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Physicochemical Evaluation and Phytochemical Screening of Siddha formulation, *Narasinga Rasayanam*.

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Abstract

Pharmacognostical standardization of herbal formulation is essential in order to assess the quality of drugs, based on the concentration of their active principles. The present study was attempt to evaluate the physicochemical and phytochemicals parameters of *Narasinga Rasayanam* which is a poly herbal Siddha preparation. This medicine is used in traditional Siddha system of medicine to treat Gunmam (Peptic ulcer), *Paandu* (Anaemia), 18 types of *Kuttam* (Skin diseases), *Magotharam* (Ascites), *VaiyitruKatti* (Abdominal tumour). But there is no standardization work reported on *Narasinga Rasayanam*. Physicochemical parameters, preliminary characterization and phytochemical analysis were carried out. Heavy metal analysis, microbial load, aflatoxin analysis were also determined. There finding will be useful to world establishing quality control parameters for the standardization of Siddha medicine *Narasinga Rasayanam* and can be used as reference standards for the preparation of a standardized pharmaceutical product and further quality control researches.

Keywords: *Narasinga Rasayanam,* Physicochemical Parameters; Phytochemicals screening.

Introduction

Indian systems of medicines have been widely used for thousands of years in India[1]. Siddha medicine is a unique one as it is not only a curative but also preventive and to achieve the healthy body and mind. Siddha medicines revitalize and rejuvenate the body[2]. As per the estimates of World Health Organization (WHO), more than 80% of global population uses plants or their products as the primary source of medicinal agents[3]. Most of the medicines are mixture of compounds and because of its synergistic action; toxicity is being diminished, thereby increasing bioavailability through the cells of the body[4].

Though Siddha system has its own value it has been latent due to the modern medicine which has its way of immediate healing and treatment. In recent years the Siddha system has its dawn among worldwide for its natural inheritance, holistic approach, healthy lifestyle and preventive treatment[2].

Herbal drugs have found wide spread use in many countries not only because they are easily available and are cheaper but an important reason has been the notion that they are safer than synthetic drugs which may not always be true[5].

The Siddha medicine requires standardization though it has been practiced for many years. Standardization of the drug brings the validation to be used as a medicine by subjecting the drug to many analysis and determining its quality and effectiveness[2]. Pharmacognostical standardization of herbal formulation is essential in order to assess the quality of drugs, based on the concentration of their active principles.

Thus, the present study deals with standardization of Siddha herbal preparation, *Narasinga Rasayanam* mentioned in siddha text "*Sarabenthira Vaithiya Muraigal -Gunma Roga Sigitchai"*, has been used for the treatment of 5 types of *Gunmam* (Peptic ulcer), *Paandu* (Anaemia), 18 types of *Kuttam* (Skin diseases), *Magotharam* (Ascites), *VaiyitruKatti* (Abdominal tumour)[6].

Systematic protocols for standardization of *Narasinga Rasayanam* is not available, hence it was decided to evaluate the qualitative and quantitative analysis for *Narasinga Rasayanam* scientifically to prevent its adulteration. For the standardization of this drug Organoleptic properties, Phytochemical screening and Physicochemical parameters, were carried out. In addition, residue analyses such as heavy metal analysis, microbial load, Aflatoxin analysis were also examined to strength the standardization process

Materials and Methods

Procurement of Raw Drugs:

The raw drugs were procured from a well reputed country shop in Parrys corner, Chennai. All the ingredients were purified and the medicine was prepared in the *Gunapadam* laboratory of National Institute of Siddha.

Identification and Authentication of the drug:

All the plant materials were identified and authenticated by the Botanist, Department of Medicinal Botany, National Institute of Siddha.

Purification of the drugs:

All the drugs mentioned here were purified as per the Siddha literature.

Serankottai [7]

The nose like projection of the seed was cut down and boiled with decantation of cow dung, decoction made of tamarind leaves and aloes juice.

Kodiveli [8](*Plumbago zeylanica*):

The inner root of *Plumbago zeylanica* root was removed and the root bark was powdered well. The powdered drug was placed in a cloth and tied in the mouth of a pot filled with milk. Then, the pot was closed with suitable lid and boiled for 3 hours. Then it is allowed to dry completely and grounded well.

