Research Protocol

DOI: 10.7860/JCDR/2026/79861.22546

Ayurveda Section

Comparative Quality Control and In-vitro Antioxidant Evaluation of *Shramahara-Mahakashaya* and its Modified Anti-fatigue Drink: A Research Protocol

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ABSTRACT

Introduction: Nutraceuticals, derived from medicinal plants, marine organisms, vegetables, and fruits, are gaining popularity due to their health benefits, including antioxidant properties that combat oxidative stress. In India, the Food Safety and Standards Act of 2006 regulates this industry, promoting nutraceuticals over conventional medicine due to their fewer side effects. Ayurvedic formulations, such as the ten dravyas of *Shramahara Mahakashaya* (SMK), are known for relieving fatigue by providing essential nutrients. Fatigue, often linked to depressive disorders, responds slowly to traditional treatments, highlighting the need for new therapeutic approaches. Electrolytes are vital for cellular functions, and imbalances can lead to serious health issues.

Need of the study: There is an ongoing need to explore ways to expand the range of available therapeutic alternatives. Consumer demand for foods containing physiologically active compounds, particularly antioxidants that help the body combat oxidative stress, has increased due to rising health awareness. Improved therapeutic outcomes can be obtained by combining several medicinal components in a single formulation. *Shramahara* Drink (SD), also known as the "Antifatigue Drink," can be recommended to patients of all ages, from young children to the elderly, as an alternative to other unappealing supplements on the market because of its fruity flavour.

Aim: To modify the dosage of SMK into a drinkable form (SD) to alleviate fatigue and to conduct comparative quality control assessments along with in-vitro evaluations of Antioxidant Activity (AA).

Materials and Methods: This in-vitro study will be conducted at the Central Research Laboratory of JNMC, Datta Meghe Institute of Higher Education and Research, Sawangi (Meghe), Wardha, Maharashtra, India, from July 2025 to November 2025. The drugs required for the preparation of the Shramahara (Antifatigue) Drink will be procured from Dattatraya Ayurved Rasashala, Wardha, and the drink will be prepared in the Department of Rasa Shastra and Bhaishajya Kalpana, Mahatma Gandhi Ayurved College Hospital Research Centre, Salod (H), Wardha. Organoleptic and physicochemical parameters will be examined, and AA of the Shramahara Drink will be assessed using in-vitro methods. One-way Analysis of Variance (ANOVA) will be employed for statistical analysis, and a p-value of <0.05 will be considered statistically significant.

Keywords: Electrolyte imbalance, Fatigue, Nutraceuticals, Oxidative stress

INTRODUCTION

Rasashastra and *Bhaishajya Kalpana*, essential branches of Ayurveda, involve the preparation of various dosage forms [1], including *Mahakashaya*, a subclass of *Kaas-haradi Varga* [2]. Ayurveda frequently utilises polyherbal formulations, combining multiple medicinal plants to enhance therapeutic effects and reduce toxicity [3]. *Bhaishajya Kalpana* focuses on processing drugs to make them more palatable, potent, and longer-lasting. The *Charak Samhita*, a foundational Ayurvedic text, classifies medicinal plants into pharmacotherapeutic groups based on their primary actions [4]. In the chapter *Shadavirechana shatashiritiya*, *Charak* categorises plants into 50 classes known as *Dashamani* and *Mahakashaya*. The ten dravyas of SMK, including grapes and other fruits, are rich in minerals and antioxidants, offering a synergistic effect that helps balance electrolytes [2].

Among the main Ayurvedic dosage forms in *Bhaishajya Kalpana*, *Kwatha Kalpana* is the most frequently used, followed by *Hima*, *Phanta*, *Kalka*, and *Swarasa*. *Kwatha*, a liquid made by boiling the coarse powder of specific *dravyas* with water, serves as a base for several dosage forms, including syrups and pills [5]. Sugar is often used as a preservative in *Sharkara Kalpana*, which provides sweetness and viscosity while inhibiting microbial growth due to its high osmotic pressure [6].

