WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 4, 1-15. Research Article ISSN 2277– 7105

PHYSICO-CHEMICAL STANDARDIZATION OF THE DRUG "NANDUKKAL BHASHMAM"

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Article Received on
22 Jan. 2020,

Revised on 12 Feb. 2020,
Accepted on 03 March 2020,

DOI: 10.20959/wjpr20204-16997

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ABSTRACT

The drugs used in Siddha Medicine is classified as internal and external medicines each consisting of 32. One efficacious nano medicine that is used in "Kalladaippu" (Urolithiasis) is "Nandukkal Bhashmam" which denotes the "bhashmam" class drug in internal medicines. The main objective of the study is to standardize the drug "Nandukkal Bhashmam" through physico-chemical analysis. As per to that "Nandukkal Bhashmam" was prepared and the physico-chemical analysis was carried out including traditional "Bhashmam pareekshai" (bhashmam examinations). According to the results obtained the organoleptic characteristics of "Nandukkal Bhashmam" was solid in nature, grey color in daylight and the smell, sound and taste were not to be found characteristic. The drug sample fulfilled important bhasmam pareekshai(Examination of bhashmam) as Nischandratvam, Rekhapurnatvam, Varitaratvam and Amila pareekshai. It was observed that, 0.4328%, a negligiable loss on drying, 99.8249% of high total ash value, 0.99% water soluble ash value and 82.26% acid soluble ash value. pH of the drug with de-ionized water is 7.91 (28.3^{o}C), 7.20 (29.5 ^{o}C) with dry *Aerva lanata* decoction and 7.04 (29.5 ^{o}C) with fresh *Aerva lanata* decoction. The water soluble extractive value of the drug "Nandukkal Bhashmam" is 0.88%, alcohol soluble extractive value of the formulation is, 0.704%, and no significant value was observed in ether soluble extractive. The bottomline of the study is that, the analysis of physico-chemical characteristics of Nandukkal Bhashmam

indicates the consequence of drug standardization before introduce into the market. Further studies should be executed to increase the safety and efficacy of the drug Nandukkal Bhashmam through sophisticated analytical instruments.

KEYWORDS: Nandukkal Bhashmam, Physico-chemical analysis, Kalladaippu.

1. INTRODUCTION

In Siddha, herbo-mineral drugs are commonly used comparatively equal to other medicines. The drugs used by "Siddhars" were classified as "Thavaram" (Herbs), "Thaathu" (Minerals), "Jeeva vaguppu" (Live stocks and their products). According to the mode of application of the drugs they are divided as Internal and External Medicines each consisting of 32. Bhashmam is one of Internal medicine thats active ingredients considered to be herbo-mineral/metals/non-metals. The part of Siddha that deals with bhashmas is called as "Rasa-shasthiram"(Vedic chemistry).[3]

In Siddha there are so many bhasmams conveyed by "Siddhars". One of the bhasmam class drug and most often used for urinary tract disorders is the "Nandukkal Bhashmam" (NKB). Main ingredients of the drug "Nandukkal Bhashmam" (NKB) are "Nandukkal" (Fossil stone crab), *Raphanus sativus* and *Aerva lanata.* It is administrated to "Neeradaippu" (Acute glomerular nephritis), "Kalladaippu" (Urolithiasis), "Sathai Adaippu" (Renal tumors) and "Neerkattu" (Anuria).[3]

As per to the Siddha medical dictionary, "kalladaippu" is defined as the large concretion of stone in the bladder or kidney calculus or gravel which results in difficulty in passing urine. The drugs that used for "Kalladaippu" and decreases the recurrence of the disease tend to be less in any medical system. It's limelight that drugs like "Nandukkal Bhashmam" should be studied further to obtain standardized drug for the wellbeing of the public.[2]

1.1. Background of the study

A large number of people are suffering from urinary stone problem all over the globe. Calcium oxalate and/or phosphate stones account for almost 70% of all renal stones observed in economically developed countries.[10]

