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Email: sljim@fim.cmb.ac.lk

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Cover story

Hatawariya

Botanical name: ***Asparagus racemosus***
Family: ASPARAGACEAE
Vernacular names: Sinhala: *Hatawariya;* **Sanskrit:** *Satavari;*
English: Wild Asparagus; **Tamil:** *Shatavali, Thaneervittan, Kilangu;*
Hindi: *Shatavari;* **Urdu:** *Satavar*

Asparagus racemosus, often referred to as *Shatavari*, is a valued medicinal herb in both Ayurvedic and Unani traditional practices. This climbing, prickly bush flourishes in tropical and subtropical regions, typically located in sandy or loamy soil. It features small, white blossoms and tuberous roots, which serve as the main source of its healing qualities[1].

A. racemosus is common throughout Sri Lanka, India and the Himalayas. *A. racemosus* is a woody climber growing to 1-2 m in height. The leaves are like pine needles, small and uniform and flowers are white and have small spikes[1]. *A. racemosus* and has been specially recommended in cases of threatened abortion and as a galactogogue. It is also beneficial in female infertility to increases libido and cures inflammation of sexual organs, Enhance folliculogenesis and ovulation, prepares the womb for conception, prevents miscarriages, acts as post-partum tonic by increasing lactation and normalizing the uterus and the changing hormones[2]. Root of *A. racemosus* has been referred as bitter-sweet, emollient, cooling, nervine tonic, constipating, galactogogue, and aphrodisiac, diuretic, rejuvenating, carminative, stomachic, and antiseptic and as tonic[3].

Phytochemically, ethanolic root extract of *A. racemosus* revealed the presence of alkaloids, flavanoids, tannins, phytosterols, glycosides, carbohydrates, proteins and fats. This plant also contains vitamins (A, B1, B2, C, E) and minerals (Mg, P, Ca, Fe) and folic acid[4].

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Effects of *Shatavari* (*Asparagus racemosus*) root powder of Sri Lankan origin on Seminal Fluid Parameters; A double-blind placebo-controlled randomized clinical study

Karunarathne Y.A.U.D.[1*] and Weerasooriya T.R.[2]

Abstract

Male factor infertility is a multifactorial disorder that affects a significant percentage of infertile couples; however, many of them remain untreated. In recent years, considerable numbers of infertile men have pursued 'herbal remedies' as an effective treatment. Among 'herbal remedies', *Shatavari – Asparagus racemosus* Willd is recommended for male sub-fertility in Ayurveda medicine. The effect of *Shatavari – Asparagus racemosus* root powder of Sri Lankan origin was compared with a placebo for the male reproductive potential. The study included 150 subfertile men with Oligospermia, Asthenozoospermia, Teratozoospermia, and Oligoasthenoteratozoospermia (OAT) who were randomized to receive Shatavari 24 g/day (group T) or a similar regimen of placebo (group C) for 6 months, administered daily in the morning before breakfast with cow's ghee. The two groups were compared for changes in semen parameters. At the end of the study, statistically significant improvements were observed in the Shatavari-admitted group in semen parameters (volume, sperm concentration, motility, and morphology). At the end of the trial, patients in group T had a mean volume of 2.69 ± 0.14 mL, concentration of 53.28 ± 5.50 million/mL and rapid motility of 26.75 ± 1.68% which was statistically significant from the mean of volume 2.10 ± 0.12 mL, concentration of 32.09 ± 2.88 million/mL and rapid motility on 16.84 ± 1.63% in the placebo group ($p < 0.001$). Normal sperm morphology was 41.12 ± 3.00% and 32.87 ± 2.71% in groups T and C, respectively ($p < 0.001$). Shatavari statistically significantly improved semen parameters in sub-fertile men with decreased seminal parameters.

Keywords: *Shatavari*, male reproductive potential, Seminal Fluid parameters

Introduction

Sub-fertility is both a clinical and a public problem, affecting the life of the couple, the healthcare services, and the social environment. Subfertility affects an estimated 15% of couples globally, and males are found to be solely responsible for 20-30% of cases, contributing to 50% of cases overall[7]. A decrease in the quality and quantity of sperm is one of the main factors responsible for reducing male reproductive potential. A wide range of strategies has been implemented to address this issue. Treatment of human chorionic gonadotropin and follicle-stimulating hormones and assisted reproductive technologies, including intrauterine insemination (IUI), *in vitro* fertilization (IVF), intracytoplasmic sperm injection (ICSI), etc., are examples. However, high expenditure, invasive, and may increase the risk of birth defects, the whole world is seeking effective natural remedies to enhance male reproductive potential. According to Ayurveda properties of the *Shatavari* root enhance male reproductive potential. Classical compendia of Ayurveda mention that Shatavari has properties of *Shukrala* (Increasing sperm concentration), *Shukra pravartaka* (Increasing

[1]*Department of Shalya Shalakya, Prasutitantra and Kaumarabhrithya, Faculty of Indigenous Medicine, University of Colombo, Sri Lanka.*
[2]*Faculty of Medicine, Kothalawala Defense University, Sri Lanka.*

Correspondence: Karunarathne Y.A.U.D., Department of Shalya Shalakya, Prasutitantra and Kaumarabhrithya, Faculty of Indigenous Medicine, University of Colombo, Sri Lanka. Email: drdkarunarathne@yahoo.com

motility), *Shukra shodhana* (Purifying action of seminal fluid), *Rasayana* (Rejuvenating action), and *Balya* (Promoting strength)[2,5]. The roots of *A. racemosus* have been extensively used by Ayurveda physicians in Sri Lanka and India for treating enhanced seminal parameters. However, the effects of *A. racemosus* on enhancing the seminal parameters have not yet been proven scientifically. Recently published research reports revealed some beneficial effects of both alcoholic and aqueous extracts of the *Shatavari* roots on aphrodisiac activity[10]. Thus, the present study aims to evaluate the effect of *A. racemosus* on male reproductive potential with respect to seminal fluid parameters to demonstrate the scientific validity of the usage *in vivo*.

Materials and methods

Study design

This study was a randomized double-blind clinical trial conducted in the gynecology clinic of the National Ayurveda Teaching Hospital, Borella, Sri Lanka. The ethics approval for this clinical trial was obtained from the Research Approval Committee of the Faculty of Graduate Studies at the University of Colombo (FGS/ERC/2016/22). The study was conducted adhering to good clinical practice guidelines. Written informed consent was obtained from each participant before conducting the trial. The participants were given sufficient time to ask questions and decide whether they wished to participate in this study or not.

Study Population

The sample size was calculated with the main outcome parameters as the enhancement of concentration, motility, and morphology of the seminal fluid (each group, 75+, loss to follow-up 10).

Sample size

150, Convenient Sampling

Randomization Technique

The eligible males were identified, and they were randomly allocated to the test group or the placebo group. Envelopes were made, including groups by using computer-generated random sampling (SPSS), and were kept with the medical officer in the clinic who delivered the drug and placebo.

Both the test drug and the placebo were prepared, with the test drug labeled as A and the placebo as B. An equal number of each was kept with the medical officer, who delivered the drugs without revealing the meaning of the labels. A separate register was maintained by the medical officer providing the drugs, noting the name and label of each. The register was stored in a secure location and only reviewed during data analysis and interpretation.

Inclusion and exclusion criteria

Inclusion criteria for recruitment of the male participants

- 25 to 45 years of age
- Semen parameters – men with Oligoasthenoteratozoospermia (OATS) syndrome, oligozoospermic, asthenozoospermic, and teratozoospermic abnormal semen parameters – total motile sperm count < (1.5 x 15 x 35) (volume x concentration x motility) as per reference ranges of the 5th edition of WHO laboratory manual for the examination and processing of human semen, 2010.