Nutraceuticals, including dietary supplements and herbal bioactive compounds, are used to enhance health, delay ageing, prevent chronic diseases, and support bodily functions [7]. Their popularity is driven by increased awareness of the health benefits of biologically active compounds, particularly antioxidants, which stabilise free radicals and protect cells from oxidative stress, thereby improving overall health [8].

The ten dravyas of SMK can alleviate fatigue by providing essential sugars and nutrients [2]. Fatigue is a common issue in both community and clinical settings and often responds slowly to traditional treatments [9]. It is associated with neurotransmitters like serotonin, norepinephrine, dopamine, and histamine. Existing therapies for conditions like cancer-related fatigue are often inadequate, highlighting the need for new strategies.

Electrolytes are crucial for life, maintaining electrical neutrality in cells. Imbalances can disrupt bodily functions and lead to severe complications [10]. SMK is being adapted into a drinkable form to enhance bioavailability and convenience. Liquid formulations are generally absorbed more rapidly than solid forms, allowing for a quicker

onset of therapeutic effects, making SMK drinks particularly suitable for individuals seeking immediate relief from fatigue and stress.

Integration into daily routines: Drinkable SMK can be easily incorporated into daily wellness routines, such as post-exercise recovery or stress-relief rituals, enhancing adherence and consistent usage. Integrating this formulation into contemporary research can further substantiate its therapeutic potential, particularly in addressing fatigue and anxiety [11].

Individual components and benefits:

Draksha (*Vitis vinifera L.*): Known for its antioxidant properties, it helps reduce oxidative stress, thereby mitigating fatigue and promoting overall wellbeing.

Kharjura (*Phoenix dactylifera L.*): Rich in natural sugars and essential nutrients, it acts as a quick and effective energy enhancer, restoring depleted energy levels and alleviating fatigue.

Priyala (*Buchanania latifolia Roxb.*): Exhibits rejuvenative properties, supporting tissue repair and enhancing stamina.

Badara (*Zizyphus mauritiana Lamk.*): Possesses soothing properties that help ease anxiety and support deep, restful sleep.

Dadima (*Punica granatum Linn.*): Acts as a natural cardiac tonic, enhancing heart health and circulation, essential for maintaining optimal energy and vitality.

Phalgu (*Ficus carica Linn.*): Provides natural cooling effects that calm the nervous system and relieve stress-related fatigue.

Parushaka (*Grewia asiatica Linn.*): Also known as *Phalsa*, it is valued for its nutritive and rejuvenating properties, enhancing stamina and physical resilience.

Ikshu (*Saccharum officinarum L.*): Delivers instant energy and serves as a natural revitaliser.

Yava (*Hordeum vulgare Linn.*): A nourishing grain that promotes digestive health and ensures a steady, sustained release of energy.

Shashtika (*Oryza sativa Linn.*): A soft and easily digestible rice variety that supplies vital nutrients supporting overall vitality and wellbeing [12].

The specific benefits of the drinkable SMK include anti-anxiety and neuroprotective effects.

REVIEW OF LITERATURE

Fatigue is a complex symptom with multiple potential causes, including poor sleep habits, lack of physical activity, excessive exertion, mental health issues, and nutritional deficiencies, all of which can reduce energy levels. SMK reduces oxidative stress in neuronal cells, decreases apoptosis, and enhances antioxidant enzyme levels, leading to improved behaviour in anxiety models [13]. Its immunomodulatory properties, due to its rich nutrient and antioxidant content, contribute to enhanced immune response, supporting overall wellbeing and resilience against stressors [14].

In Ayurvedic terms, SMK is believed to restore *ojas*, the vital energy essential for maintaining health and vitality. By replenishing *ojas*, SMK combats fatigue and promotes overall vigour [15]. Additionally, the herbs in SMK support digestive health and metabolic balance, further enhancing its anti-fatigue effects.

Transforming SMK into a drinkable form represents a harmonious blend of ancient wisdom and modern convenience. This adaptation preserves the formulation's traditional therapeutic benefits while enhancing its applicability in today's fast-paced lifestyle, offering a natural solution for managing fatigue, stress, and supporting overall health [16]. Proper evaluation and treatment can help alleviate fatigue and improve general wellbeing [17].

