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Research Article

# ESTIMATION OF NUTRITIVE VALUE OF "NANNARI KANJI" AND EVALUATION OF ITS ANTI-BACTERIAL ACTIVITY AGAINST *STAPHYLOCOCCUS AUREUS*, A RESPIRATORY PATHOGEN BY *IN VITRO* METHOD

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**ABSTRACT**

**Objective:** To estimate the nutritive value of Nannari Kanji. To evaluate the beneficial effect of Nannari Kanji against the respiratory pathogen, *Staphylococcus aureus*. To analyze the minerals present in Nannari Kanji.

**Methods:** Nannari Kanji is a polyherbal formulation prepared using Nannari, Karunguruvai rice, and coconut milk by simple boiling method. In the present study, the Nutritive value of Nannari Kanji was analyzed and its antimicrobial activity was studied in terms of minimum inhibitory concentration against the respiratory pathogen, *S. aureus*. In addition to that mineral analysis was performed using the ICP-OES instrument.

**Results:** In the nutritive analysis, protein (26.83%) was present in large amounts. The antimicrobial activity showed an inhibitory effect against *S. aureus* at the concentration of 527.02 µg/mL. The mineral analysis confirms the presence of magnesium, iron, and zinc of which magnesium shows a higher value (192.06 ppm).

**Discussion and Conclusion:** Nannari Kanji has potent antimicrobial activity against *S. aureus.* It has more protein and fat content, which is useful in suppressing respiratory infections. Nannari Kanji contains good magnesium content which is considered to be a good antimicrobial agent.

**Keywords:** Nannari Kanji, Respiratory infection, Nutritional value, Siddha medicine.

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## INTRODUCTION

The Siddha system is a unique system of medicine that ultimately aims to maintain three vital humors and seven physical constituents in equilibrium. In the Siddha system of medicine, diet and way of life assume a significant part in treating and forestalling illnesses [1]. Food serves as a vital nutritional and healing source. It is valuable in recapturing the decreased strength during the post-treatment time. A number of disorders occur due to improper dietary practices that affect the balance of the three humors (uyir thaathukkal) and physical elements (udal thaathukkal).

*"Unavae marunthu, marunthae unavu"*

This verse says that diet can act as medicine and explains the importance of diet. *Kanji* is one of the most popular and regular foods which has been in use since olden times obtained by boiling rice with different kinds of grains from which they derive their names and accordingly, there are 27 types of *Kanji* [2]. In particular for all children, kanji is a very well-balanced diet. It is a very good immune system builder. It contributes to the maintenance of a healthy lifestyle and helps protect us from diseases by boosting immunity [3]. It promotes healthy digestion and cleans the body inside. A polyherbal formulation Nannari Kanji mentioned in *Brahmamuni Maruthuva Vilakam* is a dietary supplement indicated for respiratory and gastric ailments. Nannari Kanji was made using Nannari, Karunguruvai rice, and coconut milk by simple boiling method [4].

According to disability-adjusted life years, which measure the burden of sickness around the world, 41% of it is still related to infectious diseases. Nearly 545 million people worldwide had a chronic respiratory condition in 2017, an increase of 39.8% since 1990, according to the Lancet Respiratory Medicine. About 3.9 million people died from chronic respiratory disorders in 2017 (an increase of 18% from 1990) [5].

*Staphylococcus aureus* is a Gram-positive, spherical-shaped bacterium that is typically found in the body's microbiota and on the skin and upper respiratory tract . Despite being a frequent source of food poisoning, sinusitis, and other respiratory illnesses as well as skin infections such as abscesses [6]. There had previously been no other work done in Nannari Kanji. Hence, this study is aimed to evaluate the nutritive value of Nannari Kanji, exploring its antimicrobial activity against the respiratory pathogen, *S. aureus,* and analyzing the elements present in Nannari Kanji.