Kidney stones are quite common and usually affect people who are between 30 and 60 years of age affect both gender, about 10-20% of men, and 3-5% of women.[10]

In most countries with a relatively high incidence of renal calculi due to climate, diet habits, local geology with hydro mineralogy and sanitation by affecting geo minerology. The researchers discovered that stone formers had a 60% greater risk of developing chronic kidney disease (CKD) and a 40% increased risk of developing end-stage renal disease (ESRD), the most severe form of CKD.[10]

1.2. Justification of the study

Bhashmams are claimed to be biologically produced nanoparticles, which are prescribed with several other medicines of Siddha. But the most important challenges faced by these formulations are the lack of complete standardization by physiochemical parameters.[3]

"Nandukkal bhashmam" is a combination of siddha drug consisting of three constituents, one of which is reported to have anti -urinary calculus properties. The safety, acceptability and efficacy of various drugs and formularies need to be documented before they can be promoted for human consumption. World Health Organization (WHO) has emphasized the need to ensure quality control of Siddha formulations by using modern techniques and by applying suitable parameters and standards (WHO, 2007). Evaluations of physicochemical parameters are essential to standardize the siddha formulations. Analytical specifications of bhashmam are given in Protocol for testing Ayurvedic, Unani and Siddha medicines. According to this, "Nandukkal bhashmam" was subjected to Standardization through classical and physico-chemical analysis.

2. OBJECTIVES

2.1. General Objective

- To standardize the drug "Nandukkal Bhashmam" through Physico- Chemical analysis.

2.2. Specific Objectives

- To prepare the drug "Nandukkal Bhahsmam".
- To analyze Physico-Chemical properties of the drug "Nandukkal Bhashmam".

3. LITERATURE REVIEW

3.1. "Nandukkal" (Fossil crab stone)

"Gunapadam" (Siddha Pharamacology) deals with detailed study of Siddha drugs. Based on their origin, raw drugs are categorized as of plant ("Mooligai Vakuppu"), mineral ("Thathu Vakuppu") and animal origin ("Jeeva Vakuppu"). Siddha literature describes 220 minerals

under four main groups "Ulogam"(Metals) – 11, "Karasaram" (Salts) -25, "Patanam" (Arsenic and Mercurial compounds) -64 and "Uparasam" (Other minerals) -120. The usage of fossils in Siddha medicine is found under the classification of "Uparasam". Fossil crab stone is treated as one of the minerals in Siddha medicine and it is grouped under other minerals category. "Kal nandu soothiram"- "கல் நண்டு சூத்திரம்" is the manuscript that describes "Nandukkal(Fossil crab stone)" and the Siddha drug "Nandukal Bhashmam" in curing various diseases mainly urinary tract disorders.[11],[3]

Fig 3.1.1. "Nandukkal" (Fossil crab stone).

3.1.2. Nandukkal Purification

Equal parts of limestone and fuller's earth are mixed in water and allowed to settle for some time. The clear solution is then taken out. Fossil stone crab is then placed in this solution and heated for three hours. The fossil stone crab is then taken out and washed in water to get purified.[11],[3]

Fig 3.2.1 Limestone.

Fig 3.2.2 Fuller's earth.

Fig 3.2.3. Purification of "Nandukkal".

3.3. "Nandukkal Bhashmam-(NKB)" Preparation

"Nandukkal" should be purified using "Kalchunnam" (Limestone) and "Pooneeru" (Fuller's earth) before the preparation of "Nandukkal Bhashmam". Purified "Nandukkal" will be put into "kalvam" and ground well for 3 days using "sirupeelai charu" (*Aerva lanata leaf* extract) and made small cakes("Villai"), dried and calcinated using 400 cowdungs for 1Kg of "Nandukkal".