The study conducted by Jayaprakasha GK et al., reported that grape seed extracts are rich in phenolic compounds, including catechins, epicatechins, and procyanidins, which contribute to potent AA [18].

Biglari F et al., concluded that among eight date fruit varieties from Iran, the dry date variety exhibited the highest AA, Total Phenolic Content (TPC), and Total Flavonoid Content (TFC) [19]. Singh RP et al., demonstrated that antioxidant-rich fractions extracted from pomegranate (*Punica granatum*) peels and seeds using ethyl acetate, methanol, and water showed significant AA in in-vitro models such as β-carotene-linoleate and 1,1-Diphenyl-2-Picryl Hydrazyl (DPPH) [20]. Aljane F et al., found that figs, particularly dark varieties, contain high levels of bioactive substances and antioxidants, making them a promising alternative for pharmaceutical and food applications [21]. Duarte-Almeida JM et al., reported that natural phenolic antioxidants present in sugarcane juice could be useful in mitigating oxidative stress [22].

While antioxidant-rich beverages are associated with various health benefits, further research is needed to fully understand their efficacy and safety for clinical applications. This study aims to develop a new, palatable formulation rich in antioxidants and minerals to promote overall health and address fatigue and electrolyte imbalances.

Primary objectives:

- To transform the traditional SMK into a drinkable form (SD) that is palatable and maintains the therapeutic efficacy of the original formulation.
- To conduct comparative quality control evaluations between the traditional SMK and the newly developed SD to ensure consistency, safety, and efficacy.

Secondary objectives:

- To assess the AA of the SD using in-vitro methods such as DPPH assays to determine its potential in alleviating fatigue and oxidative stress.

Hypotheses:

Null Hypothesis (H0): The modified SMK drink does not exhibit significantly enhanced in-vitro AA and does not meet established quality control standards, rendering it ineffective as an anti-fatigue intervention.

Alternate Hypothesis (H1): The modified SMK drink exhibits significantly enhanced in-vitro AA and meets established quality control standards, making it an effective anti-fatigue intervention.

MATERIALS AND METHODS

The experimental in-vitro study will be conducted in the Central Research Laboratory of JNMC, Datta Meghe Institute of Higher Education and Research, Sawangi (Meghe), Wardha, Maharashtra, India, from July 2025 to November 2025. Ethical approval was obtained from the Institutional Ethics Committee (IEC) (MGACH&RC/IEC/June-2024/856).

The research drugs will be procured from Cotex Laxmi Private Limited, Ayurveda Rasashala Salod (H) Wardha, or another authentic source. Identification of raw drugs will be performed by the Dravyaguna Department, MGACHRC, and authentication of the raw herbal drugs will be conducted at FRLHT, Bengaluru. Raw drugs will be standardised according to the Ayurvedic Pharmacopoeia of India (API), ensuring the authenticity, purity, and quality of herbal ingredients used in Ayurvedic medicine. A list of ingredients used for SMK and the *Shramahara* (Antifatigue) Drink, along with their proportions and properties, is illustrated in [Table/Fig-1,2] [23-26].

Drug preparation method:

1. **Preparation of SMK [27]:** *Shramahara Kwatha* will be prepared as described in the *Charaka Samhita* (*Purvardha*). Equal amounts (5 grams each) of the ten specified raw materials will be pounded into a coarse powder. In a stainless-steel vessel, 16 times the total weight of the powdered ingredients (i.e., 800 mL of potable water) will be added. The mixture will be heated and allowed to boil over a medium flame until the volume reduces to one-eighth (approximately 100 mL) of its original volume. After boiling, the decoction will be filtered through a sterile cotton cloth. The resulting filtrate will be utilised as *Kwatha* (herbal decoction) for all medicinal purposes.