## METHODS

### Drug profile

The raw drugs for the Nannari Kanji preparation were purchased from a well-reputed country raw drug shop and drugs were authenticated by the Assistant Professor, Department of Medicinal Botany, National Institute of Siddha, Chennai, Tamil Nadu. After that the drugs were purified separately then the Nannari Kanji was prepared in the Gunapadam Laboratory of the National Institute of Siddha as per the literature. The ingredients of Nannari Kanji are Nannari root (*Hemidesmus indicus*), old *Karunguruvai* rice (black paddy rice), water, and coconut milk (Fig. 1). The root of Nannari was ground to the size of a lemon and kept aside (Fig. 2a). In an earthen pot, old Karunguruvai rice was cooked with water without the addition of salt. The ground Nannari root was added to the pot after the rice boiled up and then added the It was heated until it attains that equals the weight of *Nannari Karkam* (Fig. 2b). It was heated until it attained the consistency of porridge (Fig. 2c). It is indicated for Irumal, Illaippu, Uppisam, and Yengal [4].

**Nutritive value analysis**

*Estimation of carbohydrates by anthrone method*

From the supplied stock solution (10 mg/mL), samples of different concentrations of glucose (200, 400, 600, 800, and 1000 µg) (Table 1) were pipetted out and made up to a volume of 1 mL with distilled water (Fig. 3a). 5 mL of the anthrone reagent (2 g of anthrone dissolved in 1 L of concentrated $H_2SO_4$) was added to each tube and mixed thoroughly by vortexing. The tubes were cooled. The tubes were then placed in a boiling water bath for 10 min or incubated at 90°C for 17 min with marbles or caps on top. At normal temperature, the optical density at 660 nm was evaluated in comparison to a blank. A standard curve of glucose concentration and absorbance was created (Fig. 3b) [7].

*Estimation of protein by Lowry's method*

Reagent

a. 2% $Na_2CO_3$ in 0.1 N NaOH
b. 1% NaK Tartrate in $H_2O$
c. 0.5% $CuSO_4$.5 $H_2O$ in $H_2O$
d. Reagent I: 48 mL of A, 1 mL of B, and 1 mL of C
e. Reagent II: 1 part Folin-Phenol (2 N):1 part water.

*Procedure*

Different concentrations (20, 40, 60, 80, and 100 µg) (Table 2) of BSA working standard (1 mg/mL) were taken in five test tubes and samples (50 and 100 µg) and made up to 1 mL using distilled water. A test tube containing 1 mL of distilled water was used as a control. After adding reagent I, 4.5 mL was incubated for 10 min. 30 min of incubation followed by the addition of 0.5 mL of reagent II. The standard graph was used to measure and illustrate the absorbance at 660 nm (Fig. 4). The standard graph was used to estimate the amount of protein in the sample that was provided [8].

*Estimation of fat content*

The sample 2 g was weighed. While the sample was still heated, 0.5 mL of hexane was added. The two layers of oil and hexane (two phases) were separated by a thorough shaking. Hexane was evaporating while the tubes were held on a heated mantle. The remaining oil content was weighed and calculated [9].

*Estimation of crude fiber*

200 mL of 0.128 M sulfuric acid up to the 150 mL notch was added to 2 g of ground sample. It was preheated by the hot plate. Then it was boiled for 30 min. The solution was filtered onto a separate apparatus

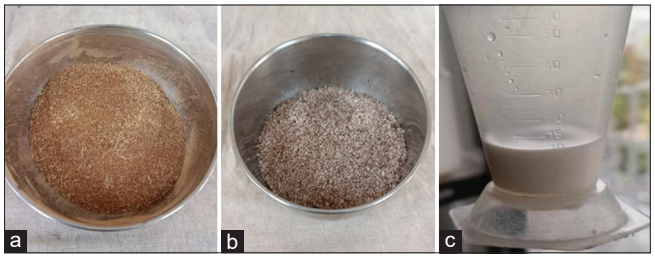


**Fig. 1: Ingredients of Nannari Kanji (a-c). (a) Nannari root powder. (b) Karunguruvai rice powder. (c) Coconut milk**

**Table 1: Absorbance at different concentrations of carbohydrates**

| Concentration (µg/ml) glucose | Absorbance at 660 nm |
|---|---|
| 20 | 0.09 |
| 40 | 0.14 |
| 60 | 0.24 |
| 80 | 0.34 |
| 100 | 0.40 |

