as Vaṭi, Cūrṇa, Ghṛta and Taila. However, comparatively limited pharmaceutical and analytical information is available regarding fermented preparations containing Guggulu. Standardisation is particularly important in Sandhāna Kalpanā because natural fermentation is influenced by the quality of raw materials, fermentable sugar concentration, microbial population, temperature, humidity, pH, oxygen availability, fermentation vessel and environmental conditions.

Hence, the present study was undertaken to systematically prepare and evaluate Guggulvāsava and to document the pharmaceutical behaviour, difficulties encountered during fermentation and analytical characteristics of the available experimental batches.

AIM AND OBJECTIVES

The study was undertaken with the following objectives:

1. To compile classical references regarding Sandhāna Kalpanā with special reference to Guggulvāsava.
2. To prepare Guggulvāsava according to an SOP developed on the basis of the classical reference.
3. To document pharmaceutical changes occurring during the preparation and fermentation of Guggulvāsava.
4. To evaluate the available samples using relevant physicochemical parameters.
5. To perform qualitative phytochemical and chromatographic evaluation using TLC and HPTLC.
6. To generate preliminary pharmaceutical and analytical reference data for future standardisation of Guggulvāsava.

MATERIALS AND METHODS

Study Design

The study comprised three components:

1. Conceptual study: Classical and contemporary literature related to Sandhāna Kalpanā, Asava-Ariṣṭa and Guggulvāsava was reviewed.

2. Pharmaceutical study: Pharmaceutical processing of raw materials and preparation of Guggulvāsava were carried out according to the adopted classical procedures.

3. Analytical study: Raw materials and available experimental samples were evaluated using appropriate physicochemical, phytochemical and chromatographic parameters.

Procurement and Authentication of Raw Materials

All ingredients required for the preparation of Guggulvāsava were procured from reliable sources. The raw materials were identified and assessed for their suitability before pharmaceutical processing.

Śodhana of Guggulu

Āsuddha Guggulu was subjected to Śodhana using Triphalā Kwātha. Triphalā Cūrṇa and water were taken in the prescribed proportion and heated to prepare the Kwātha. Guggulu was tied in a cloth Pottalī and suspended in the Triphalā Kwātha using Dola Yantra. Mild heating was continued until the soluble fraction of Guggulu passed through the cloth into the liquid medium. The residual impurities were discarded, and the dissolved Guggulu was further processed and dried. The initial quantity of Guggulu was 200 g and 172 g of Śuddha Guggulu was obtained after purification.

Śodhana of Citraka

Citraka was subjected to Śodhana using freshly prepared Cūrṇodaka. The drug was immersed in Cūrṇodaka according to the adopted classical procedure for three consecutive days, with fresh medium being used each day. After completion, the drug was washed and dried. The weight decreased from 100 g to 93.2 g following Śodhana.

Preparation of Guggulvāsava

Guggulvāsava was prepared according to the classical reference described in *Gada Nigraha*. Triphalā Kwātha was initially prepared, after which purified Guggulu was incorporated into the warm Kwātha and appropriately processed. Madhura Dravyas were dissolved in the prepared liquid after cooling.

The formulation was transferred to a suitably prepared Sandhāna Pātra. Dhātakī Puṣpa was incorporated as the Sandhānakara Dravya. Prakṣepaka Dravyas, including Drākṣā and Dādima, were incorporated according to the adopted pharmaceutical procedure. The vessel was maintained under appropriate conditions and periodically monitored for signs of fermentation.

Three experimental batches designated GA1, GA2 and GA3 were prepared.

Preparation of Ikṣu Śukta

Ikṣu Śukta was prepared separately from Ikṣu Rasa. During its fermentation, the initially sweet liquid developed a sour-sweet taste, mild alcoholic odour and mild effervescence. Its pH decreased from 5.2 to 3.4, specific gravity from 1.061 to 1.017 and refractive index from 1.395 to 1.343.

The intended final mixing of Guggulvāsava with Ikṣu Śukta could not be performed because complete fermentation of Guggulvāsava was not achieved. Therefore, subsequent stability evaluation of the intended final mixed formulation could not be undertaken.

ANALYTICAL EVALUATION

Organoleptic Evaluation

The samples were evaluated for:

- Colour
- Appearance
- Odour
- Taste
- Consistency
- Effervescence
- Hissing sound
- Behaviour of Prakṣepaka Dravyas

Physicochemical Evaluation

The available samples were evaluated for:

- pH
- Specific gravity
- Refractive index
- Viscosity
- Total solids
- Alcohol content
- Reducing sugars
- Non-reducing sugars
- Methanol
- Total acidity
- Heavy metals

Phytochemical Screening

Qualitative phytochemical screening was performed to detect major classes of secondary metabolites, including alkaloids, flavonoids, tannins, saponins, glycosides, phenolic compounds and terpenoids.