Exclusion criteria

- Patients with a history of chronic illnesses such as diabetes mellitus, hypertension, renal and liver dysfunction, fatty liver, hyperlipidemia, gall bladder stones, genetic disorders, and testicular atrophy.
- Patients on chemotherapy and any hormonal treatments.
- Men having a history of any surgeries for reproductive tract disorders.
- Varicocele, hydrocele, and testicular pathology

Data Collection and Intervention

A validated questionnaire collected data

Intervention

Group A - The test group was treated with 24 g *Shatavari* root powder with 10 mL of cow's ghee in the morning before breakfast.

Group B – The Control group was treated with a placebo, a form of bolus of 24 g powder of brown rice with 10 mL of cow's ghee in the morning before breakfast.
Treatment period – 6 months
Follow-up period – 6 months

Collection and Preparation of the test drug and placebo

Collection of Asparagus racemosus

Roots of *A. racemosus* of Sri Lankan origin were collected from Hambantota, Anuradhapura, and Colombo districts in Sri Lanka.

***Authentication of the Asparagus racemosus* plant**

The curator of the botanical garden in Peradeniya identified and authenticated plant material of Sri Lankan origin. A specimen was deposited at the National Herbarium, Botanical Garden, Peradeniya, Sri Lanka.

Preparation of a research drug for a clinical study

Ingredients of the research drug

Root powder of *A. racemosus* – 24 gm Cow's ghee – 10 mL. Dose of the research drug and *Anupana* were selected according to Kashyapa Samhita[8].

Preparation of root powder of Asparagus racemosus

The roots were de-stoned and cleaned with running tap water, followed by rinsing with deionized water, and cut into small pieces. Fine powder was made and packed in 24g sterilized vacuum sachet packets under hygienic conditions.

Cow's ghee

Highland cow's ghee was purchased and packed as 10 mL weight sterilized vacuum sachet packets under hygienic conditions. Specification of "Highland" cow's ghee was taken from MILCO Pvt Ltd, Sri Lanka.

Preparation of a placebo for a clinical study

Red rice was purchased from the Sri Lankan market and washed with running tap water, followed by rinsing with deionized water, and dried in direct sunlight. The fine powder was made and 24 g weight, sterilized, and vacuum sachet packets were packed under hygienic conditions.

Red rice powder and *Shatavari* root powder had almost the same appearance; therefore, the researcher could not identify a placebo from the trial drug.

Instructions for the participants in the clinical study

Instructions were given to the participants to make a bolus of one sachet packet mixed with 10 mL of cow's ghee, which was taken before breakfast.
red as statistically significant.

Efficacy Assessment

Seminal fluid analysis

Seminal fluid analysis (SFA) was done before treatment, after three months of treatment, and end of the treatment. During the follow-up period, SFA was analyzed twice with a gap of three months. Semen collection, analysis, and interpretation were carried out as per WHO guidelines[12].

Methodology to enhance compliance

All participants were asked to visit the clinic every 14th day. Telephone contacts, SMS, and emails were used as reminders.

Safety analysis

Each patient underwent the hematological investigations (full blood count (FBC), aspartate aminotransferase (ALT), alanine aminotransferase (AST), serum creatinine, glomerular filtration rate (GFR), urine full report (UFR) and USS of the abdomen and scrotum before and after the treatment. The vital signs were measured and recorded in a patient diary at each visit. The safety endpoints were considered as the number and proportion of patients withdrawing from treatment early for safety reasons, changes in hepatic and renal safety parameters, and the number and proportion of patients experiencing adverse effects.

Statistical analysis

The results of experiments were expressed using mean ± standard error of the mean. Differences

between the two groups were compared by using Student's t-test, and statistical comparisons between experimental groups were made by analysis of variance and Tukey's posttest, as offered by Minitab 18 Stat version (Minitab, Inc., USA)

Statistical significance was determined as $P < 0.05$.

Results and observations

An epidemiological study encompassed age, religion, education, socio-economic status, occupation, dietetic pattern, addictions, BMI, sub-fertility history, sexual history, and history of environmental and occupational factors that affect reproductive potential, and the factors were non-significant in both groups. Therefore, participants of both the trial and control groups had similar backgrounds in the present study sample.

Liquefaction time, viscosity, appearance, and pH were almost similar in both groups before and after the treatments, and after the follow-up period. Liquefaction time was normal within 60 minutes, appearance was opaque, and pH was alkaline. According to trial group data, viscosity was normal in 85% of cases 1% of cases reported low viscosity and 15% of cases were in high viscosity before treatment. After 3 months of treatment, 11% in the trial group had high viscosity and 4% of cases had low viscosity. After the end of the treatment, all cases were reported to have normal viscosity in the trial group. Considering the viscosity of the control group 7% of cases were in high viscosity, 1% of cases were low viscosity and 92% of cases were normal. After the end of the six months, 94% of cases were in the normal category.

Trial group seminal fluid analysis is shown in Table1. Seminal fluid analysis, before, end of the treatment and end of the follow up period of the trial group is shown in Table 2.

At present study volume, concentration, motility and morphology were mainly considered and compared before the treatment, end of the treatment and after the follow-up period between trial and control groups. Seminal fluid analyses were taken after 3 days of abstinence. Before the treatment mean volume of trial group participants was 2.38 mL and it was increased to 2.5 mL and 2.69 mL after three months and the end of the treatment, respectively. After the follow-up period volume of sperm of participants in the trial group was 2.55 mL; however, this difference was insignificant. Tables 1 and 2 revealed that concentration also significantly increased before and after treatment. Further, that increment remained for another 6 months of the follow-up period.

Sperm motility and reproductive potential have a positive relationship. For fertility, it is required to have rapid motility of more than 25%. Tables 1 and 2 revealed that the mean value of rapid motility of the trial group was 11.23 at the beginning of the treatment of *Shatavari* root powder. It increased up to 17.68 and 26.75 after 3 months of duration and end of treatment, respectively. The difference was highly significant. Though there was no significant difference during the follow-up period, it was observed that rapid motility was maintained beyond the normal limit. The percentage of slow motility also significantly increased before treatment and at the end of the treatment. The mean value of slow motility was 18.34, 20.45, and 21.15 before the treatment, at the end of the treatment and end of the follow-up period. There was no significant difference shown between before and after treatment and after the follow-up period. However, the percentage of immotile sperm was dramatically reduced in the trial group between before and after the treatment. Reduction needed to be maintained during the follow-up period, and the percentage of immotile sperm was almost the same as at to end of the treatment i.e., 30.16%.

According to tables 1 and 2, the mean value of normal morphology was 29.91%. It was expressively increased at the end of the treatment, i.e., 41.12%. And abnormal morphology percentage pointedly reduced from 69.97% to 58.29% during 6 months of the treatment period and it persisted same another 6 months of the follow-up period.

Seminal fluid analysis of the control group is shown in Table 3. Seminal fluid analysis, before, end of the treatment and end of the follow up period of control group is shown in Table 4. Table 5 is shown the

seminal fluid analysis of Trial Group (TG) vs Control Group (CG).