**Table 2: Absorbance at different concentrations of protein**

| Concentration (µg/mL) | Absorbance at 660 nm |
|---|---|
| BSA | |
| 20 | 0.02 |
| 40 | 0.05 |
| 60 | 0.07 |
| 80 | 0.09 |
| 100 | 0.13 |

**Table 3: Percentage viability of different**

| Concentration (µg/mL) | Percentage viability |
|---|---|
| 200 | 59.41 |
| 400 | 59.31 |
| 600 | 46.59 |
| 800 | 38.19 |
| 1000 | 32.79 |


**Fig. 2: Nannari Kanji preparation (a-c). (a) Nannari Karkam. (b) Boiling stage. (c) Final stage**

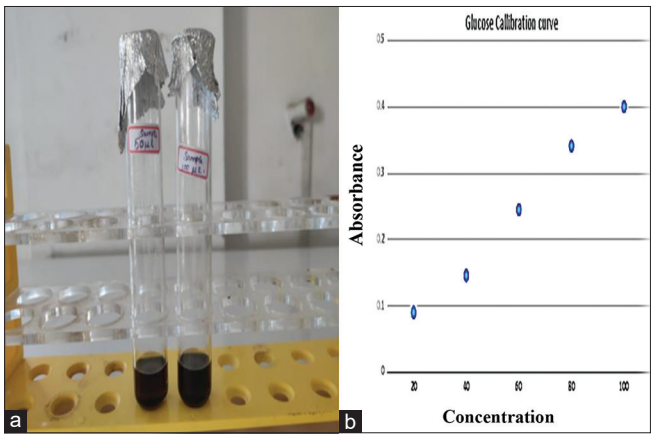


**Fig. 3: (a) Carbohydrate estimation. (b) Calibration of glucose**

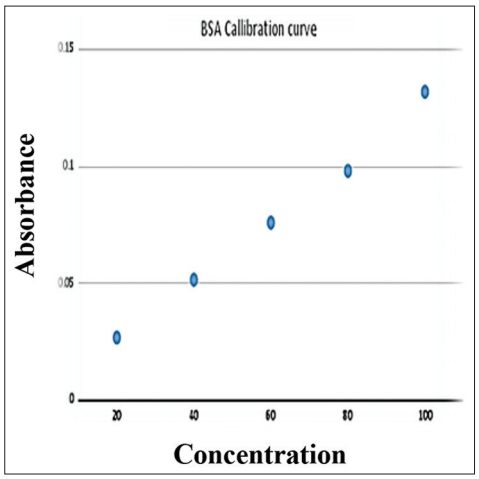


**Fig. 4: Calibration of BSA**

using a cloth. Washed 3 times with 30 mL (crucible filled up to the top) of hot deionized water. After draining the last wash, 200 mL of 0.313 M pre-heated sodium hydroxide (NaOH) was added to that. Again, it was boiled for 30 min. Then it was filtered and washed. Finally, the crucibles were cleaned with cold, deionized water to help them cool. The crucibles were removed and the dry weight after drying in an oven at 105°C for an hour or up to constant weight was determined. Cooled in a desiccator. This weight, relative to the initial weight shows the crude fiber plus ash content [10].

*Antimicrobial activity*

The sample was tested for antibacterial activity against *S. aureus* using the microdilution method. Luria broth (Himedia, Mumbai) was prepared and sterilized by autoclaving for 15 min at 121°C, 15 lbs. Each test tube received 3 mL of broth. Each sample (200 µg/mL, 400 µg/mL, 600 µg/mL, 800 µg/mL, and 1000 µg/mL) (Fig. 5a) was put in each test tube at three different concentrations. For every well, 5 µL of log phase culture was added. Azithromycin (100 µL from 10 mg/mL) was also added to the broth. After that, 5 µL of log phase culture was added (Fig. 6). This functioned as a positive control. As a control, sterile broth is used (Fig. 5b). The broth was incubated at 37°C for 24 h. The absorbance of the broth that was incubated was measured using a spectrophotometer that was set to 600 nm (Table 3). At the lowest concentration of the sample, the minimum inhibitory concentration (MIC) was determined by calculating the entire suppression of growth [11].