S. No.	Name of drugs [23]	Latin Name	Part Used	Proportion
1.	*Badar*	*Zizyphus mauritiana Lamk.*	Fruit *(Phala)*	1 Part
2.	*Dadima*	*Punica granatum Linn.*	Fruit *(Phala)*	1 Part
3.	*Draksha*	*Vitis vinifera L.*	Fruit *(Phala)*	1 Part
4.	*Ikshu*	*Saccharum officinarum L.*	Stem *(Vrunta)*	1 Part
5.	*Kharjura*	*Phoenix dactylifera L.*	Fruit *(Phala)*	1 Part
6.	*Phalgu*	*Ficus carica Linn.*	Fruit *(Phala)*	1 Part
7.	*Phalsa*	*Grewia asiatica Linn.*	Fruit *(Phala)*	1 Part
8.	*Priyal*	*Buchanania latifolia Roxb*	Seed *(Beeja)*	1 Part
9.	*Shashtika rice*	*Oryza sativa Linn.*	Seed *(Beeja)*	1 Part
10.	Water	*Aqua pura*	16-times added	1/8 reduced
11.	*Yav*	*Hordeum vulgare Linn.*	Seed *(Beeja)*	1 Part

[Table/Fig-1]: The ingredients and proportions of *Shramahara Mahakashya* and antifatigue drink [23].

S. No.	*Dravya*	*Rasa* (Taste)	*Guna* (Qualities)	*Virya* (Potency)	*Vipaka* (Post-digestive taste)	*Karma* (Therapeutic action)
1.	*Badar* [24] *(Zizyphus mauritana Lamk)*	*Madhura* (Sweet), *Amla* (Sour), *Kashaya* (Astringent)	*Picchila* (Slimy), *Guru* (Heavy), *Snigdha* (Unctuous)	*Sheeta* (Cold)	*Madhura* (Sweet)	*Vata-pittashamak, Brimhana, Shukrala, Dahashamak, Grahi, Trishnahara, Ruchikara, Malabhedaka, Agnivardhaka.*
2.	*Dadima* [24] *(Punica granatum Linn.)*	*Madhura* (Sweet), *Amla* (Sour), *Kashaya* (Astringent)	*Laghu* (Light), *Snigdha* (Unctuous)	*Ushna* (Hot)	*Madhura* (Sweet), *Amla* (Sour)	*Tridosha shamak, Ruchya, Grahi, Kanthya, Lekhana, Vishaghn and Vatanulomaka.*
3.	*Draksha* [24] *(Vitis vinifera L.)*	*Madhura* (Sweet)	*Snigdha* (Unctuous), *Guru* (Heavy).	*Sheeta* (Cold)	*Madhura* (Sweet)	*Vaatpittashamak, Deepana, Pachana, Stambhan, Dahaprashaman, Chardinigraha.*
4.	*Ikshu* [25] *(Saccharum officinarum L.)*	*Madhura* (Sweet)	*Guru* (Heavy), *Snigdha* (Unctuous)	*Sheeta* (Cold)	*Madhura* (Sweet)	*Vaatpittashamak. Saarak,Hrudya, Raktapittashamak, Vrushya, Stanyajanan, Mutral, Balya, Bruhana.*
5.	*Kharjura* [24] *(Phoenix dactylifera L.)*	*Madhura* (Sweet)	*Guru* (Heavy), *Snigdha* (Unctuous)	*Sheeta* (Cold)	*Madhura* (Sweet)	*Vata-pittashamak Anulomana, Raktashodhak, Mutral, Vrushya, Balya, Bruhana and Dahaprashmana*
6.	*Phalgu* [24] *(Ficus carica L.)*	*Madhura* (Sweet)	*Guru* (Heavy), *Snigdha* (Unctuous)	*Sheeta* (Cold)	*Madhura* (Sweet)	*Vaatpittashamak Vranshothahara, Raktashodhak, Varnya, Daahprashaman, Visphotshamak, Mutral, Vrushya, Balya, Bruhana.*
7.	*Phalsa* [24] *(Grewia asiatica Linn.)*	*Madhura* (Sweet)	*Laghu* (Light), *Snigdha* (Unctuous)	*Sheeta* (Cold)	*Madhura* (Sweet)	*Pittavaathara, Hridya, Pittahara, Brihana, Vishtambhi.*
8.	*Priyal* [24] *(Buchanania latifolia Roxb)*	*Madhura* (Sweet)	*Snigdha* (Unctuous), *Guru* (Heavy) *Sara* (Flowing)	*Sheeta* (Cold)	*Madhura* (Sweet)	*Vaatpittashamak Kushthaghna, Varnya, Shothahara Raktaprasadak, Hrudya*
9.	*Shashtik Rice/Sali [fruit]* [26] *(Oryza sativa Linn.)*	*Madhura* (Sweet), *Anuras Kashaya* (Astringent)	*Snigdha* (Unctuous), *Laghu* (Light)	*Sheeta* (Cold)	*Madhura* (Sweet),	*Pittahara. Hrudya, Ruchikara, Vrushya, Mutral, Vishaghna, Bhramma, Swarya.*
10.	*Yava* [25] *(Hordeum vulgare Linn.)*	*Kashaya* (Astringent), *Madhur* (Sweet)	*Ruksha* (Dry), *Guru* (Heavy), *Picchila* (Slimy), Mrudu (Soft)	*Sheeta* (Cold)	*Katu* (Pungent)	*Pittakaphahara,Medahara,Balya, Vrushya,Swarya, Varnya, Sthairyakara, Purishakruta, Mutrahara, Lekhana*