Thanneervittan (*Asparagus racemosus*):

Tuber of *Asparagus racemosus* was washed thoroughly and the outer skin was peeled off and also the center part was removed.

Nerunjil (*Tribulus terrestris*):

Tribulus terrestris was washed in the running tap water to remove the soil and impurities.

Nilapanai Kizhangu (*Curculigo orchioides*):

Curculigo orchioides was washed in the running tap water to remove the soil and impurities

Preparation of *Narasinga Rasayanam*:

Procedure [6]:

All the ingredients in Table.No.1 were purified and dried in sun shade, and then it was made into fine powder separately and finally mixed together. After that in the mixture of all herbal powder, the ghee, honey and palm jiggery were added little by little and ground in a *Kalvam* (stone mortar) until it attained waxy consistency. Then it was stored in an air tight container.

Table. No: 1. Ingredients of *Narasinga Rasayanam*

Botanical name	Siddha name	English name	Family	Quantity
Plumbago zeylanica	Kodiveli Vaer	Ceylon lead-wort	Plumbaginaceae	560g
Semecarpus anacardium	Serankottai	Marking nut tree	Anacardiaceae	560g
Asparagus racemosus	Thannervittan Kizhangu	Wild asparagus	Liliaceae	2240g
Tribulus terrestris	Nerunjil	Small caltrops	Zygophyllaceae	280g
Curculigo orchioides	Nilapanai Kizhangu	Black musale	Hypoxidaceae	700g
Borassus flabellifer	Panai vellam	Palm jiggery	Arecaceae	875g
	Tean	Honey		560g
	Nei	Cow's Ghee		280g

Standardization Parameters:

The various standardization parameters organoleptic properties, physico-chemical investigations, preliminary phytochemical analysis, Heavy metal analysis, microbial load analysis and aflatoxins investigations were studied.

.Organoleptic Evaluation: [9,10]

The organoleptic characters of the samples were evaluated based on the method described by Siddiqui et al. Organoleptic evaluation refers to evaluation of the formulation by colour, odour, taste and texture etc (Table.No: 2).

Physico-chemical evaluation: [11,12]

Physicochemical Properties of *Narasinga Rasayanam* was analyzed at Regional Research Institute of Unani Medicine (RRIUM), Royapuram, Chennai-600013.

Physico-chemical studies of the plant drugs are necessary for standardization, as it helps in understanding the significance of physical and chemical properties of the substance being analyzed in terms of their observed activities and especially for the determination of their purity and quality. The analysis includes the determination of ash value, Loss on drying of the sample at 105ºC, pH value and Extractive value. These were carried out as per guidelines.

1. Loss on drying at 105ºC

4g of *Narasinga Rasayanam* was weighed in a previously weighed 100ml beaker and heated in an oven at 105ºC for 5hours. Cooled in a dessicator and weighed. Repeated the procedure till constant weight was obtained. The percentage loss in weight of the test drug was calculated by the following formula.

Calculation

Percentage of Loss on Drying at 105°C =

$$\frac{\text{Loss in weight of the sample}}{\text{Weight of the test drug taken}} \times 100$$

2. Ash content

2. a Total ash content

4g of *Narasinga Rasayanam* was weighed accurately in a previously ignited and tared silica dish. The material was evenly spread and ignited in a muffle furnace at 600°C until it became white indicating the absence of carbon. The dish was cooled in a dessicator and weighed. As carbon free ash cannot be obtained in this manner, the dish was cooled and the residue moistened with sufficient quantity of water. Dried on a water bath and then ignited in the electric furnace to get the constant weight. Cooled the dish in a dessicator and then weighed. The percentage of total ash of air-dried materials was calculated as per the formula given below.

Calculation

Percentage of total ash =

$$\frac{\text{Weight of the ash}}{\text{Weight of test drug taken}} \times 100$$

2.b. Acid-insoluble ash

The total ash of *Narasinga Rasayanam* was found out as described above. To the dish containing the total ash was added 45 ml of 1: 5 hydrochloric acid in three portions of 13 ml each time. Boiled gently for 5 minutes and filtered. Collected the insoluble matter on an ashless filter paper (Whatman No.41) and washed with distilled water until the residue was free from acid. Transfer the filter paper containing the insoluble mater to the original dish. Dried and ignited to the constant weight. Cooled the dish in a dessicator, and then weighed. Calculation was made by given formula.

