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PHYSICO CHEMICAL ANALYSIS AND ANTI MICROBIAL ACTIVITY OF A SIDDHA HERBO MINERAL DRUG *SILASATHU PAAVANAI*

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ABSTRACT

Indian systems of medicine are Siddha, Ayurveda and Unani. Among them, Siddha system of medicine is being practiced in southern parts in India. Siddha has its strong foundation of science laid by siddhars and other sages who aimed at attaining salvation and hence formulated medicines for a longer and healtier life span. Among the 4448 diseases mentioned by them, Kalladaippu (Urolithiasis) is one of the reasons for obstruction in the urinary system and hence producing low urinary output. Many medicines have been prescribed for this disease and *Silasathu paavanai* is one among them. The present study aims to analyze drug physico chemically and to explore its anti microbial activity. The drug *Silasathu paavanai* contains essential elements which are considered to be stone inhibitors and the aqueous extract has good anti microbial activity against *E.coli* which is the commonest urinary tract infection among patients with urolithiasis.

KEY WORDS: Siddha, Silasathu paavanai, Kalladaippu, Anti microbial activity, Physico chemical analysis

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INTRODUCTION

The quest for a healthy body and healthy mind has been an incessant urge in human beings all over the world. The present millennium is searching for natural and safe treatment method for the same. The WHO has estimated that approximately 60 to70 % of the worlds' population rely on traditional medicine for their health needs[1]. Siddha system of medicine, a rich tradition of ancient India, is unique and timeless gift for mankind providing radiant health from time immemorial. The philosophy of Siddha gives us an understanding of the connection between body, mind and soul offering advice for a more natural healthy living. In Siddha, well being of any human is based on the harmony and balance in Biodynamics of the three humors namely Vali, Azhal and Iyyam based on the muthodam theory Kalladaippu (Urolithiasis) comes under Neerinai arukkal noigal[2], producing low output of urine due to various aetiological factors. It is a clinical manifestation where the Azhal kuttram is increased. This kuttram reduces the body fluids and dries up the urine resulting in concentration of urinary salts[2] Urinary tract infection is considered to be one of the factors for formation of urinary stones. The commonest organism isolated from urine of patients suffering from urolithiasis is *E.coli* (62.5%)[3] In the Siddha system of medicine, the drug "*Silasathu Paavanai*[3]**"** is reported to be useful in the treatment of Kalladaippu. *Silasathu* in Siddha Medicine is a famous drug used in Genito urinary disorders especially in calculi disorder. *Silasathu* available in market is the mineral selenite which is calcium sulphate dihydrite by its chemical name. *Silasathu* by itself has direct indications for urolothiasis. By the literature review we came to know that the drug is used in various preparations indicated fro Kalladaippu. Hence it has strong evidence in Siddha literature for urolithiasis. By the name it indicates that *"Silasathu paavanai"* is a preparation where one main drug is processed in other drugs. Paavanai by the name itself indicates that one drug of target is processed in other drugs to improve it efficacy and potency in treating the disease. *Silasathu paavanai* is the drug prepared by processing silasathu in tender coconut water containing other herbal mixtures. In this paavanai the method adopted was Dhula Iyandhiram method. Heat is applied to this paavanai method so that there may be ionic changes taking place in the drug. The present study aims to evaluate the physico chemical and anti microbial properties of the drug.

MATERIALS AND METHODS

Ingredient of *Silasathu paavanai*[4]

- Main Ingredient – Karpoora *Silasathu* (Selenite) : 1 palam (35g)

Ingredients used for processing

- *Glycyrrhiza glabra*(Adhimadhuram) : 1 palam (35g)
- *Santalum album* (Sandhana thool) : 1 palam (35g)
- *Vitis vinifera* (Thirakshai pazham) : 1 palam (35g)
- *Tender Coconut water* (Ilaneer) : 1 padi (1.4litres)

Procurement and authentication of drugs

Silasathu and other raw drugs were procured from raw drug store from Chennai. The identity and authenticity of the drugs were confirmed by Dr. E. Sasikala, pharmacognosist, Siddha Central Research Institute, Chennai and Dr. M. Allimuthu, Prof & HOD, Dept of Gunapadam, Govt Siddha Medical College, Arumbakkam, Chennai

Purification of Silasathu

The *silasathu* was purified by boiling it in tender coconut water until the water evaporates. The drug is removed washed and dried.