[Table/Fig-2]: The ingredients and properties of *Shramahara Mahakashya* [24-26].

2. **Preparation of *Shramahara* Drink (SD) [28]:** All ingredients will be taken in equal proportions. Sixteen times the quantity of potable water will be added, and the mixture will be boiled over a medium flame until the volume reduces to one-eighth. The decoction will then be filtered. To the filtered decoction (*Kwatha*), 67% sugar will be added and dissolved. This mixture will be heated again over a medium flame until it reaches the *Tantupaka Avastha*, characterised by a one- to two-thread-like consistency, resulting in a syrupy texture similar to honey. After cooling, the syrup will be filtered and stored in dry, sealed bottles at a temperature not exceeding 25°C, in a cool, dark location.

Primary outcomes: Quality control parameters: Both SMK and SD will be evaluated and compared based on the following analytical parameters:

A. Organoleptic characters:

1. Colour
2. Taste
3. Odour
4. Touch
5. Visual appearance

B. Physico-chemical parameters [29]:

1. Viscosity: Measures a liquid's resistance to flow, influencing the consistency and stability of herbal formulations. Consistent viscosity ensures uniform dosing and affects the mouthfeel of liquid preparations.
2. Density: Mass per unit volume of a substance. Determining density helps ensure batch-to-batch consistency and detect adulteration or variations in composition.
3. Solubility: Ability of a substance to dissolve in a solvent. Assessing solubility is essential for understanding bioavailability and selecting appropriate extraction solvents.
4. pH: Measures the acidity or alkalinity of a solution. pH can influence the stability of active constituents and shelf life, and maintaining an appropriate pH ensures the efficacy and safety of the formulation.
5. Moisture content: Indicates the amount of water present in a substance. High moisture levels can promote microbial growth, leading to spoilage. Controlling moisture is vital for product preservation and longevity.
6. Water-soluble extract: Measures the number of constituents in a plant material soluble in water, providing an estimate of water-soluble active compounds such as glycosides, tannins, and sugars, indicating the potential efficacy of aqueous extracts.
7. Alcohol soluble extract: Determines the quantity of constituents soluble in alcohol, evaluating the presence of alcohol-soluble compounds like resins, alkaloids, and certain glycosides, which contribute to the therapeutic action.
8. Total ash: Represents the total amount of inorganic residues remaining after complete incineration of the plant material. It indicates mineral content and helps detect adulteration with inorganic substances such as sand or soil.

Total solids: Represents the combined content of all solid substances in the beverage, including sugars, minerals, and other

soluble and insoluble components. It is determined by evaporating the water content and weighing the residue.

Brix Value (°Bx): Measures the total soluble solids, primarily sugars, in the beverage. One degree Brix corresponds to 1 gram of sucrose in 100 grams of solution. A refractometer will be used for this measurement.