Again it will be ground using "mullangi charu" (*Raphanus sativus* extract) for 3 days, made cakes, dried and calcinated as above. The final product will be 'ASH WHITE'. It must be kept in air tight container to avoid absorbing moisture content from the air.[11],[3]

Fig 3.3.2. *Raphanus sativus.*

Fig 3.3.1. *Aerva lanata.*

3.4. Preparation procedures of "Nandukkal Bhashmam"

Fig 3.4.1. Grinding Nandukkal in Kalva yantram ❶

Fig 3.4.2. Griding with *Aerva lanata* leaf extract ❷

Fig 3.4.3. Making pellets /cakes (Villai seithal)❸

Fig 3.4.4. Seelai man seithal (7 times the utensil containing pellets covered with soft cotton cloth and mud) ❹

Fig 3.4.5. Pudam iduthal – (the pellets will be calcinized using 400 cow dungs per Kg)❺

Fig. 3.4.6. Final product is Ash white – called Bhashmam❻

1-5 procedures continued same with the *Raphanus sativus* juice to obtain final bhashma ❻.

4. MATERIALS AND METHODS

The ingredients for the preparation of the drug "Nandukkal Bhashmam" was procured from local raw drug selling stores from Jaffna. The "Nandukkal" was purified and prepared the Siddha drug "Nandukkal Bhashmam" as mentioned in the Siddha literatures. Then the drug was analyzed for traditional parameters and physico-chemical parameters partly at Bandaranayake Memorial Ayurvedic Research Institute, Navinna, Maharagama and another part at Department of Chemistry, University of Jaffna.

4.1. Traditional Parameters - Organoleptic, physical and chemical tests for "Nandukkal Bhashmam".

Table 4.1.1. Organoleptic characters of Bhashmam.

Organoleptic Characters		
1.	Varnam (Color)	Observed for color
2.	Sparsham (Touch)	A pinch of bhasmam was taken in fingers and observed for fineness.
3.	Rasa (Taste)	A pinch was taken in mouth and tasted.
4.	Gandham(Odor)	Certain amount of bhasmam was smelled.
5.	Shabdam(Sound)	A pinch of bhasmam was taken in fingers and observed for sounds when crushing it with fingers.

Table 4.1.2. Physical tests of Bhashmam.

Physical tests		
1.	Varnam (Color)	Observed the color
2.	Varitaram (Float)	Small amount of prepared bhasma is sprinkled over the cold and stagnant water in a beaker. Properly incinerated bhasma will float on water surface which states that prepared bhasma is light and fine.
3.	Rekhapurnatam (Furrow filling)	Bhasmam was spread between thumb and index finger and rubbed and observed whether it get easily into the lines and crevices of the finger.
4.	Unamam	A grain of rice is carefully placed on the floated layer of

		bhasmam and observed whether the grain floats or sinks.
5.	Anjana Sannibham(softness)	When the bhasmam was applied as anjanam observed for whether it caused irritation or not.
6.	Nischandram(Lustreless)	Bhasmam was taken in petri dish and observed under bright sunlight.
7.	Sukshmatwam(Fineness)	Observed through varitaram and rekhapurnatvam for the presence of fineness.

Table 4.1.3. Chemical tests of Bhashmam.

Chemical tests		
1.	Gatarasatwam (Tastelessness)	A pinch of bhasmam was taken in tongue and observed whether any taste present.
2.	Nirdhumam (Absence of fumes)	Bhasmam was put in the fire to see whether fumes are coming out.
3.	Amla pareekshai / Dadhi pareekshai (Sour test)	Bhasmam was added with lime juice or curd and observed for color change.

Table 4.1.4. Physico –Chemical tests of Bhashmam.