Purification of other drugs

The other raw drugs were dried under shade and the dust and foreign matter removed.

Preparation of the drug

The requisite amount of *silasathu* is made into small pieces and tied in cloth loosely. The powder of Adhimadhuram, Sandhanam and fresh Thiraksha were ground finely and dissolved in the said amount of tender coconut water. The cloth containing the *silasathu* is mounted in the tender coconut water containing the mixture in such a way that it does not touch the bottom of the pot. The distance between the cloth and the bottom may vary from 1 – 2 inches. Then it is heated until all water evaporates. The process adopted was Dhula Iyandram method. The *silasathu* in the cloth is removed, wasted, dried under shade and powdered finely. The finished drug is stored in an airtight glass container. Physico chemical characterization of *Silasathu paavanai*[5] The physico chemical characterization was done according to PLIM guidelines[6]

Determination of total ash

About 2 grams of the dried crude drug were weighed accurately in a tarred platinum or silica dish and was incinerated at a temperature not exceeding 550^{o}c until free from carbon. It was then cooled and weighed. The percentage of ash was calculated with reference to air dried drug.

Determination of water soluble ash

The total ash was boiled for five minutes with 25ml of water. The insoluble matter was collected on a Gooch crucible (or) an ash less filter paper. It was washed with hot water and ignited for 15 minutes, at a temperature not exceeding 550^{o}c. The weight of the insoluble matter was subtracted from the weight of the ash, the difference in the weight of the ash represents the water soluble ash. The percentage of water soluble ash was calculated with reference to the dried drug.

Determination of acid insoluble ash

The total ash was boiled with 25ml of 2 M HCL for 15 minutes. The insoluble matter was collected on a Gooch crucible (or) an ash less filter paper. It was washed with hot water and ignited. It was then cooled in a desiccator and weighed. The percentage of acid insoluble ash was calculated with reference to the dried drug.

Loss on drying

5gm of the drug is heated in a hot oven at 40 degree C to constant weight. The percentage of loss of weight was calculated. Quantitative analysis of essential elements in *Silasathu paavanai* by atomic absorption spectrometer [7] In this method the quantitative analysis of essential elements Calcium, Magnesium and Zinc was estimated. The study was done at Chennai Mettex lab pvt ltd, Guindy.

Apparatus and Equipment

500 ml Glass beakers, graduated pipettes, Hot plate, Watch glass.

Instrument

Atomic Absorption Spectrometer.

Chemicals

Nitric acid, Hydrochloric acid

Sample Preparation

Weigh approximately 1g sample in a 250ml beaker, add 2ml of 1+1 HNO_3 and 10ml of 1+1HCl and digested on a hot plate. Cool and filter to remove insoluble material. Transfer sample to volumetric flask, adjust volume to 50ml and mix. Take all precautions to avoid contamination at all stages. Prepare a reagent blank containing the same amounts of acids used in the preparation of sample. Aspirate the sample in Atomic Absorption spectrometer against standard solution and measure the absorbance.

Calculation (mg/kg)

Sample concentration x volume made up / weight of sample

Antimicrobial study of Silasathu paavanai

Media used

1. Nutrient Agar per litre/gram (NA)

Peptone	-5.0g
Yeast extract	-2.0g

NaCl	-5.0g
Agar	-18.0g
Distilled water	-1000ml
pH	- 7.0

Culture collection and maintenance

The organisms used for the study namely *E.Coli*, *Staphylococcus aureus, Salmonella typhii, Vibrio cholera, Pseudomonas spp* were obtained from CAS in Botany and Department of Biochemistry University of Madras, Guindy Campus, Chennai 600 025.

Preparation of culture for antibacterial studies

Twelve hours old bacterial suspension was adjusted to 0.5 OD and 1.0ml from the above was inoculated into 50 ml of nutrient broth and incubated at 37 ^{0} C in an orbital shaker at 150 rpm. To determine the growth rate, culture was removed at 4h interval and the growth was monitored by measuring the optical density at 540nm in spectrophotometer. The growth curve was drawn by plotting OD value against the incubation time.