Calculation:

Percentage of acid-insoluble ash=

$$\frac{\text{Weight of the acid-insoluble residue}}{\text{Weight of test drug taken}} \times 100$$

2. c. Water Soluble Ash

The above obtained ash was boiled for 5minutes with 25mL water. The insoluble ash was collected using filter paper and washed with hot water and transferred to the silica crucible then ignites for 15minutes at temperature not exceeding 450°C. The silica crucible and residue were weighed until constant weight was attained for determination of weight of insoluble ash. The weight of the water soluble ash was determined by subtracting the weight of insoluble ash from the weight of total ash.

3. Extractive value of the test drug

4 g of *Narasinga Rasayanam* was weighed accurately in a glass stoppered flask. Added 100 ml of distilled water and shacked occasionally for 6 hours and then allowed to stand for 18 hours. Filtered rapidly taking care not to lose any solvent and pipette out 25 ml of the filtrate in a preweighed 100 ml beaker and evaporated to dryness on a water bath. Kept in an air oven at 105ºC for 6 hours. Cooled in a dessicator and weighed. Repeated the experiment twice, and taken the average value. The percentage of water soluble extractive was calculated by the formula given below.

Calculation:

Percentage of water soluble extract =

$$\frac{\text{Weight of the extract}}{\text{Weight of sample taken}} \times \frac{100}{25} \times 100$$

3. a. Water-soluble extractive of the test drug

3g of *Narasinga Rasayanam* was taken in a glass stoppered flask and add 100 mL of distilled water, shake occasionally for 6 h and then allow standing for 18 h. filter rapidly taking care not to lose any solvent and pipette out 25 mL of the filtrate in a pre weighed 100 mL beaker and evaporate to dryness on a water bath. Keep it in an air oven at 105°C for 6 h, cool in a desiccator and weighed. Repeat the experiment twice and take the average value.

3. b. Alcohol-soluble extractive of the sample

4 g of *Narasinga Rasayanam* was weighed accurately in a glass stoppered flask. Added 100 ml of distilled alcohol (approximately 95%) and shaken occasionally for 6 hours and then allowed to stand for 18 hours. Filtered rapidly taking care not to lose any solvent and pipette out 25 ml of the filtrate in a pre weighed 100 ml beaker and evaporated to dryness on a water bath. Kept in an air oven at 105ºC for 6 hours and cooled in a dessicator and weighed. Repeated the experiment twice, and taken the average value. The percentage of alcohol soluble extractive was calculated by the formula given below.

4. Determination of pH:

Five grams of *Narasinga Rasayanam* was weighed accurately and placed in clear 100 ml beaker. Then 50 ml of distilled water was added to it and dissolved well. After 30 minutes it was then applied in to pH meter at standard buffer solution of 4.0, 7.0, and 9.2. Repeated the test four times and average was recorded. The results were tabulated in Table No: 3.

Preliminary Phytochemical Analysis: [13]

Preliminary qualitative phytochemical analysis of all the extracts was carried out by employing standard conventional protocols (Table. No: 4).

1. Detection of alkaloids

Extracts were dissolved individually in diluted hydrochloric acid and filtered.

Mayer's test

2 ml of extract was treated with few drops of Mayers‟ reagent; formation of yellow colored precipitate indicates the presence of alkaloids.

Wagner's test

2 ml of filtrate was treated with Wagner's reagent. Formation of brown /reddish precipitate indicates the presence of alkaloid

2. Detection of carbohydrate

Extract was dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for presence of carbohydrates.

Molisch’s test

2 ml of filtrate was treated with few drops of alcoholic Alpha naphthol solution in a test tube. Formation of the violet ring at the junction indicates presence of carbohydrates

Benedict’s test

Filtrate was treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

3. Detection of Glycosides

Liebermann's test

2ml of extract was treated with 2ml chloroform and 2ml of acetic acid, Violet color change into blue and green indicates presence of Glycosides.