Table 1: Seminal fluid analysis of Trial group

Parameter	Mean Values of before treatment (Mean ± SE)	Mean Values of after 3 months of treatment (Mean ± SE)	Mean Values of end of the treatment (Mean ± SE)	Mean Values of after 3 months of follow up (Mean ± SE)	Mean Values of end of the follow up period (Mean ± SE)	T value of before & after 3 months of Treatment	T value of after 3 months & end of the Treatment	T value of end of the treatment & after 3 months of Follow up	T value of after 3 months of Follow up & end of the follow up	Probability value of before & after 3 months of Treatment	Probability value of after 3 months & end of the Treatment	Probability value of end of the treatment & after 3 months of Follow up	Probability value of after 3 months of Follow up & end of the follow up
Volume	2.38 ± 0.14	2.50 ± 0.14	2.69 ± 0.14	2.44 ± 0.12	2.55 ± 0.11	-1.13	-2.19	2.69	-1.32	0.262	*0.032	**0.009	0.192
Concentration	31.60 ± 3.66	37.61 ± 3.48	53.28 ± 5.50	50.58 ± 4.72	53.70 ± 3.88	-3.42	-5.03	0.96	-1.12	**0.001	**0.000	0.340	0.268
Rapid Motility	11.23 ± 1.34	17.68 ± 1.30	26.75 ± 1.68	27.16 ± 1.64	29.52 ± 1.63	-5.14	-5.92	-0.35	-1.82	**0.000	**0.000	0.726	0.073
Slow Motility	18.34 ± 11.49	20.15 ± 12.61	20.45 ± 1.17	21.40 ± 1.22	21.15 ± 1.15	-1.02	-0.22	-0.67	0.21	0.309	0.831	0.502	0.834
Non-Motile	18.79 ± 1.58	19.99 ± 1.33	18.07 ± 1.11	18.51 ± 1.04	19.20 ± 1.12	-0.88	1.59	-0.38	-0.66	0.381	0.116	0.701	0.510
Immotile	50.44 ± 2.57	42.20 ± 2.31	34.57 ± 2.09	32.99 ± 1.98	30.16 ± 1.93	3.44	4.06	0.84	1.81	**0.001	**0.000	0.401	0.070
Normal Morphology	29.91 ± 3.04	35.52 ± 2.90	41.12 ± 3.00	39.41 ± 3.04	41.97 ± 2.96	-2.71	-3.29	1.01	-1.61	**0.008	**0.002	0.314	0.113
Abnormal Morphology	69.97 ± 3.03	64.48 ± 2.90	58.75 ± 2.99	60.45 ± 3.03	58.29 ± 2.95	2.64	3.38	-1.01	1.35	*0.010	**0.001	0.314	0.181

Table 2: Seminal fluid analysis of Trial group, before, end of the treatment and end of the follow up period

Parameter	Mean Values of before treatment (Mean ± SE)	Mean Values of end of the treatment (Mean ± SE)	Mean Values of end of the follow-up (Mean ± SE)	T value of before treatment & end of the Treatment	T value of before treatment & end of the Follow-up	The probability value of before treatment & end of the Treatment	Probability value before treatment & end of the follow-up
Volume	2.38 ± 0.14	2.69 ± 0.14	2.55 ± 0.11	-2.83	-3.12	**0.006	**0.003
Concentration	31.60 ± 3.66	53.28 ± 5.50	53.70 ± 3.88	-6.48	-6.37	**0.000	**0.000
Rapid Motility	11.23 ± 1.34	26.75 ± 1.68	29.52 ± 1.63	-7.80	-9.42	**0.000	**0.000
Slow Motility	18.34 ± 11.49	20.45 ± 1.17	21.15 ± 1.15	-1.23	-1.64	0.223	0.104
Non-motile	18.79 ± 1.58	18.07 ± 1.11	19.20 ± 1.12	0.47	-0.28	0.643	0.784
Immotile	50.44 ± 2.57	34.57 ± 2.09	30.16 ± 1.93	5.48	7.16	**0.000	**0.000
Normal Morphology	29.91 ± 3.04	41.12 ± 3.00	41.97 ± 2.96	-4.80	-4.40	**0.000	**0.000
Abnormal Morphology	69.97 ± 3.03	58.75 ± 2.99	58.29 ± 2.95	4.80	4.20	**0.000	**0.000

Table 3: Seminal fluid analysis of Control group

Parameter	Mean Values of before treatment (Mean ± SE)	Mean Values of after 3 months of treatment (Mean ± SE)	Mean Values of end of the treatment (Mean ± SE)	Mean Values of after 3 months of follow up (Mean ± SE)	Mean Values of end of the follow up period (Mean ± SE)	T value of before & after 3 months of Treatment	T value of after 3 months & end of the Treatment	T value of end of the treatment & after 3 months of Follow up	T value of after 3 months of Follow up & end of the follow up	Probability value of before & after 3 months of Treatment	Probability value of after 3 months & end of the Treatment	Probability value of end of the treatment & after 3 months of Follow up	Probability value of after 3 months of Follow up & end of the follow up
Volume	2.48 ± 0.13	2.27 ± 0.12	2.10 ± 0.12	2.06 ± 0.11	1.84 ± 0.12	1.82	1.33	0.31	1.69	0.074	0.187	0.760	0.096
Concentration	45.56 ± 4.43	37.21 ±.19	32.09 ± 2.88	26.09 ± 2.48	22.24 ± 2.30	2.45	1.83	2.29	1.93	0.017	0.072	*0.025	0.057
Rapid Motility	24.89 ± 2.27	21.57 ± 1.99	16.84 ± 1.63	16.28 ± 1.39	16.45 ± 1.50	2.15	2.73	0.37	-0.12	0.035	**0.008	0.715	0.909
Slow Motility	19.01 ± 1.20	19.56 ± 1.12	20.76 ± 1.20	21.52 ± 1.18	20.03 ± 1.09	-0.50	-0.98	-0.58	1.29	0.621	0.329	0.567	0.201
Non-motile	18.73 ± 0.99	21.75 ± 0.97	21.84 ± 0.98	23.76 ± 0.96	25.99 ± 1.07	-2.77	-0.09	-1.86	-1.92	**0.007	0.929	0.068	0.059
Immotile	37.89 ± 2.49	37.19 ± 1.92	40.68 ± 1.87	38.24 ± 1.82	37.36 ± 1.95	0.37	-1.97	1.20	0.47	0.715	0.053	0.234	0.637
Normal Morphology	28.31 ± 2.84	29.71 ± 2.77	32.87 ± 2.71	32.96 ± 2.82	33.51 ± 2.98	-0.69	-1.98	-0.08	-0.49	0.494	0.052	0.939	0.623
Abnormal Morphology	71.69 ± 2.85	70.29 ± 2.77	67.25 ± 2.72	67.04 ± 2.82	66.41 ± 2.97	0.69	1.85	0.18	0.57	0.494	0.068	0.862	0.572

Table 4: Seminal fluid analysis of before, end of the treatment and end of the follow up period of the Control group

Parameter	Mean Values of before treatment (Mean ± SE)	Mean Values of end of the treatment (Mean ± SE)	Mean Values of end of the follow up (Mean ± SE)	T value of before treatment & end of the Treatment	T value of before treatment & end of the Follow up	Probability value of before treatment & end of the Treatment	Probability value of before treatment & end of the follow up
Volume	2.48 ± 0.13	2.10 ± 0.12	1.84 ± 0.12	3.18	4.34	**0.002	**0.000
Concentration	45.56 ± 4.43	32.09 ± 2.88	22.24 ± 2.30	3.78	5.76	**0.000	**0.000
Rapid Motility	24.89 ± 2.27	16.84 ± 1.63	16.45 ± 1.50	4.31	4.04	**0.000	**0.000
Slow Motility	19.01 ± 1.20	20.76 ± 1.20	20.03 ± 1.09	-1.23	-0.61	0.222	0.541
Non-motile	18.73 ± 0.99	21.84 ± 0.98	25.99 ± 1.07	-2.44	-5.31	*0.017	**0.000
Immotile	37.89 ± 2.49	40.68 ± 1.87	37.36 ± 1.95	-1.27	0.19	0.207	0.846
Normal Morphology	28.31 ± 2.84	32.87 ± 2.71	33.51 ± 2.98	-2.00	-2.03	*0.049	*0.046
Abnormal Morphology	71.69 ± 2.85	67.25 ± 2.72	66.41 ± 2.97	1.92	2.06	0.059	*0.043