	Physico –Chemical tests	
1.	**LOD (Loss on drying at 105^{o})**	The sample was dried in an electrical oven at 105^{o} until reaches constant weight.
2.	**pH of Aqueous Solution**	The powdered drug was suspended in glass distilled water, fresh and dry *Aerva lanata* extracts. After 2hrs, filtered and the clear solution was measured for pH.
3.	**Total Ash (% of ash)**	5 gm of test sample was ignited in an electric furnace at 500 – 5500C in silica crucible until the sample reaches a constant weight.
4.	**Water Soluble Ash Value**	The water insoluble matter was collected in an ashless filter paper and ignited in an electric furnace at 450^{o}C in silica crucible until reaches a constant value. The weight of insoluble matter was subtracted from the weight of the total ash to indicate the weight of water soluble ash.
5.	**Acid Insoluble Ash Value**	Total ash obtained was heated with addition of 25 ml of dilute HCL for 10 min. It was filtered in an ash less filter paper (Whatman No. 41) and the residue was ignited in the furnace to get a constant weight.
6.	**Alcohol soluble extractive**	Macerated 5gm of powdered drug with 100ml of 90% alcohol in a 100ml stoppered flask for 24 hrs filtered with filter paper and evaporated 25ml of water extract to dryness in flat bottomed shallow dish. Dried the residue at 105^{o} upto constant weight.
7.	**Water soluble extractive**	Same procedure as 5.2.6 water used instead of alcohol
8.	**Ether soluble extractive**	Same procedure as 5.2.6 Ether used instead of alcohol

Table 4.1.5. Qualitative chemical analysis of Bhashmam.

	Qualitative Chemical Analysis	
1.	**Qualitative Tests**	5gm of "Nandukkal Bhashmam" was weighed accurately and placed in a 250ml clean beaker and added with 50ml of distilled water. Then it was boiled well for about 10 minutes. Then it was cooled and filtered in a 100ml volumetric flask and made up to 100ml with distilled water.
2.	**Test for Chloride**	2ml of the extracts was added with 2ml of dil.HNO_3 until the effervescence ceased. Then 2ml of Silver Nitrate solution was added. Presence of Cloudy appearance indicated the presence of chloride.
3.	**Test for Phosphate**	2ml of the extract was treated with 2ml of Ammonium Molybdate solution and 2ml of con.HNO_3. Presence of yellow cloudy appearance indicated the presence of phosphate.
4.	**Test for Iron**	2ml of extract was added with 2ml of Thiocyanate Ammonium solution. Appearance of mild red colour indicated the presence of Iron. With this additional 2ml of Thiocyanate Ammonium solution and 2ml of con.HNO_3 was added. Appearance of blood red colour indicated the presence of Iron.
5.	**Test for Calcium**	2ml of the extract was added with 2ml of 4% dil. Ammonium Oxalate solution. Cloudy appearance and white precipitate indicated the presence of calcium.
6.	**Test for Alkaloids**	Solvent free extract, 50 mg was stirred with few ml of dilute Hydrochloric Acid and filtered. To a few ml of filtrate, 1 – 2 ml of Dragendorff's reagent was added. A prominent yellow precipitate indicated the test as positive.

5. RESULTS

5.1. "Bhasmam Pareekshai" (Examination of Bhasmam according to traditional measures).

Table 5.1.1. Organoleptic Characters.

Organoleptic Characters		
1.	Varnam (Color)	Grey
2.	Sparsham (Touch)	Soft & smooth
3.	Rasa (Taste)	Tasteless
4.	Gandham(Odor)	No specific
5.	Shabdam(Sound)	Soundless

Table 5.1.2. Physical tests.

Physical tests		
1.	Varnam (Color)	Grey
2.	Varitaram (Float)	Floats on water
3.	Rekhapurnatam (Furrow filling)	Filling the spaces between the finger lines
4.	Unamam	Lightness
5.	Anjana Sannibham(softness)	No irritation caused
6.	Nischandram(Lustreless)	Lustreless
7.	Sukshmatwam(Fineness)	Soft, smooth(Reduced particle size)

Fig 5.1.1. Varitaram (Floating).

Fig 5.1.2. Unamam (Rice floating).

Fig 5.1.3. Rekhapurnatam (Furrow filling).

5.1.5. Amla pareekshai.

Fig 5.1.4. Varnam & Nischandram

Table 5.1.3. Chemical tests.

Chemical tests		
1.	Gatarasatwam (Tastelessness)	Tasteless
2.	Nirdhumam (Absence of fumes)	No fumes present
5.	Amla pareekshai / Dadhi pareekshai (Sour test)	No color change observed

Physico-chemical Analysis

Table 5.1.4. Physico-Chemical Analysis.