4. Detection of Saponins

Froth test

Extracts was diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 centimeter layer of foam indicates the presence of Saponins.

Foam test

0.5-gram extract was shaken with 2 ml of water. If foam produced persists for 10 minutes, it indicates the presence of saponins.

5. Detection of phytosterols

Salkowski's test

Extracts was treated with chloroform and filtered; the filtrates were treated with few drops of concentrated sulphuric acid, shaken and allowed to stand for few minutes. Golden yellow color indicates the presence of triterpene

6. Detection of phenols

Ferric Chloride test:

2 ml of extracts was treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

7. Detection of tannins Gelatin test

Detection of tannins

To the extracts, 1% of gelatin solution containing sodium chloride was added; formation of white precipitate indicates the presence of tannins

8. Detection of flavonoids

Alkaline reagent test

Extract was treated with few drops of 10% sodium hydroxide, formation of intense yellow color then on addition of diluted hydrochloric acid it becomes colorless, and it indicates the presents of flavonoids.

Lead acetate test

Extract was treated with few drops of lead acetate solution; yellow color precipitate indicates presence of flavonoids.

9. Detection of proteins and aminoacids

Xanthoproteic Test:

The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

10. Detection of diterpenes

Copper Acetate test

Extracts were dissolved in water and treated with 3-4 drops of copper Acetate solution; formation of emerald green color indicates the presence of diterpenes

11. Test for gum and mucilage

The extract was dissolved in 10 ml of distilled water and to this 2ml of absolute alcohol with the constant stirring white cloudy precipitate indicates the presence of gum and mucilage.

12. Test for Quinones

Extract was treated with sodium hydroxide blue or red precipitate indicates the presence of Quinones. The results were tabulated in table number 5.

Heavy Metal Analysis [14,11]:

Heavy metal analysis was carried out for determination of heavy metals as per procedures WHO, 1998 and AOAC, 2005. Thermo Fisher M Series, 650902 V1.27 model Atomic Absorption Spectrometer (AAS) was used for the analysis. The Hallow cathode lamp for Pb, Cd, Hg and As analysis were used as light source to provide specific wavelength for the elements to be determined.

Microbial Analysis:

Microbial analysis was carried out for determination of microbial contamination as per procedures of Indian pharmacopoeia [11] 2010 and WHO Guideline [14]. The test included total bacterial count, total fungal count and identification of specified organisms such as Escherichia coli, Salmonella sp., Staphylococcus aureus and Enterobacteriaceae, (Table 6).

Aflatoxin:

The Aflatoxin B1, Aflatoxin B2, Aflatoxin G1 and Aflatoxin G2 in *Narasinga Rasayanam* were also analyzed.

Results and Discussion

The field of the herbal drugs and formulations is very vast and there is still lot to explore on the subject of standardization of these. So, while developing an herbal drug formulation it is must to have all the related knowledge of that particular drug including all its organoleptic characters, phyto-constituents, pharmacological action and its standardization in respect to various parameters via various techniques.

Organoleptic parameters

The Organoleptic characters of the *Narasinga Rasayanam* were as shown in Table. No: 2. *Narasinga Rasayanam* was dark brown with pleasant odour and sweet taste.

Table.No:2. Organoleptic character of *Narasinga Rasayanam*

S.No	Organoleptic characters	Observation
1.	Colour	Dark brown
2.	Odour	Pleasant
3.	Taste	Sweet
4.	Texture	Semisolid

Physicochemical parameters

The Physicochemical parameters of the *Narasinga Rasayanam* are tabulated in Table. N: 3.

Table. No: 3. Physico- chemical Analysis of *Narasinga Rasayanam*

S.No	Parameters	Results
1.	Loss on Drying at 105 °C	4.54%
2.	Ash value a. Total ash (w/w) b. Acid insoluble ash (w/w) c. Water Soluble ash (w/w)	8.48% 0.766% 6.48%
3.	Extractive values a. Alcohol soluble (w/v) b. Alcohol successive soluble (w/v)	33.03% 11.11%
4.	pH values (1% solution)	5.9

The loss on drying at 105°C in *Narasinga Rasayanam* was found to be 4.54%. It indicates that very low quantity of selected plant material was loss after drying. The ash values of *Narasinga Rasayanam* such as total ash, water soluble ash and acid insoluble ash were found to be 8.48%(w/w),6.48%(w/w),and 0.766%(w/w) respectively. Total ash value of plant materials indicate the amount of minerals and earthy materials attached to the plant material. The extractive values such as alcohol soluble and alcohol successive soluble values were found to be 33.03% (w/v) and 11.11 % (w/v) respectively which indicating the presence of polar constituents like organic and inorganic compounds. The pH of 1% w/ v solution was found to be 5.9.

