



Research Article

Quality Characterization and Bio accessibility Profiling of *Sitadi Agada* by diffusion method

Houserao Patil¹, Nilima Wadnerwar^{2*}

1. PhD Scholar, Department of Agadtantra, Mahatma Gandhi Ayurved College, Hospital and Research Centre, Salod(H), Datta Meghe Institute of Higher Education and Research (DU), Wardha, Maharashtra, India. Professor and Head, Department of Agadtantra, L.R.P.A.M.C.H.PG.I & R.C. Islampur (Ishwarpur), Maharashtra, India.
2. Professor, Department of Agadtantra, Mahatma Gandhi Ayurved College, Hospital and Research Centre, Salod(H), Datta Meghe Institute of Higher Education and Research (DU), Wardha, Maharashtra, India.

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Abstract

Sitadi Agada is a classical herbo-mineral formulation indicated in the management of *Shankavisha*. Scientific validation of its pharmaceutical characteristics and bio accessibility is necessary to substantiate its traditional therapeutic claims in emergency conditions. Aim: To evaluate the pharmaceutical parameters and in vitro bio accessibility of *Sitadi Agada* using the Franz diffusion cell method. Materials and Methods: *Sitadi Agada* was prepared using authenticated raw materials following modified classical procedures. The formulation was subjected to organoleptic evaluation, physicochemical analysis (loss on drying, ash values, extractive values, and pH), tablet evaluation (hardness, friability, and disintegration time), and microbiological testing as per standard guidelines. Bio accessibility was assessed using the Franz diffusion cell diffusion technique to study the release and diffusion profile of active constituents. Results: The formulation exhibited acceptable organoleptic characteristics with a sweet taste and pleasant odour. Physicochemical analysis revealed low moisture content (0.59%), acceptable ash values, higher water-soluble extractive value (31.42%) compared to alcohol-soluble extractive value, and near-neutral pH (6.8). Tablet evaluation showed rapid disintegration (3 min 30 s), adequate hardness (3.5 kg/cm²), and low friability (0.5%). The Franz diffusion study demonstrated effective diffusion of water-soluble phytoconstituents, indicating favourable bio accessibility. Conclusion: *Sitadi Agada* was found to be pharmaceutically stable, microbiologically safe, and bio accessible. The predominance of water-soluble constituents and rapid disintegration support its potential oral bioavailability. The study provides preliminary scientific evidence supporting the traditional use of *Sitadi Agada* in the management of *Shankavisha* (pseudo-snakebite) and fear-induced manifestations.

Keywords: *Sitadi Agada*, *Shankavisha*, Pseudo-snakebite, Bioaccessibility, Franz diffusion cell, Herbo-mineral formulation

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Introduction

Snakebite envenoming remains a major public health concern, particularly in tropical and subtropical regions, where it is associated with considerable morbidity and mortality. The World Health Organization has classified snakebite as a neglected tropical disease, underscoring the need for accessible, safe, and effective therapeutic interventions. While antivenom therapy remains the cornerstone of modern management, its limitations

including restricted availability, cold-chain requirements, variable efficacy against different species, and potential adverse reactions highlight the need to explore complementary and supportive therapeutic approaches. Recent estimates suggest that between 2000 and 2019, India accounted for almost half of all global deaths due to snakebite, with an average of 58,000 fatalities annually (1). Beyond confirmed envenomation, a substantial number of patients present with non-venomous bites, dry bites, or even suspected bites without actual exposure. In some hospital-based series, up to 20–46% of cases show no systemic signs of envenoming (2,3). These presentations often referred to as “pseudo-snakebites” reflect the profound psychological and somatic responses triggered by fear of envenomation, which can mimic true toxic manifestations.

Ayurveda describes such phenomena under the concept of *Shankavisha*, elaborated by Acharya Charaka. *Shankavisha* arises

* Corresponding Author:

Nilima Wadnerwar

Professor, Department of Agadtantra, Mahatma Gandhi Ayurved College, Hospital and Research Centre, Salod(H), Datta Meghe Institute of Higher Education and Research (DU), Wardha, Maharashtra, India.

Email Id: dr.nilima_wadnerwar@rediffmail.com

not from actual venom but from *udvega* (mental agitation) caused by fear of poisoning. This may occur when a rope or stick is mistaken for a snake in dim light, or when a thorn prick is perceived as a bite. Despite the absence of venom, the afflicted person may develop clinical features resembling snakebite such as agitation, fever, vomiting, fainting, burning sensation, malaise, mental confusion, and diarrhea (4). *Sitadi Agada*, a classical herbo-mineral antitoxic formulation, is specifically indicated in *Shankavisha*. It comprises *Sharkara* (sugar candy), *Vaigandhika* (sulphur), *Draksha* (*Vitis vinifera* Linn.), *Payasya* (*Asparagus racemosus* Willd.), *Madhuka* (*Glycyrrhiza glabra* Linn.), and honey.