Antibacterial susceptibility test[8]

Brain heart infusion (BHI) agar was used for susceptibility testing. Disks of 6mm in diameter were punched from a sheet of Whatman filter paper, sterilized, and impregnated with 25 micro L each of 0.2 g/ml extract or solvent alone and dried at 30–35 degree C for 12–24 h. The bacterial inoculums were prepared from subcultures of bacteria as follows: four to five colonies of the isolates were emulsified in sterile distilled water and the turbidity adjusted to 1.5×10^{8} CFU/ml (corresponding to 0.5 McFarland standards). A sterile cotton swab was dipped into the standardized bacterial suspension and used to evenly inoculate the BHI agar plates. The plates were allowed to dry for 3-5 min. Thereafter, all disks were placed on the plates and pressed gently to ensure complete contact with agar. A distance of at least 15mm was maintained from the edges of the plates to prevent overlapping of inhibition zones. Fifteen minutes following placement of the disks, the plates were incubated at 37°C for 2–5 days. They were then examined and the diameter of the zone of inhibition measured.

Well-in agar method[9]

Anti-bacterial activity of aqueous extract of *silasathu paavanai* was tested by a modified well-in agar method. The inoculum suspension was spread uniformly over the agar plates using sterile cotton swab spreader, to get a uniform distribution of bacteria. Subsequently, using a sterile borer, well of 0.5 cm diameter was made in the inoculated media. Different concentration drug 25, 50, 75 and 100 µg/ml of each extract was aseptically filled into the well. Later the plates were placed at room temperature for an hour to allow diffusion of extract into the agar. Then the plates were incubated for 24 h at 37°C. The results were recorded by measuring the diameter of inhibition zone at the end of 24-48 h

RESULTS

Physical properties of Silasathu paavanai

Table – 1

SL.NO	Parameter	Results
	Physical properties	
1	Ph at 25°C (1:10 Ratio)	5.48
2	Ash Value @ 550°C (%)	82.5
3	Water soluble (%)	58.5
4	Alkalinity as $CaCO_3$ in water soluble ash (%)	0.16
5	Acid Insoluble Ash, (%)	1.20
6	Loss of drying @ 105°C (%)	0.72

Quantitative analysis of essential elements in *Silasathu paavanai* by atomic absorption spectrometer

Sample Description: Silasathu

Table – 2

SL. NO	PARAMETER	RESULT
1	Calcium as Ca	17.58 %

Sample Description: Silasathu paavanai

Table - 3

SL. NO	PARAMETER	RESULT
1	Calcium as Ca	20.74 %
2	Magnesium as Mg	68.3 mg/kg
3	Zinc as Zn	4.4 mg/kg

Anti microbial study of Silasathu paavanai

The antibacterial activity of crude ethanol extract of *Silasathu paavanai* was tested against various bacterial pathogens and inhibition was determined by disc diffusion method.

Table – 4

Test micro organism	Zone of inhibition (mm)			
	Different concentrations of ethanol extract of SP			
	25µl	50µl	75µl	100µl
E.Coli	6	7	7	8
Staph.aureus	8	9	10	12
Salmonella typhii	7	8	8	8
Vibrio cholera	7	9	10	12
Pseudomonas spp	5	6	6	7

The anti bacterial activity of aqueous extract of SP was tested against bacterial pathogens and effective inhibition was determined by afar well diffusion method.

Table – 5

Test micro organism	Zone of inhibition (mm)			
	Different concentrations of aqueous extract of SP			
	25µl	50µl	75µl	100µl
E.Coli	15	17	19	20
Staph.aureus	9	10	11	13
Salmonella typhii	13	14	15	17
Vibrio cholera	11	13	14	16
Pseudomonas spp	9	10	14	15

DISCUSSION

Physical characterization of the drug was carried out to specify the features of the drug and its authenticity. The pH of *Silasathu paavanai* is 5.48. It is reported that Calcium