No	Physico-Chemical Analysis	Values
1.	Loss on drying	0.4328%
2.	Total ash value	99.8249%
3.	Acid insoluble ash value	82.26%
4.	Water soluble ash value	0.99%
5.	Solubility Water Alcohol Ether	 0.88% 0.704% Insoluble
6.	pH NKB dissolved in water NKB dissolved in dry *Aerva lanata* decoction NKB dissolved in fresh *Aerva lanata* decoction	 7.91 (28.3^{o}C) 7.20 (29.5 ^{o}C) 7.04 (29.5 ^{o}C)

Table 5.1.5. Assay of elements.

No	Assay of elements	
1.	**Anions** Carbonate (CO_3^{2-}) Sulphate (SO_4^{2-})	 Present Present
2.	**Elements** Aluminium Strontium Calcium Sodium	 Present Present Present Present
3.	Group IV	Few black spots were observed

6. RESULTS AND DISCUSSION

The organoleptic characteristics of NKB(“Nandukkal Bhashmam”) was solid in nature, grey color in daylight and the smell, sound and taste was not to be found characteristic.

As far as *bhashmam* is concerned, ancient scholars of siddha have described various parameters for its qualitative evaluation, but all those are subjective in nature and cannot be evaluated numerically for reproducibility in the result. So, in the present study, tests as prescribed in traditional science, namely color, taste, touch, odor, “*Niścandratvam”* (lustrelessness), “*Rekhāpūrṇatām”* (quality of entering into grooves of fingers) and “*Vāritaratvam”* (quality of floating on water) were performed. The drug sample fulfilled important “bhashmam pareekshai” (Examination of bhashmam) as “Nischandratvam”, “Rekhapurnatvam”, “Varitaratvam” and “Amila pareekshai.

The values of the physico-chemical parameters depicted in the **Table 5.1.4.** It was observed that, negligiable moisture content of 0.4328% w/w% loss on drying was found in the drug NKB which indicates that the moisture content of the formulations within the range. Moisture content / LOD of the sample are less than 5% w/w%, so it could prevent microbial growth. LOD suggests that the presence of moisture, organic matter/ volatile substance present in the drug. Therefore, results obtained were within the required level of LOD and these values of LOD are an indication of moisture in final product.

Total ash in the given sample was found 99.8249% (w/w%) where as water soluble ash is 0.99% (w/w%). The value of total ash in the formulation is high because of the method of preparation of this drug is calcination procedure. Though most of the percentage of the bhasmam was in ash form. Total ash was determined to check proper incineration of the drug. High total ash contents of bhashmam indicates the presence of very high inorganic contents that are useful in bio-available form.

Acid insoluble ash indicates the presence of silica and oxalates in the drug. Acid insoluble ash is a direct marker of digestibility of a substance. Thus lower the value of acid insoluble ash implies higher bio- availability of the substance. But in the analysis it is found that acid insoluble ash present is, 82.26% (w/w%), may be its because of presence of silica as the assay of elements showed that there is no oxalates present in anions but casts of group IV(Group of silica) present that is difficult to do separation.

pH of the drug NKB was observed in single dose administration with its anupanams. NKB dissolved in de-ionized water showed 7.91(28.3^{o}C), with dry *Aerva lanata* decoction showed 7.20(29.5 ^{o}C), and with fresh *Aerva lanata* decoction showed 7.04(29.5 ^{o}C). The change in the pH may be due to the chemical contituents present in *Aerva lanata.*

The water solubility extractive value of the drug NKB is 0.88%, alcohol soluble extractive value of the formulation is, 0.704%, and no significant value was observed in ether soluble extractive.

Qualitative chemical analysis (Table 6.1.5) of the drug revealed that NKB possesses the anions carbonate (CO_3^{2-}), sulphate(SO_4^{2-}) and elements as aluminium, iron, strontium, calcium, sodium and few traces of group IV elements. The presence of alkaloids in the drug is may be due to it includes the juices of *Aerva lanata* and *Raphanus sativus*. In addition to

the qualitative analysis the formulation doesnot contain mercury, arsenic, ammonium, zinc and unsaturated compounds.