When a drug is administered in body, its pharmacokinetic and mechanism must be known. Especially when it is intended for emergency, fast absorption is required. Understanding the bio accessibility of herbal formulations is a critical step in establishing their pharmacological relevance. Bio accessibility refers to the fraction of bioactive constituents that are released from a formulation in the gastrointestinal environment and become available for absorption. It provides an early indication of the potential *in vivo* efficacy of orally administered preparations. Franz diffusion cell methodology, though conventionally applied for dermal and transdermal delivery studies, has been adapted for assessing drug release and permeability profiles under controlled conditions. Its application offers a reproducible *in vitro* model to investigate the diffusion characteristics of phytoconstituents, thereby bridging traditional concepts with contemporary pharmacokinetic insights.

The present study was undertaken to evaluate the bio accessibility of *Sitadi Agada* using the Franz cell diffusion method. By investigating the release and diffusion profile of its active constituents, the study aims to generate preliminary experimental evidence supporting its antitoxic claims and to provide a scientific basis for further pharmacological exploration.

Methodology

The present work is a part of the study which was started after the Institutional Ethics Committee approval (Ref MGACHRC/IEC/JULY-2021/259 Dated 10 July 2021).

Collection, Identification, and Authentication of Drugs

Out of the ingredients of *Sitadi Agada*, *Payasya* (*Shatavari*) and *Madhuka* (*Yashtimadhu*) were procured from Essen Herbs India Pvt Ltd. Nagpur, Maharashtra, a vendor that cultivates and supplies medicine using good manufacturing practices. *Vaigandhika* (*Gandhaka*) was procured from Shiva Herbals, Haryana. *Sita* (rock sugar), dried fruits of *Draksha* and *Madhu* (honey) were sourced from local market. Fruits of *draksha* were checked for physical impurities and quality. Honey was tested for its adulteration. The authentication of all ingredients was carried out at Foundation for Revitalization of Local Health Traditions (FRLHT), Bangalore and certified the same with a letter dated 19th October 2022. The voucher number is mentioned in table 1.

Preparation of *Sitadi Agada*

The *Sitadi Agada* was prepared at Dattatray Rasashala, MGACHRC, Salod (H), Wardha. The pharmaceutical process involves *shodhana* of *Gandhaka*, preparation of *Draksha kwatha* and final product.

Table 1: Drugs and their quantity of *Sitadi Agada*

Sr. No.	FRLHT Acc. No.	Name of Drug (IUPAC)	Scientific/Latin Name	Part used	Quantity
1	5483	<i>Sita</i>	<i>Saccharum officinarum</i>	-	2 kg
2	5482	<i>Vaigandhika</i>	Sulphur	-	2 kg
3	5481	<i>Draksha</i>	<i>Vitis vinifera</i> L.	Fruit	2 kg
4	5480	<i>Payasya</i>	<i>Asparagus racemosus</i> Willd.	Root	2 kg
5	5479	<i>Madhuka</i>	<i>Glycyrrhiza glabra</i> L.	Root	2 kg
6	5478	<i>Madhu</i> (<i>Anupana</i>)	Honey	-	q.s.

Purification of *Gandhaka* (5)

Gandhaka 2kg and *ghrita* were heated in a pan until the *Gandhaka* melted and dissolved in the *ghrita*. This mixture was poured into milk, converting it into a solid mass. This solid mass was washed with hot water and dried in sunlight. This procedure was repeated for seven times to obtain *Shuddha Gandhaka* (purified sulphur) for medicinal use (6).

Preparation of Decoction:

2kg Coarse raisins (*Draksha/Manuka*) were soaked in 16 litres of water overnight to extract their beneficial compounds. This mixture was boiled and then simmered until it reduced to one-eighth of its original volume. This *Kwatha* was strained through a cotton cloth to remove solid particles (7).

Final Preparation:

Sita (Candy Sugar), *Payasya* (*Shatavari*), and *Madhuka* (*Yashtimadhu*) and purified *Gandhaka* (sulphur) were powdered individually till they become fine. Each drug was taken in equal quantity (2kg) in a vessel. Purified sulphur was mixed with *Sita*, *Shatavari*, *Madhuka*. The decoction was taken in a casserole, and the mixture of powdered drugs was added. It was mixed properly until it becomes homogenous. This semi-solid preparation was poured into a large casserole and dried in sunlight until all the water evaporates. This resulting product was a coarse powder or granules, intended for tablet preparation in 250 mg doses.