Table 5: Seminal fluid analysis of the Trial Group (TG) vs Control Group (CG)

Parameter	Mean Values of before treatment (Mean ± SE) - TG	Mean Values of before treatment (Mean ± SE) -CG	Mean Values of after 3 months of treatment (Mean ± SE) - TG	Mean Values of after 3 months of treatment (Mean ± SE) - CG	Mean Values of end of the treatment (Mean ± SE) - TG	Mean Values of end of the treatment (Mean ± SE) - CG	T value of before Treatment	T value of before & after 3 months of Treatment	T value of after 3 months & end of the Treatment	Probability value of before Treatment	Probability value of before & after 3 months of Treatment	Probability value of after 3 months & end of the Treatment
Volume	2.38 ± 0.14	2.48 ± 0.13	2.50 ± 0.14	2.27 ± 0.12	2.69 ± 0.14	2.10 ± 0.12	-0.52	1.28	3.20	0.601	0.204	**0.002
Concentration	31.60 ± 3.66	45.56 ± 4.43	37.61 ± 3.48	37.21 ± 3.19	53.28 ± 5.50	32.09 ± 2.88	-2.43	0.08	3.42	*0.016	0.932	**0.001
Rapid Motility	11.23 ± 1.34	24.89 ± 2.27	17.68 ± 1.30	21.57 ± 1.99	26.75 ± 1.68	16.84 ± 1.63	-5.18	-1.63	4.23	**0.000	0.105	**0.000
Slow Motility	18.34 ± 11.49	19.01 ± 1.20	20.15 ± 12.61	19.56 ± 1.12	20.45 ± 1.17	20.76 ± 1.20	-0.37	0.32	-0.18	0.709	0.750	0.855
Non-Motile	18.79 ± 1.58	18.73 ± 0.99	19.99 ± 1.33	21.75 ± 0.97	18.07 ± 1.11	21.84 ± 0.98	0.03	-1.07	-2.55	0.977	0.287	*0.012
Immotile	50.44 ± 2.57	37.89 ± 2.49	42.20 ± 2.31	37.19 ± 1.92	34.57 ± 2.09	40.68 ± 1.87	3.50	1.67	-2.18	**0.001	0.097	*0.031
Normal Morphology	29.91 ± 3.04	28.31 ± 2.84	35.52 ± 2.90	29.71 ± 2.77	41.12 ± 3.00	32.87 ± 2.71	0.38	1.45	2.04	0.701	0.149	0.149
Abnormal Morphology	69.97 ± 3.03	71.69 ± 2.85	64.48 ± 2.90	70.29 ± 2.77	58.75 ± 2.99	67.25 ± 2.72	-0.41	-1.45	-2.10	0.680	0.149	*0.037

Parameter	Mean Values of after 3 months of follow up (Mean ± SE) - TG	Mean Values of after 3 months of follow up (Mean ± SE) - CG	Mean Values of end of the follow up period (Mean ± SE) - TG	Mean Values of end of the follow up period (Mean ± SE) - CG	T value of end of the treatment & after 3 months of Follow up	T value of after 3 months of Follow up & end of the follow up	Probability value of end of the treatment & after 3 months of Follow up	Probability value of after 3 months of Follow up & end of the follow up
Volume	2.44 ± 0.12	2.06 ± 0.11	2.55 ± 0.11	1.84 ± 0.12	2.35	4.45	*0.020	**0.000
Concentration	50.58 ± 4.72	26.09 ± 2.48	53.70 ± 3.88	22.24 ± 2.30	4.59	6.97	**0.000	**0.000
Rapid Motility	27.16 ± 1.64	16.28 ± 1.39	29.52 ± 1.63	16.45 ± 1.50	5.07	5.89	**0.000	**0.000
Slow Motility	21.40 ± 1.22	21.52 ± 1.18	21.15 ± 1.15	20.03 ± 1.09	-0.07	0.71	0.944	0.482
Non-Motile	18.51 ± 1.04	23.76 ± 0.96	19.20 ± 1.12	25.99 ± 1.07	-3.72	-4.37	**0.000	**0.000

Table 3 and 4 represented control group sperm analyzing data of the present study. Mean volume of control group before treatment was 2.48 mL. It was significantly reduced up to 2.1 mL and 1.84 mL at the end of the treatment and end of the follow up period. However, changes of volume were in normal limits. According to data sperm concentration also reduced from 45.56 million to 32.09 million during 6 months of treatment period and up to 22.24 million during 6 months of follow up period. Though above value different was significant statistically, all three were above to normal limit according to WHO. Control group of study participants were shown the decrease of rapid motility. At the beginning mean percentage of rapid motility was 24.89% and it was in minimum requirement of rapid motility. At the end of the treatment rapid motility was reduced 16.84% and it was remained in same level during the follow up period too. When considered slow motility, non-motility, normal morphology and abnormal morphology there were no any changes during the treatment and follow up period except nonmotile percentage at the end of follow up.

Table 5 shows the comparison of seminal fluid parameters between the trial and control groups. According to the data, it was revealed that the difference in volume was statistically significant at the end of the treatment and follow-up period. The

sperm concentration of the control group was higher than the trial group, and that increase was statistically significant. But, at the end of the treatment and during the follow-up period, that was reversed and concentration was higher in the trial group than the control group; the difference was statistically significant.

At the beginning of treatment mean rapid motility of the trial group was 11.23% and it was lower than the mean rapid motility percentage of the control group. Even though, in the trial group, rapid motility was increased remarkably and the mean percentage of rapid motility of the trial group at the end of treatment and end of follow-up period was 26.75 and 29.52, respectively. The difference was highly significant. There was no significant change in slow motility percentages between the groups of the study sample. Considered in non-motile sperm percentage, it was significantly increased in the control group at the end of the treatment and during the follow-up period. Immotile sperm percentage was dramatically reduced in the trial group and the difference was statistically significant. There was not much difference in normal and abnormal morphology between the trial and control groups (Table 6).

Table 6: Semen Classification of trial and control group participants before and after treatment and after follow up

Semen Classification	Before Treatment				After Treatment				After Follow-up			
	Trial		Control		Trial		Control		Trial		Control	
	No	%	No	%	No	%	No	%	No	%	No	%
Normal	02	2.7	25	33.3	38	50.7	12	16	51	68	09	12
Hypospermia	10	13.3	01	1.3	03	4	00	0	02	2.7	01	1.3
Hyperspermia	01	1.3	00	00	01	1.3	00	0	00	0	00	0
Oligozoospermia	16	21.3	06	8	08	10.7	20	26.7	05	6.7	22	29.3
Asthenozoospermia	35	46.7	32	42.7	21	28	35	46.7	13	17.3	31	4.3
Teratozoospermia	01	1.3	05	6.7	03	4	02	2.7	03	4	03	4
OATS	10	13.3	06	8	01	1.3	06	8	01	1.3	09	12

For before treatment; Chi-Square value is 36.303 df, Asym. Significant (2 sided) 0.000

At the end of the treatment; Chi-Square value is 29.934 df, Asym. Significant (2 sided) 0.000

After follow up period; Chi-Square value is 54.201 df, Asym. Significant (2 sided) 0.000

According to seminal fluid analysis of the study sample, participants were categorized into 07 groups normal, hypospermia, hyperspermia, oligo-zoospermia, asthenozoospermia, teratozoospermia, and OATS syndrome. It was statistically significant in a normal category before the treatment between trial and control groups. In the trial group, there were 2.7% of participants belonged to a normal category, and it was 33.3% in the control group. Participants who suffered from *asthenozoospermia* were high in both groups, i.e., 46.7% and 42.7% in the trial and control groups, respectively. There were 21.3% of *oligozoospermic* clients in the trial group and 8% in the control group before the treatment. A total of 21.3% of clients suffered from OATS syndrome before the treatment.