Preliminary phytochemical screening

The phytochemical active compounds of *Narasinga Rasayanam* were qualitatively analyzed and the results are presented in Table. No: 4. The preliminary phytochemical analysis of aqueous extracts of *Narasinga Rasayanam* indicated the presence of carbohydrates, glycosides, saponins, phytosterols, phenols, flavonoids, proteins & amino acids, diterpenes, gum & mucilage and quinone.

Table. No: 4. Phytochemical Screening of *Narasinga Rasayanam*

S.No	Phytochemicals	Test Name	H_2O ext.
1	Alkaloids	Mayer's test	Absent
		Wagner's test	Absent
2	Carbohydrates	Molisch's test	Absent
		Benedict"s test	Present
3	Glycosides	Libermann Burchard's test	Present
4	Saponins	Froth test	Present
		Foam test	Present
5	Phytosterols	Salkowski's test	Present
6	Phenols	Ferric chloride test	Present
7	Tannins	Gelatin test	Absent
8	Flavonoids	Alkaline Reagent test	Present
		Lead acetate test	Present
9	Proteins and Amino acids	Xantho proteic test	Present
10	Diterpenes	Copper acetate test	Present
11	Gum & mucilage	Extract + alcohol	Present
12	Quinone	NAOH + Extract	Present

Heavy metal analysis:

Heavy metals may be present in crude drugs through the soil and atmospheric pollution. Moreover minerals and metals are also used in preparing indigenous medicine. However, heavy metals have been associated with various adverse effects. Hence, heavy metals need to be detected in such preparations. Heavy metal analysis of *Narasinga Rasayanam* revealed that the presence of heavy metals (Pb, As, Cd and Hg) in study drug was below the WHO/FDA permissible limits. (Table. No: 5).

Table. No: 5. Heavy Metal Analysis of *Narasinga Rasayanam*

S.No	Heavy Metals	Results	Reference Limits
1.	Lead	Not detected	Not more than 10ppm
2.	Arsenic	04.3760ppb	Not more than 3.0ppm
3.	Cadmium	0.0063ppm	Not more than 0.3ppm
4.	Mercury	18.51586ppb	Not more than 1.0ppm

Microbial Load:

For detection of such microorganisms, colonies obtained on specific media were subjected to suitable microbial tests along with pure strains to detect their presence or absence. The results obtained revealed that the absence of these microorganisms thereby confirming the non toxic nature of the formulations (Table. No: 6).

Table. No: 6. Microbial load Analysis of *Narasinga Rasayanam*

S.No	Parameters	Results	Reference Limits
1.	Total Bacterial Count (TBC)	7×10^3cfu/gram	10^5CFU/gm
2.	Total Fungal Count (TFC)	3×10^2 cfu/ml	10^3CFU/gm
3.	Enterobacteriaceae	Absent	10^3
4.	*Escherichia coli*	Absent	10
5	*Salmonella* spp	Absent	Absent
6	*Staphylococcus aureus*	Absent	Absent

Aflatoxin:

Aflatoxins B_1, B_2 and G_1, G_2 were analyzed for *Narasinga Rasayanam* and they were absent and the results were tabulated in (Table. No: 7).

Table.No:7. Aflatoxin Analysis of *Narasinga Rasayanam*

S.No	Test Parameters	Results
1.	Aflatoxin B1	Absent
2.	Aflatoxin B2	Absent
3.	Aflatoxin G1	Absent
4.	Aflatoxin G2	Absent

Conclusion

The present study on physicochemical parameters and preliminary phytochemical analysis provides important information which can be used as a fingerprint of poly herbal Siddha medicine *Narasinga Rasayanam*.

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