7. CONCLUSION

According to the results obtained the prepared drug of "Nandukkal Bhashmam" fulfilled all the traditional and physico- chemical parameters that probably justifies that the drug is good to use in Renal calculi.

Renal calculi is one of the pressing concerns of the low and middle socio economic groups of the world. The epistemology of Siddha is experimental, intuitive and holistic. This review work on the drug NKB from the authentic texts and justified journals reveals that the drug is very efficacious particularly in urinary calculi. The use of bhasmam drug in renal calculi have good preventive, curative and rejuvenating potential. The present busy world led to the decline in the qualities of herbo- mineral drugs. Physical and chemical properties help develop quality in the drug product. There should be fixed standards of physico-chemical features to ensure the desired action. This becomes more important for medicines of Siddha and Ayurveda, due to vast variation in sources of raw drugs and wide diversity in the manufacturing procedures. The present effort to develop analytical profile of "*Nandukkal Bhasmam"* deals with loss on drying, total ash value, acid insoluble ash value, water soluble ash value, solubility in water, alcohol, ether, pH and chemical assay of elements in NKB. The analysis of physico chemical characteristics of" Nandukkal bhasmam" indicates the consequence of drug standardization before introduce into the market. This piece of work is just a preliminary study which can serve as a step towards standardization of a herbo-mineral drug "*Nandukkal Bhasmam"*. Further study is necessary to explore other parameters related to standardization to be carried out in different batches to set the limit for the reference standards for the quality control and quality assurance of "*Nandukkal Bhasmam"*.

8. RECOMMENDATIONS

Siddha herbo-mineral formulations like NKB have been practised since ancient times, therefore it is suggested and concluded that the manufacturing of these formulations should be regulated strictly before launching them into the market and more scientific studies need to be conducted to establish the final verdict on the safety of this and several other similar formulations.

The evidence of use of fossils in Siddha system prevails only on fossil crab. The source of fossil crab is still mysterious. Even though, the studies on "Nandukkal" proves that it is efficacious particularly in urinary calculi. More research studies should be initiated in focus on "Nandukkal".

An effort has been made to characterize the prepared "*Nandukkal bhashmam"* using sophisticated analytical tools as a step towards standardization of the drug. To assure the quality of the prepared bhashmam, apart from classical and physical characterization techniques as mentioned in the siddha and ayurvedic texts, modern characterization techniques like XRD (X-Ray Diffraction), DLS (Dynamic light scattering), Zeta potential, SEM (Scanning electron microscopy) and EDAX (Energy-Dispersive X-ray spectroscopy), analysis of bhashmam samples should be carried out for the advanced safety and efficacy of the original drug.

It is also suggested that there is a need of systematic and well organized coordination of allied sciences along with adequate infrastructure and facilities to solve various problems related to the standardization of herbo-mineral drugs like bhashmams.

9. ACKNOWLEDGEMENT

First of all we are ineffably indebted to our research supervisor, Dr.Mrs.R.Thatparan, for her conscientious guidance and encouragement to accomplish this research work.

We give our plenteously gratitude to Dr.Mr.K.Velauthamoorthy, Senior Lecturer, Department of Chemistry, University of Jaffna for assisting us in the part of physico-chemical analysis.

We express our gratitude towards the workers at Pharmacy, Bandaranaiyake Memorial Ayurvedic Research Institute, Navinna, Maharagama for helping us in "Nandukkal Bhashmam" preparation.

We are highly thankful to the analytical lab assistant, who helped us in executing part of the physico chemical analysis at Bandaranaiyake Memorial Ayurvedic Research Institute, Navinna, Maharagama.

At last but not least, gratitude goes to all of our friends who directly or indirectly helped us to complete this research work.

Any omission in this brief acknowledgement does not mean lack of gratitude.

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