Analytical Study of *Sitadi Agada*

A detailed physicochemical evaluation of the formulation was performed, including Loss on drying, Total ash value, Acid insoluble ash, Alcohol extractive value, Water extractive value, Tablet hardness, tablet disintegration time and friability.

Diffusion study for oral bio accessibility of *Sitadi Agada*: (8-10)

Procedure:

The fresh gut of goat was collected. The 5cm goat gut was taken and it was cut open with blade. Fat layer was separated by blade so as to observe the thin skin layer which is to be placed on lower flat flange joint. A buffer solution was prepared in a beaker by adding one buffer tablet of 500mg in water of 100ml and stirred. Solution was poured in receptor chamber. The donor chamber was placed at top and tied with the rubber to avoid the movement. The drug was loaded in donor chamber. The beaker filled with water was placed on the Magnet stirrer and 37° C temperatures was set on the stirrer as that of body temperature. The stirrer was started. The drug in donor chamber was allowed to diffuse from the goat intestinal membrane in the recipient chamber. Sample was collected at 15 and 30 min by maintaining pH of 4.0, at 60 and 120 min by maintaining pH of 7.0, at 180 min and at 240 min by maintaining pH of 9.2. All the samples were run under SL244 Double Beam UV-VIS Spectrophotometer (ELICON®) on 240nm Wavelength. The readings were taken and plotted against time and absorbance on x and y axis.

Observations and Results

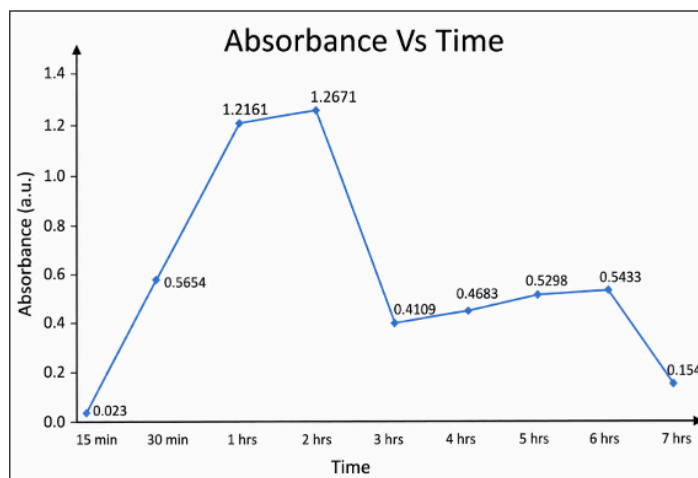
Results of Analytical Study of *Sitadi Agada*

The analysis was conducted at the Analytical Laboratory, MGACHRC, Salod (H), Wardha.

Table 2: Analytical Study of *Sitadi Agada*

Organoleptic Parameter		
1	Color	Buff color
2	Odor	Characteristic
3	Taste	Sweet
Physicochemical Parameter		
4	Loss on drying at 1050c	0.59%
5	Total Ash Value	4.82%
6	Water Soluble Ash	2.16%
7	Acid Insoluble Ash	0.5%
8	Water Extractive value	31.42%
9	Alcohol soluble extractive	14.53%
10	pH	6.8
11	Disintegration time	3min 30 second
12	Hardness Test	3.5 kg/cm2
13	Friability Test	0.5%
Microbiological contamination		
14	Total viable count	Absent
15	Enterobacteriaceae	Absent
16	Total fungus count	Absent
17	<i>E-coli</i>	Absent
18	Salmonella	Absent
19	<i>Staphylococcus aureus</i>	Absent
20	<i>Pseudomonas aeruginosa</i>	Absent

Figure 7: Diffusion study for oral bio accessibility of *Sitadi Agada*



On oral administration of *Sitadi Agada*, its absorption starts at 15 minutes at pH 4 which is present in saliva. Hence it can be interpreted that the absorption of *Sitadi Agada* starts from mouth. The absorption of the drug increases after 30 minutes and attains a peak level at 2 hours. Hence the best effect of *Sitadi Agada* can be observed in 2 hours after its administration at pH 7. Hence it can be stated that the maximum absorption of the drug takes place in stomach. After 2 hours the absorption is reduced and the level is maintained up to 6 hrs at pH 9.2. It indicates the absorption from intestine which is minimum. After 6-7hrs, the drug is eliminated. Hence from this diffusion study, it shows that overall effect of *Sitadi Agada* lasts for 6hrs. Hence the drug can be administered three times a day to maintain the effect of the drug in body to achieve desired results.