Chromatographic Evaluation

TLC and HPTLC studies were performed to generate chromatographic fingerprints that could assist in identification and future quality-control evaluation of the formulation.

RESULTS

Pharmaceutical Observations

The experimental batches demonstrated organoleptic and physicochemical changes suggestive of initiation of fermentation. Development of mild alcoholic odour, effervescence, hissing sound, changes in consistency and progressive reduction in pH, specific gravity and refractive index were observed during processing. However, none of the experimental batches achieved satisfactory complete fermentation within the study period.

GA1: Fermentation remained incomplete.

GA2: Fermentation remained incomplete.

GA3: Extensive fungal contamination developed, producing a thick white fungal mat over the formulation. The batch developed an offensive odour and was considered spoiled and discarded.

Organoleptic Findings

GA1 and GA2 showed deep dark-brown colour, turbid and opaque appearance with suspended particles, mild alcoholic odour, astringent-sour-sweet taste and slightly viscous consistency. GA3 developed extensive white fungal growth and a sour, musty and offensive odour; therefore, taste evaluation was not performed.

Physicochemical Findings

Parameter	GA1	GA2
pH	2.85	3.72
Specific gravity	1.135	1.133
Refractive index	1.390	1.362
Viscosity (cP)	5.80	5.62
Total solids (% w/v)	24.8	24.2
Alcohol (% v/v)	1.0	1.0
Reducing sugars (% w/v)	18.6	19.2
Non-reducing sugars (% w/v)	6.41	5.80
Total acidity (% w/v)	0.68	0.61
Methanol	Not detected	Not detected

The low alcohol concentration accompanied by relatively high residual sugar levels supported the observation that fermentation remained incomplete.

Heavy Metal Analysis

Lead, cadmium, arsenic and mercury were not detected in the evaluated samples, supporting their safety with respect to the heavy metals assessed.

Phytochemical Screening

Qualitative phytochemical evaluation demonstrated the presence of alkaloids, flavonoids, tannins, saponins, glycosides, phenolic compounds and terpenoids, indicating the presence of multiple phytochemical classes derived from the constituent drugs.

TLC Profile

TLC produced characteristic bands at R_f 0.27 and 0.58 under 254 nm and at R_f 0.11, 0.29, 0.72 and 0.89 under 366 nm. Following derivatization, a characteristic dark-green band was observed at R_f 0.58. These findings may serve as preliminary chromatographic fingerprinting data for further studies on Guggulvāsava.

HPTLC Profile

HPTLC evaluation produced multiple chromatographic peaks, including peaks around R_f 0.32, 0.69, 0.71, 0.76, 0.85 and 0.90. These peaks demonstrated the presence of multiple phytochemical constituents in the samples and provide a chromatographic profile that may be useful for future comparative quality-control studies.

DISCUSSION

Pharmaceutical Significance of Sandhāna Kalpanā

Sandhāna Kalpanā represents a unique Ayurvedic pharmaceutical process involving microbial transformation of medicinal substrates. Madhura Dravyas provide fermentable sugars, while Dhātākī Puṣpa traditionally functions as a Sandhānakara Dravya. The self-generated alcohol formed during successful fermentation contributes to extraction and preservation of phytoconstituents.

The process is nevertheless biologically complex. Unlike manufacturing processes in which every parameter can be precisely controlled, natural fermentation depends on the interaction between raw materials, fermentative microorganisms and environmental conditions.

Significance of Guggulu Śodhana

Śodhana of Guggulu using Triphalā Kwātha resulted in a reduction in weight from 200 g to 172 g. The process facilitated removal of insoluble and extraneous material and provided purified Guggulu suitable for subsequent pharmaceutical processing.

Significance of Citraka Śodhana

Citraka was purified using Cūrṇodaka, with a reduction in weight from 100 g to 93.2 g. The procedure constituted an important preliminary pharmaceutical step before incorporation of Citraka into the formulation.

Fermentation Behaviour of Experimental Batches

GA1 and GA2 demonstrated initial features associated with fermentation but failed to attain complete maturation. The progressive changes in pH, specific gravity, refractive index, odour and effervescence suggested biological activity within the fermentation medium. Nevertheless, the persistence of high total solids, residual sugars and only 1% alcohol demonstrated inadequate conversion of the available sugars.

GA3 developed extensive fungal contamination and could not be considered suitable for analytical assessment. This observation emphasizes the susceptibility of naturally fermented Ayurvedic preparations to contamination when optimal fermentative conditions are not maintained.