*Mineral analysis*

In a Teflon microwave digestion vessel, 50 mg of sample was inserted, and 1 mL of ultrapure nitric acid was added for 45 min of digestion using Anton Paar microwave digestion equipment. In a standard measuring flask, the substance was diluted to 50 mL. Using ultrapure nitric acid and a blank, the calibration standard solution was created in concentrations ranging from 0.25 g/mL to 10 g/mL (Figs. 7and 8). The RF power of the Agilent ICP-OES 5100 VDV apparatus is 1.2 kW, the plasma gas flow rate is 12 L $min^{-1}$, and the nebulizer gas flow rate is 0.70 L $min^{-1}$. A nebulizer and spray chamber are used to inject the samples into the plasma for analysis.

## RESULTS

The amount of glucose in the unknown sample was calculated by plotting a standard curve of A620 on the Y-axis and µg of glucose on the X-axis.

**Estimation of fat content**

The percentage fat (wet weight basis) was calculated as follows:

$$\text{Yield} = \frac{\text{Grams of extracted obtained}}{\text{Grams of sample used for extraction}} \times 100$$

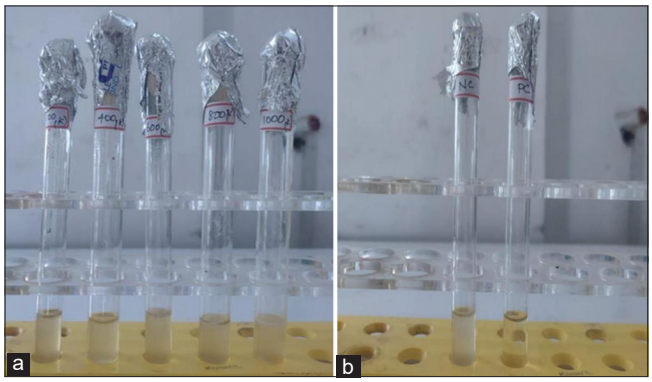


**Fig. 5: (a) Different concentrations of samples treated with *Staphylococcus aureus.* (b) Percentage viability of *Staphylococcus aureus* treated with sample**

**Estimation of crude fiber**

The percentage of crude fiber (wet weight basis) was calculated as follows:

$$\text{Yield} = \frac{\text{Grams of extracted obtained}}{\text{Grams of sample used for extraction}} \times 100$$

**Antimicrobial activity**

$$\text{Percentage viability} = \frac{\text{Number of unstained cells}}{\text{Total number of cells}} \times 100$$

**Mineral analysis**

Calibration curves should be made with standards that match the expected concentration range of the elements in your sample.

## DISCUSSION

Kanji is high in nutrition, primarily carbohydrates, proteins, fats, fiber, vitamins, and minerals, all of which aid in digestion. It is an excellent source of energy, which boosts immunity and helps to prevent disease [12]. Nannari Kanji is simple to prepare and can be included in our diet, especially for children. Nannari Kanji is suitable for children because it has no taste. Respiratory infections are more common in