At the end of the treatment highly significant difference was seen in normal participants between the two groups. There were 50.7% and 68% normal SFA seen at the end of the treatment and end of the follow-up period. It was only 16% and 12% in the control group. Hypospermia, oligospermia, asthenozoospermia and OATS percentages were also dramatically reduced in the trial group at the end of the treatment and it was maintained during the follow-up period. Considering the teratozoospermia category, 2 cases were increased in the trial group and 3 cases were decreased in the control group at the end of the treatment.

Discussion

Seminal fluid analysis can identify problems with the count of sperm (quantitative) and their overall quality (qualitative). Seminal fluid analysis remains the primary test for assessing male reproductive potential and evaluates certain characteristics of semen such as volume, pH, sperm count, motility, morphology and viability of sperm. To get a better sample, participants were asked to avoid ejaculation or abstinence for 3 days (72 hours) before the test. Semen was collected by masturbation and delivered within 30 to 60 minutes for testing. Participants were asked not to apply any cream or liquid before masturbation and they were directed to a reputed hospital to undergo their investigation to minimize lab technician error.

Semen viscosity, appearance, pH and volume were macroscopically examined. All the semen samples showed a normal appearance (grey-opalescent) and alkaline pH between 6 and 10. According to the results, 85% of cases had normal viscosity in the trial group and 92% in the control group. High viscosity was found in 14% of participants in the trial group. High-viscosity seminal fluid restricts sperm motility. *A. racemosus* root powder may lower the viscosity due to its *Pitta shamaka* property. This *Pitta shamaka* property maintains the *Drava guna* (liquidity), which is inherent with *Pitta dosha*. Hence, *Shatavari* may lower the high viscosity of the seminal fluid. Though the mean volume of seminal fluid of study participants was normal (1.5 mL) before, the end of the treatment and end of the follow-up period, a significant increment (from 2.38 ± 0.14 mL to 2.69 ± 0.14 mL: p; 0.009) was recorded in the trial group (table 1). This may reflect the enhancement of secretory activity of the glands of the accessory organs, mainly the prostate and seminal vesicles.

Sperm concentration, motility and morphology are initial characteristics that are examined microscopically. Sperm concentration should be more than 15 million/mL, at least 32% of all sperm should show progressive motility, the sum of progressive and non-progressive motility should be at least 40% and at least 4% of all sperm cells should have normal morphology, indicating a normal seminal fluid sample[12]. The *Shatavari* root powder-treated group showed a highly significant ($p \leq 0.001$) enhancement in sperm concentration at the end of the treatment period of six months as compared to the sperm concentration at the beginning and at the end of the treatment in the control group (table 2 and 4). The increase in sperm concentration was from 31.60 ± 3.66 × 10^6/mL to 53.28 ± 5.50 × 10^6/mL, corresponding to an increase of 146%. A significant increment ($p\leq 0.001$) of rapid motility was also recorded from 11.23 ± 1.34% to 26.75 ± 1.68% (table 1) after 6 months, in contrast to the value before treatment in the trial group and at the end of treatment in the control group i.e.,16.84 ± 1.63% (table 3). There was a dramatic enhancement of normal morphology documented in the *Shatavari*-treated group from 29.91 ± 3.04% to 41.12 ± 3.00%; p 0.002 at the end of the treatment. Though it was normal according to WHO, that difference was not significant at the end of the treatment, in contrast to the beginning in the control group.

According to the data of the study sample, semen was classified into seven groups: normal, *hypospermia*, *hyperspermia*, *oligozoospermia*, *asthenozoospermia*, *teratozoospermia* and OATS. There was a significant increase in normal seminal fluid percentage from 2.7% to 50.7% in the *Shatavari-treated* group at the end of treatment. The number of participants in the normal category decreased to 16% from 33.3% in the control group.

Shatavari is comprised of a predominance of *Jala* and *Pritvi mahabhuta,* which are responsible for flourishing the body contents. Therefore, *Shatavari* increases the sperm too. By having this predominance of *Jal*a and *Pritvi* elements, *Shatavari* pacifies *Vata* and *Pitta* and increases *Kapha dosha*. Due to these physiological functions/attributes, *Shatavari* has an anabolic effect on the sperm. Due to the entire metabolic process described in Ayurveda, all digested food and medicinal substances ingested by humans are carried by the *Rasa dhatu*. As *Rasa dhatu* is the first *Dhatu* that is formed by the essence of *Ahara Rasa*, all the other *Dhatus* get their particular nourishment from this circulating *Rasa Dhatu* either directly or indirectly. *Shatavari*, the selected drug,

has the characteristic power of enhancing the quality of *Rasa dhatu* and *Shukra dhatu* by its *Madura rasa*, *Guru* and *Snigdha guna*, *Sheeta veerya* and *Madhura vipaka*. According to Ayurveda *Balya*, *Rasayana*, *Vajeekarana, Shukra janana, Shukra shodana* and *Shukra pravartaka* are the pharmacological properties of *Shatavari,* which strengthen *Sapta dhatu*[4]. Thus, improving reproductive function and general health and strength.

Samanya, Vishesa, Guna, Dravya, Karma, and *Samavaya* are the *Shad padartha* mentioned by *Charaka*. The treatments of Ayurveda are based on this theory of *Shad padartha*. Among them, *Samanya* and *Vishesa* are very useful in Ayurveda medicine. These six categories, *Shad padartha,* are seen to be of immense value in the applied aspect of treatment and also for maintaining health. The object of Ayurveda has been said to be maintaining homeostasis to the level of physiological equilibrium. *Samanya* and *Vishesa* are the dynamic forces that keep normal physiological conditions in the body. *Samanya* is the cause of the increase of all things at all times, whereas the application of this principle leads to enhancing the body elements. According to this theory, the pharmacological properties and functions of *Shatavari* in the aforementioned paragraph may increase the quality of semen.

Oxidative stress is one of the most common reasons for the destruction of sperm quality and the impairment of male reproductive potential. Low levels of reactive oxygen species (ROS) provide certain positive effects to sperm cells and enhance the ability to bind to the zona pellucida; high amounts of ROS alter the integrity of spermatozoa DNA and result in DNA fragmentation. Free radicals also alter the sperm structure and function, which impairs all semen parameters. The polyunsaturated lipid membrane of the mature spermatozoa is vulnerable to oxidation in the presence of ROS and causes impaired sperm morphology and motility. Antioxidants that are naturally found in semen act as free radical scavengers that help to overcome ROS. If endogenous antioxidants are not enough, exogenous antioxidant supplements can be useful to protect the sperm cell. Research studies have proven that *Shatavari* has an antioxidant action due to having various chemicals and their different pathways to combat free radicals[6]. Further, roots of *A. racemosus* are rich in vitamins C and E and trace minerals (zinc, copper, manganese, iron, cobalt, sodium, potassium, calcium, and lithium)[6, 10] help to enhance sperm quality. Animal studies have shown that *Shatavari* caused an increase in testicular size by 6.8 percent, possibly followed by an increase in spermatogenesis[9]. The size of the testis influences the total number of spermatozoa per ejaculate[11, 12]. Testicular size reflects the level of spermatogenic activity, which also affects sperm morphology[12]. The alleviating of oligospermia may be due to increasing or maintaining the actual size of the testis, and due to the spermatogenetic potential and aphrodisiac activity of *Shatavari,* which were demonstrated by previous researchers. The enhancement of semen quality and protecting the testis by improving antioxidant capacity might be useful to overcome fertility complications of diabetic individuals[1,3].