Possible Factors Responsible for Incomplete Fermentation

The failure to obtain complete and reproducible fermentation cannot be attributed to a single factor. Several interacting variables may have contributed, including seasonal variation, environmental temperature, relative humidity, quality and microbial population of Dhātākī Puṣpa, raw-material characteristics, sugar concentration, pH, oxygen availability, cleanliness of the Sandhāna Pātra and the maintenance of an appropriate Nirvāta Sthāna.

The microbial population naturally present on Dhātākī flowers may vary according to source, storage and season. Such variation could influence the initiation and progression of fermentation.

Possible Influence of Guggulu

Another factor requiring further investigation is the presence of Guggulu itself. Guggulu is an oleo-gum-resin containing resinous and volatile constituents and is associated with antimicrobial activity. Although this property is therapeutically desirable, it may potentially interfere with the growth or metabolic activity of fermentative microorganisms.

Thus, the antimicrobial characteristics of Guggulu could theoretically contribute to delayed or incomplete fermentation. However, the present investigation was not designed to establish a direct causal relationship. Therefore, Guggulu should be regarded only as a possible contributing factor, rather than the sole reason for fermentation failure.

Interpretation of Physicochemical Parameters

The acidic pH values of GA1 and GA2 indicated development of an acidic environment during fermentation. Reduction in specific gravity and refractive index during processing suggested utilization of a proportion of dissolved sugars. However, the final specific gravity remained relatively high, while substantial reducing and non-reducing sugars persisted. Furthermore, only 1% v/v alcohol was generated. Collectively, these observations strongly support incomplete conversion of sugars and incomplete maturation of the formulation.

Significance of TLC and HPTLC

Chromatographic fingerprinting provides an important approach to the quality evaluation of complex polyherbal formulations for which assessment through a single chemical constituent may be insufficient. The TLC and HPTLC profiles

obtained in the present study demonstrate multiple detectable phytochemical components and provide preliminary fingerprint data.

However, because complete fermentation and the intended post-fermentation addition of Ikṣu Śukta were not achieved, the chromatographic profile obtained in this study should be interpreted as that of the available incompletely fermented experimental material rather than a definitive fingerprint of completely matured classical Guggulvāsava.

Significance of the Present Study

The major contribution of the present investigation lies not merely in generating analytical values but also in documenting the practical challenges associated with the pharmaceutical preparation of Guggulvāsava.

Incomplete fermentation and fungal contamination are important pharmaceutical observations because they identify potential critical control points for future studies. The results emphasize the need to standardize the fermentation environment, microbial inoculum, Dhātākī quality, temperature, humidity, sugar concentration and other variables before definitive standards for Guggulvāsava can be proposed.

LIMITATIONS OF THE STUDY

Complete maturation of Guggulvāsava could not be achieved during the study period. GA3 was excluded because of extensive fungal contamination. Since the intended post-fermentation mixing of Guggulvāsava with Ikṣu Śukta could not be performed, analytical assessment and long-term stability evaluation of the final classical formulation were not possible. Therefore, the physicochemical and chromatographic findings of the present study should be considered preliminary reference data from the available experimental batches and not definitive standards for completely fermented Guggulvāsava.

CONCLUSION

The present study attempted pharmaceutico-analytical standardisation of Guggulvāsava, a classical fermented Ayurvedic formulation described in *Gada Nigraha*. Classical pharmaceutical principles were integrated with contemporary analytical techniques to systematically document its preparation and fermentation behaviour.

Śodhana of Guggulu and Citraka was successfully performed, followed by preparation of experimental Guggulvāsava batches. Although signs suggestive of fermentation were observed, complete maturation could not be achieved. GA1 and GA2 remained incompletely fermented, while GA3 developed extensive fungal contamination and was discarded.

Analytical evaluation of GA1 and GA2 demonstrated acidic pH, persistence of residual sugars, comparatively high total solids and only 1% self-generated alcohol, corroborating incomplete fermentation. Methanol was not detected, and the tested heavy metals were absent. Qualitative phytochemical screening demonstrated multiple phytochemical classes, while TLC and HPTLC provided characteristic chromatographic profiles.

The findings indicate that fermentation of Guggulvāsava is influenced by multiple interacting factors rather than a single variable. Temperature, humidity, seasonal variation, quality and microbial population of Dhātākī Puṣpa, physicochemical conditions, hygiene and the possible antimicrobial influence of Guggulu require further systematic investigation.

The present study therefore provides preliminary pharmaceutical observations, physicochemical data and chromatographic fingerprints rather than definitive standards for a completely fermented Guggulvāsava. Further controlled studies with optimization of fermentation conditions and microbiological monitoring are required to establish a reproducible manufacturing process and comprehensive standardisation profile.

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