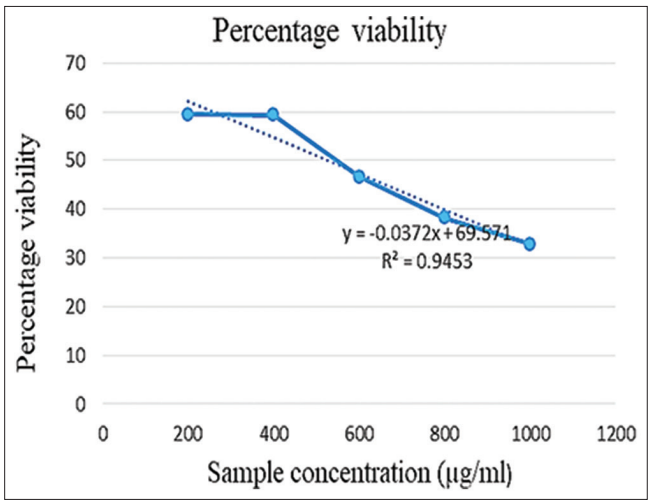


**Fig. 6: Percentage viability of *S. aureus* treated with sample**

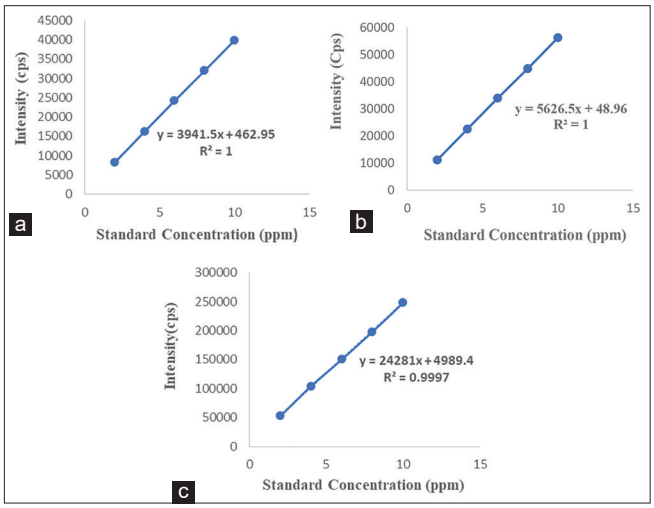


**Fig. 7: Linearity graphs of standard solutions. (a) Iron, (b) Zinc, and (c) Magnesium**

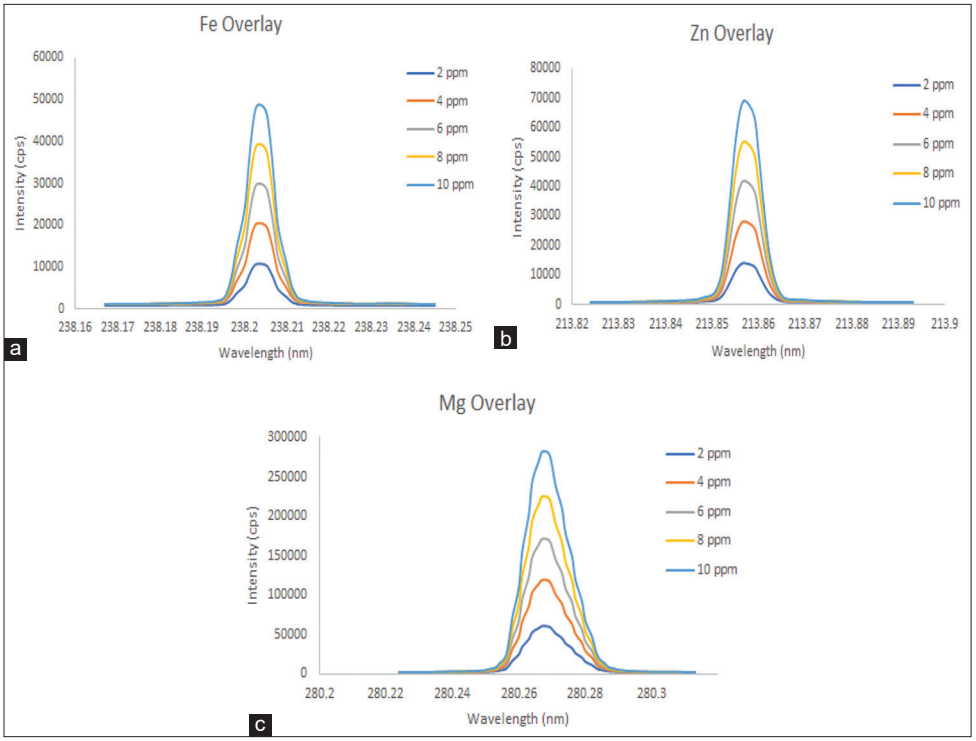


**Fig. 8: Overlaid graphs of standard solutions. (a) Iron, (b) Zinc, and (c) Magnesium**

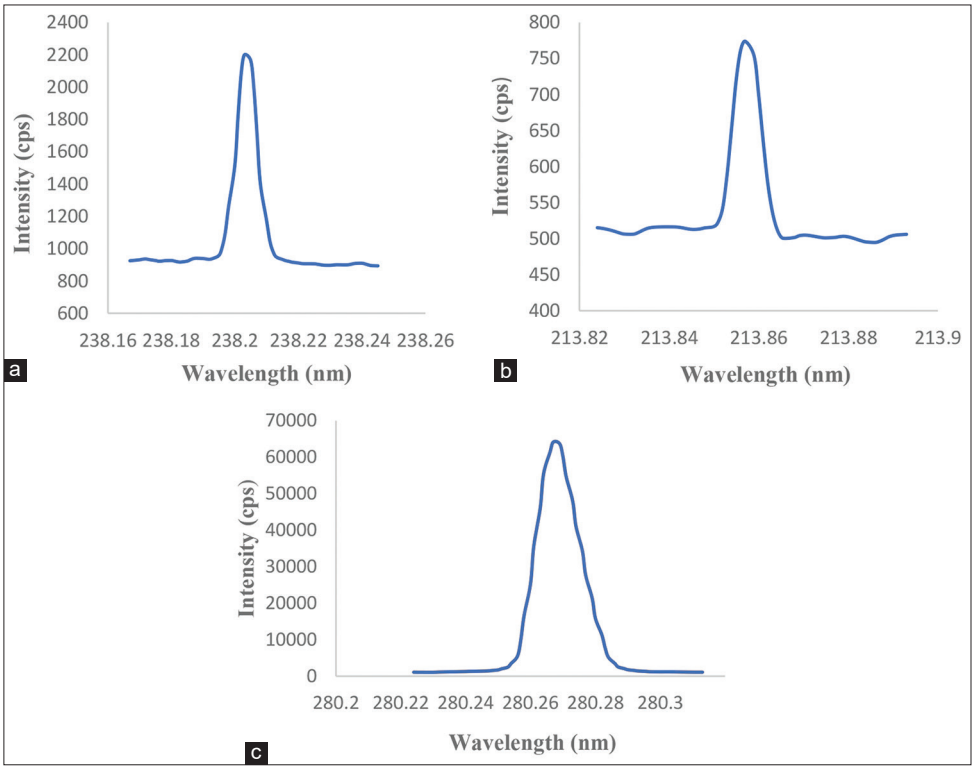


**Fig. 9: Sample Graphs for Nannari Kanji powder sample. (a) Iron, (b) Zinc, and (c) Magnesium**

**Table 4: Estimation of carbohydrate by anthrone method**

| Sample concentration | Carbohydrate concentration (µg/mg) | Carbohydrate percentage | Sample concentration | Absorbance @660 nm |
|---|---|---|---|---|
| 500µg | 72.24 | 14.44% | 500µg | 0.148 |

**Table 5: Estimations of protein by Lowry's method**

| Sample concentration | Protein concentration (µg/mg) | Protein percentage | Sample concentration | Absorbance @660nm |
|---|---|---|---|---|
| 500 µg | 134.15 | 26.83% | 500 µg | 0.087 |

**Table 6: Estimation of fat content**

| Sample concentration | Fat yield | Fat percentage |
|---|---|---|
| 3g | 0.702g | 23.4% |

**Table 7: Estimation of crude fiber**

| Sample concentration | Crude fiber yield | Crude fiber percentage |
|---|---|---|
| 2g | 0.208 g | 10.4% |

| Concentration (µg/mL) | Percentage viability |
|---|---|