Conclusion

Shatavari root powder could improve the quantity and quality of semen in a statistically significant manner in comparison to the placebo, at the given dose of 24 gm/d with 10 mL cow's ghee before breakfast for six months.

The results suggested that the *Shatavari* root powder may be a new, auspicious therapeutic amalgamation that can be used to improve the male reproductive potential of sub fertile men. This spermatogenic and aphrodisiac property may be due to the bioactive compounds of *Shatavari* root. Hence, further investigations are warranted to confirm and elucidate the effect of *A. racamosus* on semen parameters.

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Physicochemical and Phytochemical analysis of *Kottai Karanthai Chooranam* (KKC)

Shomesh V.*[1] and Soruban T.[2]

Abstract

Medicinal plants, used to treat a wide range of diseases and ailments, represent the richest natural sources of various phytochemicals. *Sphaeranthus indicus L.* commonly called "*Kottai karanthai*" in Tamil. *Kottai karanthai* chooranam (KKC) is a Traditional Siddha single-herbal formulation. The aim of the present study was to analyze physicochemical parameters and to screen the phytochemicals in KKC. It was identified as a brownish, moderately fine, non-free-flowing powder with a characteristic odor and soft consistency. Its particle size was 75.3 ± 19.95µm. KKC showed solubility in DMSO, ethanol and water. Physicochemical analysis indicated a loss on drying of 6.16 ± 0.208% at 105°C, a total ash content of 0.55 ± 0.02% and no detectable acid-insoluble ash. Water and alcohol-soluble extractable matters were 10.9 ± 0.55% and 8.9 ± 0.65%, respectively, with a pH of 6.51. Phytochemical screening of methanol extracts confirmed the presence of significant bioactive compounds, including alkaloids, flavonoids, saponins, tannins, cardiac glycosides, triterpenoids and total phenolics. The results contribute to its standardization and quality control of KKC and provide scientific support for its traditional use in Siddha Medicine.

Keywords: *Kottai karanthai chooranam*, Phytochemical Screening, Physico-chemical analysis, Siddha Herbal Formula

Introduction

Siddha Medicine is one of the oldest traditional medical systems, particularly among the Tamil people[1].Classical Siddha literatures describe many therapeutic practices and pharmaceutical preparations. Siddha treatment emphasizes both curing diseases and preventing from them, using plant, mineral, and animal based substance[2,3]. The Siddha System includes 64 categories of medicines comprising 32 types internal medicines and 32 types of external medicines[4]. *Chooranam* is one of the internal medicines, the raw materials were individually dried, purified and finely powdered, each powder was sieved through a fine cotton cloth and combined in the prescribed portion[5]. *Sphaeranthus indicus* L. from Asteraceae family commonly called East Indian Globe thistle in English and *Kottai karanthai* in Tamil[6-11]. *Kottai karanthai chooranam* (KKC) is a Traditional Siddha single herbal formulation which is mentioned in Siddha classical text book and used to cure *Vellai* (Leucorrhea), *Ul ranam* (contusion), *Karappan* (Dermatitis), *Kirani* (untreated diarrhea), and *Malaththai velipaduththum* (inducing bowel evacuation)[12]. Phytochemicals are chemical compounds naturally found in plants and that can have either positive or negative effects on health[13]. Standardization of herbal formulations is a requirement in ensuring their quality, purity, safety, and therapeutic efficacy. The establishment of a reliable and reproducible standardization system for each final product is indispensable for maintaining uniformity and scientific validation[14]. Scientific validation of traditional medicine through standardization enables the identification of bioactive constituents and elucidation of their pharmacodynamics and pharmacokinetics, thereby clarifying the mechanisms of drug action[15]. This

[1]*Department of Ayurveda, Western Province, Sri Lanka.*
[2]*Post Graduate Institute of Indigenous Medicine, University of Colombo, Sri Lanka.*

Correspondence: Shomesh V., Department of Ayurveda, Western Province, Sri Lanka. Email: shomeshvijayakumar287@gmail.com

study was mainly conducted to prepare the KKC according to Standard Operating Procedure (SOP) and to evaluate its standardization parameters. Therefore, the objectives of this study is to prepare the *kottai karanthai* chooranam (KKC) as per Standard Operating Procedures (SOP) mentioned in the classical text, and to evaluate physicochemical parameters and screen phytochemicals in KKC.

Materials and methods

Preparation of the drug

Raw drug collection, identification and authentication

Fresh raw materials of *Sphaeranthus indicus* (whole plant) were collected from Jaffna district, Sri Lanka. The plant material was identified and authenticated by experts from the Department of Gunapadam, Siddha Teaching Hospital, Kaithady, Jaffna, Sri Lanka.

Preparation of Kottai karanthai chooranam

The collected plant materials were cleaned, dried, purified, powdered and sieved through a fine cotton cloth. The final product was air tightly packed and labeled as KKC.

Evaluation of standardization parameters

Standardization parameters were assessed according to the guidelines of the Pharmacopoeial Laboratory for Indian Medicine (PLIM)[16].

Organoleptic evaluation

Description

The KKC was placed on a watch glass and its morphological characteristics were observed.

Colour

The KKC was observed against a white background under tube light to determine its colour[16,17].

Odour

The odour was assessed at two intervals with a gap of two minutes between assessments to nullify the effect of previous smelling[16,17].

Particle size determination

Particle size determination was carried out using the optical microscopic method. The samples were dissolved in sterile distilled water (approximately $1/100^{th}$ dilution). Diluted samples were mounted on a slide and fixed on the stage at the appropriate location. Light microscopic images were obtained using a scale micrometer to determine the average particle size[18].

Physicochemical analysis

Percentage loss on drying (moisture content)

4g of the sample was weighed, placed in a pre-weighed beaker and dried at 105ºC constantly for 5 hours. It was then weighed hourly until two consecutive weights did not differ by more than 0.25%. Constant weight was confirmed after drying and cooling in a desiccator for 30 minutes, until the weight difference was not more than 0.01g[16,17].

Calculation:

$$\text{Percentage of loss on drying at } 105^{o}C = \frac{\text{Loss in weight of the sample} \times 100}{\text{Weight of the sample taken}}$$

Determination of total ash

The test drug was accurately weighed in a silica dish and incinerated in a furnace at 400ºC until it turned white, indicating the absence of carbon. The percentage of total ash was calculated relative to the air-dried sample[16,17].

Calculation

$$\text{Percentage of total ash} = \frac{\text{Weight of ash} \times 100}{\text{Weight of the sample taken}}$$

Determination of acid insoluble ash

The ash obtained from total ash test was boiled with 25ml of dilute hydrochloric acid for 6 minutes. The insoluble matter was collected in a crucible, washed with hot water and ignited to constant weight. The percentage of acid insoluble ash was calculated with reference to the weight of air-dried ash[16,17].