oxalate crystals are more soluble under acidic conditions and decreases with increasing alkalinity. Here the pH of the drug is slightly acidic and this may also contribute in dissolving the calculi. The other parameters standardized will pave way for the future preparation of the drug and check with the specifications given now. The drug *Silasathu paavanai* was subjected further to the quantitative analysis of Calcium, Magnesium and Zinc by AAS method. The amount of 'Ca' in the raw drug was 17.58%. The amount of Ca, Mg and Zn in the prepared drug was found to be 20.74%, 68.3 mg/kg, 4.4 mg/kg respectively. *In vivo*, magnesium inhibits stone formation in hyperoxaluric rats, and some clinical studies suggest a protective effect of magnesium supplementation in calcium oxalate stone formers[10]. One study showed the effect of mineral water containing Calcium and Magnesium on Calcium oxalate urolithiasis risk factors. It was concluded that mineral water containing calcium and magnesium deserves to be considered as a possible therapeutic or prophylactic agent in calcium oxalate kidney stone disease[11]. Evidence suggests that consumption of low calcium diets is actually associated with a higher overall risk for the development of kidney stones. This is perhaps related to the role of calcium in binding ingested oxalate in the gastro intestinal tract. As the amount of Calcium intake decreases, the amount of oxalate available for absorption into the blood stream increases; this oxalate is then excreted in greater amounts into the urine by the kidneys. In the urine, oxalate is a very strong promoter of calcium oxalate precipitation, about 15 times stronger than calcium[12] Low dose supplementation of Zn has been shown to increase the humoral response after vaccination, demonstrating antiviral activity. Zn is thought to activate serum thymulin, which stimulates thymocyte proliferation, producing T-cells and enhancing the antibody response[13]. The aqueous and ethanol extracts were studied for their antimicrobial activity. Since urinary tract infection is commonly associated with urolithiasis this study was preferred. Among both extracts, SP in water showed greater inhibition especially against *E.Coli*. The aqueous extract of SP showed significant inhibition in all the test organisms (Zone of inhibition > 1cm). The alcoholic extract showed significant inhibition against *Vibrio cholera* and *Staphylococcus aureus*. It had moderate activity against *E.coli, Salmonella typhii* and *Pseudomonas spp* (Zone of inhibition < 1cm).

CONCLUSION

The drug *Silasathu paavanai* contains essential elements which are considered to be the inhibitors of stone formation. Moreover the aqueous extract of the drug has good anti microbial activity against *E.coli* which causes the commonest associated urinary tract infection. To conclude, the claim in the siddha system of medicine is scientifically validated.

REFERENCES

1. Veale D.J.H, Oliver, D.W., Arangies N.S and furmank, South African traditional herbal medicines used during pregnancy and childbrith, Journal of Ethnopharmacology, 36:185 – 191 ,(1992)
2. Dr. N. Kuppusamy Mudaliar, Siddha maruthuvam pothu, 6th edition, Published by Directorate of Indian medicine and Homeopathy, Pg 460-461,(2004)
3. Mukhia R, Shreshta K, Dahal P, Sharma VK, Study on chemical composition of Urinary stones and its association with Urinary tract infection, PMJN, 8(2): (2009)
4. Hakim P.M. Abdhullah Saib, Anubhoga vaidhya Navaneetham, II edition, Vol – 2, Pg 161
5. Pharmacopoeia of India, New Delhi, 1996 II edition, Manager of Publication 947
6. Protocol for testing of Ayurvedic, Siddha and Unani medicines, PLIM, Ghaziabad, MHFW, Department of AYUSH

7. Mustafa Tuzen, Determination of heavy metals in Soil, mushroom and plant samples by Atomic Absorption Spectrometry, Micro Chemical Journal, 74: 289 – 97,(2005)
8. Rabe. T and Staden J.V, Antibacterial activity of South African plants used in medicinal purposes, Journal of Ethnopharmacology ,56: 81 – 87 ,(1997)
9. Atta – ur – Rahman *et al*, Antifungal diterpenoid alkaloids from *Delphinium denudatum*, J.Nat. Prod, 60: 472 – 474 ,(1997)
10. Bradley F. Schwartz, Jere my Bruce, Stefen Leslie, Marshal L. Stoller. Journal of Endourology, Apr 1, 15(3) : 233 – 235,(2001)
11. Rodgers A.L, The influence of South African mineral water on reduction of risk of calcium oxalate kidney stone formation, South African medical journal, 88(4): 448-51,(1998)
12. Parmar, Malvinder S (2004) “Kidney Stones”. British Medical Journal 328 (7453) : 1420 – 1424
13. Guroden F. Impact of trace elements and vitamin supplementation on immunity and infections in institutionalized elderly patients. Arch. Intern. Med,159:748 – 50,(1999)