| 200 | 59.41 |
| 400 | 59.31 |
| 600 | 46.59 |
| 800 | 38.19 |
| 1000 | 32.79 |

**Table 8: MIC values of a sample**

| MIC (µg/mL) | |
|---|---|
| $IC_{50}$ | 527.02 |

children. Hence, it is important to know its nutritional values and the extent of its action. Nannari (*H. indicus*) is loaded with carbohydrates and Vitamin C. In Karunguruvai rice, carbohydrates and fibers were present in large amounts. Coconut milk is rich in fats and a moderate amount of carbohydrates. Nutritional analysis of *Nannari Kanji* revealed the presence of carbohydrates (14.44%) (Table 4), proteins (26.83%) (Table 5), fat content (23.4%) (Table 6), and crude fibers (10.4%) (Table 7).

From the analysis, it is clear that Nannari Kanji has more protein when compared with other nutrients. Kanji is often high in carbohydrates because rice is the primary source of carbohydrates [13]. However, in Nannari Kanji proteins were more abundant. Several animal studies have recommended that lack of protein induces pulmonary emphysema and impaired lung development. Furthermore, there are studies that show that protein intake is related to forced vital capacity and airflow obstruction in chronic obstructive pulmonary disease (COPD) patients. In fact, 25–40% of COPD patients are malnourished, which is associated with decreased lung function and exercise intolerance [14].