Calculation

Percentage of Acid – insoluble ash

$$= \frac{\text{Weight of the acid insoluble residue} \times 100}{\text{Weight of the sample}}$$

Determination of alcohol soluble extractive

The sample was macerated with 100 mL of alcohol in a closed flask for twenty-four hours, with frequent shaking during the first six hours and was allowed it to stand for eighteen hours. The solution was filtered rapidly, taking precautions against loss of solvent, 25ml of the filtrate were evaporated to dryness in a tared flat bottomed shallow dish, dried at 105ºC, and weighed. The percentage of alcohol-soluble extractive was calculated with reference to the air-dried drug[16,17].

Determination of water-soluble extractive

The test sample was macerated with 100ml of chloroform water in a closed flask for twenty-four hours, with frequent shaking during the first six hours and was allowed it to stand for eighteen hours. The solution was filtered rapidly, taking precautions against loss of solvent, 25ml of the filtrate were evaporated to dryness in a tared flat bottomed shallow dish, dried at 105ºC, and weighed. The percentage of water-soluble extractive was calculated with reference to the air-dried drug[16,17].

pH determination

10g of the sample was mixed with 90ml of distilled water. The mixture was stirred for three hours using a rotary shaker. Then the pH of the mixture was measured using a pH meter [16,17].

Phytochemical analysis

Phytochemical screening was carried out to identify the presence of alkaloids, flavonoids, glycosides, steroids, triterpenoids, phenols, cardiac glycoside, tannins, saponins, and total phenolics using standard protocols (Table 1).

Table 1: Phytochemical analysis tests

Phytochemical	Test Method	Observation for the presence
Alkaloids	Wagner's method	A reddish-brown precipitate
Flavonoids	Alkaline reagent test	Formation of an intense yellow color
Glycosides	Keller-Kiliani	A pink, red, or violet coloration
Steroids	Liebermann Burchard's	Show a series of colors. starting with pink or red, then turning to violet or purple, and finally to deep green or bluish-green.
Triterpenoids	Salkowski test	A reddish-brown coloration will form at the interface
Phenols	Ferric chloride test	Formation of a colored complex, typically a blue, purple, green, or red-brown color
Cardiac glycosides	Keller-kiliani	A brown ring will form at the interface between the two layers
Tannins	Ferric chloride	A change in color, which can be a greenish-black or brownish-green
Saponins	Foam test	Formation of a stable, persistent foam lasting at least 15 minutes
Total phenolic	Folin-Ciocalteu method	Formation of a blue color solution

Results and observations

The fresh materials and raw materials are shown in Figures 1 and 2.

Fig.1: Fresh raw materials of *Sphaeranthus indicus*

Fig.2: Dried and purified raw materials of *Sphaeranthus indicus*

Standardization parameters of the KKC

Table 2 shows the organoleptic parameters of KKC.

Table 2: Organoleptic parameters of KKC

Parameter	Interpretation
State	Solid
Nature	Moderately fine
Odor	Characteristic
Touch/ Consistency	Soft
Flow Property	Non-Free flowing
Appearance	Brownish

The organoleptic characters of the KKC were brownish, moderately fine, non-free-flowing powder with a characteristic odor and soft consistency.

Solubility of KKC is shown in Table 3.

Table 3: Solubility of KKC

Solvent used	Solubility / Dispensability
Chloroform	Insoluble
Ethanol	Soluble
Water	Soluble
Ethyl acetate	Insoluble
DMSO	Soluble

KKC was showed solubility in DMSO, ethanol, water and insoluble in chloroform and ethyl acetate.

Microscopic observation of particle size for the sample KKC is shown in Figure 3.

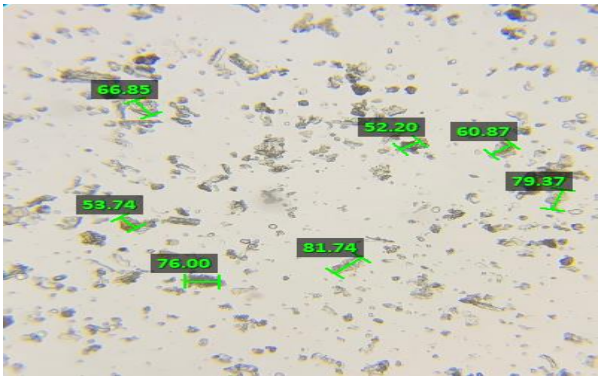

Fig. 3: Microscopic observation of particle size for the sample KKC

Microscopic observation of the particle size analysis revealed that the average particle size of the sample was found to be 75.3 ± 19.95µm further the sample has particle with the size range of lowest 52.20µm to highest 81.74µm.

Physicochemical Evaluation of KKC is shown in Table 4.

Table 4: Physicochemical Evaluation of KKC

Parameter	Mean (n=3) SD	Interpretation
Loss on Drying at 105 °C (%)	6.16 ± 0.208	**Acceptable** – Ideally < 10% to ensure low moisture (prevents microbial growth).
Total Ash (%)	0.55 ± 0.02	**Very good** – Low ash suggests minimal inorganic or contaminant material.
Acid insoluble Ash (%)	0 ± 0	**Excellent** – Indicates absence of silica, dirt, or sand.
Water soluble Extractive (%)	10.9 ± 0.55	**Normal range** – Usually 10–20% depending on formulation.
Alcohol Soluble Extractive (%)	8.9 ± 0.65	**Acceptable** – Often in the 5–15% range for herbal powders.
pH	6.51	**Good** – Near-neutral pH, compatible with oral use

Physicochemical analysis indicated a loss on drying of 6.16 ± 0.208% at 105°C, a total ash content of 0.55 ± 0.02% and no detectable acid-insoluble ash. Water and alcohol-soluble extractable matters were 10.9 ± 0.55% and 8.9 ± 0.65%, respectively, with a pH of 6.51.

Table 5 is shown the phytochemical screening of KKC

Table 5: Phytochemical screening of KKC

Phytochemical	Present / Absent
Alkaloids	Present
Flavonoids	Present
Glycosides	Absent
Steroids	Absent
Triterpenoids	Present
Phenols	Absent
Cardiac glycosides	Present
Tannins	Present
Saponins	Present
Total phenolic	Present

Preliminary phytochemical screening of KKC carried out using standard test methods, demonstrated the presence of alkaloids, flavonoids, triterpenoids, cardiac glycosides, tannins, saponins and total phenolics. In contrast, glycoside, steroids and phenols were not detected.

Discussion

Sphaeranthus indicus Linn., known as *Kottai Karanthai* in Tamil belonging to *Asteraceae* family. It is an annual herb widely distributed across India and Sri Lanka, often found as a weed in wet fields and margins. In Siddha medicine the whole plant and its parts (roots, flowers, leaves, seeds) are used for broad spectrum of ailments[19].

Organoleptic characters of KKC

Kottai Karanthai chooranam (KKC) is a single herbal formulation. Organoleptic characters of KKC are brownish, moderately fine, non-free-flowing powder with a characteristic odor and soft consistency. (Table 2)

Solubility profile

KKC is soluble in DMSO, ethanol, and water. Insoluble in chloroform and ethyl acetate. Water solubility drugs can enhance oral bioavailability and readily absorbed in gastrointestinal tract.

Physicochemical analysis

Standardization plays a vital role in the evaluation of herbal formulations as it ensures the quality, purity, and safety of the drugs through various physicochemical parameters. In the present study, parameters such as moisture content, ash values, extractive values and pH analyze for *Kottai karanthai chooranam* to establish its quality profile[11].

Moisture content

The loss on drying test is a crucial indicator of moisture and volatile matter content, which in turn influences the stability and susceptibility of herbal drugs to microbial growth and deterioration. The percentage of loss on drying for *Kottai karanthai chooranam* is 6.16 ± 0.208% (normal range: 1–20%). This relatively low value indicates minimal moisture content, suggesting a higher degree of stability and reduced microbial contamination[20].