Total/reducing sugar: Quantifies the amount of sugars present. Total sugars include all types, while reducing sugars (e.g., glucose and fructose) can participate in Maillard reactions, affecting flavour and colour. These are typically measured using titration methods.

Weight per mL (density): Determines the mass of the beverage per unit volume, which can influence mouthfeel and packaging requirements. This is measured using a densitometer or hydrometer.

Microbial contamination: Assesses the presence of microorganisms such as bacteria, yeasts, and molds to ensure the beverage's safety and shelf-life. Standard microbiological methods will be employed for this evaluation.

Shelf life: Estimates the duration the beverage remains safe and retains its desired sensory, chemical, and microbiological qualities under specified storage conditions. Shelf-life studies involve periodic testing over time.

HPTLC: High-Performance Thin-Layer Chromatography (HPTLC) plays a crucial role in identifying adulteration and assessing the quality of drugs. By separating various chemical components, the Rf values will be calculated after spot detection to establish the drug's identity, purity, and potency.

Secondary outcomes:

Experimental procedure:

A. **Antioxidant analysis (DPPH assay) [30]:** The AA of the *Shramahara* (antifatigue) drink will be evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. A 0.1 mM DPPH solution in methanol will be prepared fresh daily and protected from light. Various concentrations of the drink extract, typically ranging from 10 to 160 µg/mL, will be prepared. Each test sample (100 µL) will be mixed with an equal volume of DPPH solution and incubated in the dark at room temperature for 30 minutes.

Absorbance will be measured at 517 nm using a spectrophotometer. Controls will include a blank (methanol only), a negative control (DPPH solution without sample), and positive controls using known antioxidants such as ascorbic acid and Trolox. The percentage of DPPH radical scavenging activity will be calculated using the formula:

(RSA)% inhibition=(Absorbance of (DPPH) control-Absorbance of sample) Absorbance of (DPPH) control×100.

The IC_{50} value (half maximal inhibitory concentration), representing the concentration of the sample required to scavenge 50% of the DPPH radicals, will be determined by plotting the percentage inhibition against the sample concentrations and performing regression analysis. This method provides a reliable measure of the antioxidant capacity of the drink and ensures reproducibility across experiments.

B. **Nutritional values:** Nutritional values will be determined following the nutraceutical investigation as illustrated in [Table/Fig-3].

STATISTICAL ANALYSIS

All experimental data will be analysed using Statistical Package for the Social Sciences (SPSS) version 17.0. Mean±Standard Deviation (SD) will be calculated for all measured parameters, including organoleptic, physicochemical, and antioxidant outcomes. One-way ANOVA will be employed to compare the AA of different concentrations of SD samples. Post-hoc analysis (Tukey's HSD or Bonferroni) will be conducted if ANOVA yields statistically significant results (p-value <0.05). A p-value <0.05 will be considered statistically significant. Regression analysis will be used to determine the IC50 value from the DPPH assay data by plotting percentage inhibition against concentration and fitting a non-linear or linear regression model.

S. No.	Parameter	Unit
1.	Protein	%
2.	Fat	%
3.	Carbohydrate	%
4.	Total minerals	%
5.	Dietary fiber	%
6.	Total energy	Kcal/100 gm
7.	Folic acid (Vitamin B-9)	mg/100 mL

[Table/Fig-3]: The parameters and units of nutritional values.

Study guidelines: The study will follow the CRIS guidelines.

Acknowledgement

The authors thank the researchers whose papers were cited and discussed for their invaluable contributions. The authors also extend gratitude to all editors, journals, and publications consulted during the literature review.

Dissemination: The results of this study will be published in indexed journals.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: May 05, 2025
- Manual Googling: Aug 02, 2025
- iThenticate Software: Aug 04, 2025 (6%)

ETYMOLOGY: Author Origin

EMENDATIONS: 6

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval Obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? No
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Apr 11, 2025**
Date of Peer Review: **May 06, 2025**
Date of Acceptance: **Aug 06, 2025**
Date of Publishing: **Mar 01, 2026**