Antimicrobial activity was done in Nannari Kanji against *S. aureus*. *S. aureus* is a prevalent bacterium connected with a wide spectrum of illnesses involving the respiratory tract. It is frequently colonized in the skin or in the nose that leads to pulmonary infections [15]. The *in vitro* antimicrobial activity of *Nannari Kanji* showed that the MIC ($IC_{50}$) of the drug against the organism tested was 527.02 µg/mL (Table 8). Previous research on Nannari (*H. indicus*) revealed that the methanolic extract of *H. indicus* root has antimicrobial and free radical scavenging activity [16]. All three doshas, particularly kabam and pitham, are soothed by Nannari and Karunguruvai rice. Coconut milk is high in antioxidants, which protect the body from free radical damage [17].

Analysis of the minerals present in Nannari Kanji by the ICP-OES method showed the presence of magnesium (Mg), iron (Fe), and zinc (Zn). In which magnesium (Mg) was 1569.58 ppm, iron (Fe) was 192.06 ppm, zinc (Zn) was 25.36 ppm (Fig. 9 and Table 9). Standard

**Table 9: Minerals level in Nannari Kanji powder**

| S.no | Elements | Nannari Kanji powder (parts per million) |
|---|---|---|
| 1. | Iron (Fe) | 192.06 |
| 2. | Zinc (Zn) | 25.36 |
| 3. | Magnesium (Mg) | 1569.58 |

**Table 10: Standard linearity**

| S.no | Element (µg/g) | Wavelength | $R^2$ value |
|---|---|---|---|
| 1. | Iron (Fe) | 238.20 | 1.00 |
| 2. | Zinc (Zn) | 213.85 | 1.00 |
| 3. | Magnesium (Mg) | 280.27 | 0.99 |

Linearity of the minerals were explained in Table 10. These minerals are analyzed because they are a proven source of antioxidants, have antimicrobial activity, and play an important role in the treatment of respiratory diseases. Nannari Kanji has more amount of magnesium (Mg) when compared with iron and zinc. From the previous studies, it is obvious that magnesium has bronchodilator activity and insufficient dietary magnesium intake can contribute to asthma and worse lung function in children which is proven through airway flow rates, airway hyper-reactivity, and increased risk of wheezing. Low magnesium consumption levels may lead to the development of asthma, with several research suggesting that magnesium may even protect against the development of asthma and chronic airway obstruction [18]. Magnesium deficiency may impair the activity of our white blood cells known as neutrophils, worsening asthma attacks [19].

Iron supplementation is associated with a considerable reduction in upper respiratory tract infection morbidity [20]. Iron oxide nanoparticles were tested against various bacteria (*Escherichia coli*, *Pneumonia*, *Streptococcus*, and *S. aureus*) utilizing nutrient broth and nutrient agar techniques and demonstrated considerable antibacterial activity [21]. Zinc insufficiency has been linked to an increased risk of respiratory tract morbidity. Zinc deficiency inhibits lymphocyte production, activation, maturation, disrupts intercellular communication, and reduces host defense [22].

## CONCLUSION

According to the nutritional analysis, Nannari Kanji is high in protein and fiber when compared to carbohydrates. The antimicrobial activity of Nannari Kanji revealed that it is efficient in inhibiting *S. aureus*, the most common cause of upper respiratory tract illness. The elemental analysis showed the presence of magnesium (Mg) as a major element and other microelements. The presence of these minerals, as well as a higher protein content and antibacterial action, in Nannari Kanji, aids in the suppression of respiratory illnesses and protein deficiencies.

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## AUTHOR'S CONTRIBUTIONS

Experimental design, guidance, and results interpretation were done by Dr. Sudha Revathy. Development, selection of the formulation, and

writing of this manuscript were done by Dr. S. Sowmya. The final review was done by Dr. Meenakumari.

## CONFLICT OF INTEREST

The author declares no conflicts of interest.

## AUTHORS FUNDING

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