The samples were collected at different intervals maintaining the pH present in stomach, duodenum, small and large intestine. It is observed that the absorbance is more at 2 hrs. It indicates that *Sitadi Agada* is absorbed more from stomach in comparison with duodenum, small and large intestines.

Discussion

The analytical evaluation of *Sitadi Agada* provides crucial insights into its physicochemical stability, organoleptic acceptability, and pharmaceutical suitability as a herbo-mineral formulation. Such standardization is essential to ensure quality, reproducibility, and safety prior to subjecting the formulation to pharmacological or clinical evaluation.

The *Sitadi Agada* tablets were buff-colored, with a characteristic odor and a sweet taste. These features correspond to the presence of *Sharkara* (sugar candy), *Madhuka* (*Glycyrrhiza glabra* Linn.), and *Draksha* (*Vitis vinifera* Linn.), which are known to impart palatability. Organoleptic parameters are not only indicative of the integrity of raw materials but also enhance patient acceptability, an important consideration in formulations intended for conditions associated with anxiety and fear, such as *Shankavisha*.

Moisture content, expressed as loss on drying, was found to be 0.59%, indicating minimal residual moisture. This suggests stability against microbial growth and degradation during storage. The total ash value (4.82%) reflects the presence of inorganic constituents, consistent with the herbo-mineral composition of the formulation. Acid-insoluble ash (0.5%) remained within

acceptable limits, suggesting minimal siliceous or extraneous matter contamination.

The extractive values provide an indication of the proportion of active phytoconstituents available in different solvents. The water-soluble extractive value (31.42%) was substantially higher than the alcohol-soluble extractive value (14.53%), implying the predominance of water-soluble bioactive principles. This observation is in line with the traditional administration of the formulation with aqueous vehicles (*Kwatha*) and supports its pharmacological relevance in hydrophilic media. The pH value (6.8) suggests near-neutrality, which is favorable for oral administration, ensuring minimal gastric irritation.

Tablet evaluation revealed a disintegration time of 3 minutes 30 seconds, signifying rapid breakdown and release of active constituents in the gastrointestinal milieu. The hardness of 3.5 kg/cm² and friability of 0.5% suggest that the tablets possessed adequate mechanical strength while maintaining desirable disintegration characteristics. These findings demonstrate the balance between structural integrity and bioavailability.

Microbiological testing revealed the complete absence of bacterial and fungal contaminants, including *E. coli*, *Salmonella*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. This absence of pathogenic organisms highlights the hygienic preparation and stability of the formulation, confirming its suitability for oral administration in accordance with WHO guidelines for herbal medicines (11).

The Franz cell diffusion study (Fig. 7) demonstrated the diffusion and release profile of *Sitadi Agada*, providing a preliminary indication of its oral bio accessibility. The presence of water-soluble phytoconstituents such as flavonoids, saponins, and glycosides in *Draksha*, *Shatavari*, and *Madhuka* may have contributed to the observed diffusion. Moreover, purified sulphur (*Shuddha Gandhaka*) may enhance the stability and absorption of other constituents through synergistic interactions. The findings align with earlier reports that hydrophilic herbal compounds exhibit greater release and permeability in diffusion models, supporting their potential systemic availability. These findings are consistent with earlier experimental evidence supporting Franz diffusion models as reliable predictors of *in vitro* permeability for herbal formulations (12).

Collectively, the analytical results suggest that *Sitadi Agada* is a pharmaceutically stable, safe, and bio accessible formulation. The predominance of water-soluble constituents, rapid disintegration, and absence of microbial contamination enhance its therapeutic plausibility for clinical conditions associated with pseudo-snakebite (*Shankavisha*) and anxiety-related manifestations. Furthermore, the diffusion study provides a scientific basis for its potential systemic effects, bridging classical Ayurvedic claims with modern pharmaceutical validation.

Conclusion

The present study evaluated the organoleptic, physicochemical, microbial, and bio accessibility characteristics of *Sitadi Agada*, a classical herbo-mineral Ayurvedic formulation indicated in *Shankavisha* (pseudo-snakebite). The formulation exhibited favorable parameters, including low moisture content, acceptable

ash values, predominance of water-soluble extractives, near-neutral pH, and rapid disintegration, all of which complied with pharmacopoeial standards. Microbiological analysis confirmed the absence of pathogenic contaminants, underscoring the safety and quality of the preparation.

The Franz diffusion study further demonstrated the release of water-soluble phytoconstituents, supporting the potential bio accessibility of active components upon oral administration. These findings provide preliminary scientific validation of *Sitadi Agada* and highlight its relevance as a safe, pharmaceutically stable, and bioaccessible formulation for conditions characterized by anxiety and fear-induced manifestations resembling snakebite.

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