Ash values

Ash value determination is another important parameter in assessing the quality of herbal drugs. The total ash content reflects the presence of inorganic matter, while acid-insoluble ash indicates contamination with earthy materials such as sand and silica. In this study, the total ash value of *Kottai karanthai chooranam* is 0.55 ± 0.02% (normal range: 1–25%), and the acid-insoluble ash value is 0 ± 0 (normal range: 0.1–10%). Both values are low, thereby indicates the absence of significant contamination, substitution, or adulteration. This also supports the purity and authenticity of the formulation[20].

Extractive values

The extractive values are indicative of the number of active constituents that can be extracted in specific solvents, thereby reflecting the quality and strength of the formulation. The alcohol-soluble extractive value of *Kottai karanthai* is 8.9 ± 0.65%, while the water-soluble extractive value 10.9 ± 0.55%. The higher alcohol-soluble extractive content suggests that the bioactive constituents of the formulation are predominantly alcohol soluble, which is important for therapeutic efficacy and aligns with the nature of the ingredients used[20].

pH

pH was 6.51 and it was compatible with oral use.

Physicochemical parameters analyzed in this study confirm the quality and stability of *Kottai karanthai chooranam*. The low moisture and ash contents indicate purity and minimal contamination, while the extractive values highlight the presence of active constituents responsible for its pharmacological activity. These findings provide scientific validation and quality assurance for the traditional use of this formulation.

Phytochemical screening

Phytochemical analysis is a vital step in assessing the therapeutic potential of medicinal plants, as it enables the identification of bioactive compounds responsible for their pharmacological and biological activities[21]. The phytochemical analysis of the KKC reveals the presence of several bioactive compounds, such as alkaloid, flavonoid, triterpenoids, cardiac glycosides, tannins, saponins, and total phenolic, each of which contributes to its pharmacological activities.

Tannins are well documented for their antimicrobial properties, inhibiting the growth of bacteria, fungi, yeasts, and viruses. Their activity supports the traditional use of the plant in treating skin diseases and preventing secondary infections. They use to treat diarrhea, stomach ulcer and prevent wounds from infections[20,22]. Flavonoids are recognized for their antioxidant and hypolipidemic effects, they use to treat diseases that associated with oxidative stress[22,23]. Their presence may explain, at least in part, the plant's potential role in regulating lipid metabolism. Similarly, saponins known to exhibit antibiotic and anti-inflammatory effects and possess diverse biological properties, including hemolytic activity, hypocholesterolemic properties and a characteristic bitter taste[22,24]. Alkaloids, another major group of secondary metabolites detect, are well known for their cytotoxic properties, analgesic, antispasmodic, and antibacterial activities[24]. Cardiac glycosides are another compound present in KKC, it has beneficial effect on heart health and they enhance cardiac muscle contractility by inhibiting Na^+/K^+ ATPase enzyme, which increases intracellular calcium levels and improves the efficiency of the heart's pumping action[24].

The presence of these secondary metabolites highlights the pharmacological significance of the drug. Their combined effects contribute to the traditional uses and justify further investigations into the drugs bioactivity and therapeutic potential.

Conclusion

The present study confirms that *Kottai karanthai chooranam* prepared as per classical Siddha guidelines, meets essential physicochemical standards including low moisture content, minimal ash values, suitable extractive values, and near neutral pH indicating its purity, stability, and safety for therapeutic use. KKC contains significant bioactive phytochemicals such as alkaloid, flavonoid, triterpenoids, cardiac glycosides, tannins, saponins, and total phenolics. However, further comprehensive studies are required to establish its complete standardization and to validate its efficacy through advanced scientific approaches.

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Antibacterial activity of the aqueous rhizome extract of *Alpinia galanga* (L.) Willd (*Kaluwala*) used in Sri Lankan Indigenous Medicine

Munasinghe D.A.L.[1]* and Sudesh A.D.H.[2]

Abstract

Alpinia galanga (*Kaluwala*) is frequently used in Sri Lankan Indigenous Medicine to treat skin infections, inflammatory disorders, and respiratory conditions. Traditionally, healers employ cold-water preparations of the rhizome, but these methods have limited scientific validation. Therefore, this study was conducted to assess the antibacterial and antifungal properties of an aqueous rhizome extract of *A. galanga* prepared using traditional methods. Shade-dried rhizomes were soaked in sterile cold water, filtered, and analyzed for yield and phytochemical composition. Antimicrobial activity was evaluated using the agar-well diffusion technique against *Streptococcus pneumoniae* (ATCC 12386), Staphylococcus aureus (ATCC 25923), *Escherichia coli* (ATCC 25922), methicillin-resistant *Staphylococcus aureus* (MRSA), and *Candida albicans* (ATCC 10231). Amoxicillin and fluconazole were used as positive controls. Phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, saponins, glycosides, and phenolic compounds. The extract inhibited the growth of all tested bacteria, including MRSA, but showed no activity against *C. albicans.* The largest inhibition zones were recorded for *S. aureus* (14.3 ± 0.6 mm), followed by *S. pneumoniae* (12.4 ± 0.5 mm), MRSA (11.8 ± 0.5 mm), and *E. coli* (10.2 ± 0.4 mm). Conclusion: The aqueous rhizome extract of *A. galanga* exhibits broad-spectrum antibacterial activity, supporting its traditional use for infected skin lesions. Further pharmacological, phytochemical, and formulation studies are warranted.

Keywords: *Alpinia galanga*, *Kaluwala*, aqueous extract, antibacterial activity, Indigenous Medicine, MRSA

Introduction

Alpinia galanga (L.) Willd., commonly referred to as *Kaluwala* in Sri Lanka, belongs to the Zingiberaceae family and is traditionally used in indigenous and Ayurvedic medicine to address skin infections, abscesses, inflammatory conditions, digestive disorders, fever, and respiratory ailments[1–3]. Cold aqueous preparations of the rhizome, such as pastes, juices, and decoctions, are commonly applied to treat skin lesions thought to have microbial origins.

Phytochemical investigations have shown that *A. galanga* rhizomes contain flavonoids, phenolic compounds, essential oils, tannins, terpenoids, and diarylheptanoids. Certain constituents, including galangin, 1′-acetoxychavicol acetate, and eugenol, have exhibited antibacterial properties in vitro[4–7]. However, most studies have focused on alcoholic extracts or essential oils rather than cold-water preparations traditionally used by healers.

With the global increase in antibiotic-resistant strains such as methicillin-resistant Staphylococcus aureus (MRSA), it is crucial to explore traditional remedies as potential antibacterial agents[8–10]. The current study investigates the antibacterial and antifungal activity of a cold aqueous rhizome extract of *A. galanga* prepared according to traditional methods.

[1]*Faculty of Indigenous Medicine, University of Colombo, Sri Lanka, Sri Lanka.*
[2]*Gampaha Wickramarachchi University of Indigenous Medicine, Yakkala, Sri Lanka.*

Correspondence: Munasinghe D.A.L., Faculty of Indigenous Medicine, University of Colombo, Sri Lanka. Email: munasinghe@fim.cmb.ac.lk

Materials and Methods

Plant collection and authentication

Fresh rhizomes of *A. galanga* were harvested from Gampaha District, Sri Lanka. A botanist authenticated the plant, and a voucher specimen was deposited in the University of Indigenous Medicine herbarium.

Preparation of aqueous extract

Rhizomes were washed, sliced, and shade-dried for 7 days. Fifty grams of dried slices were soaked in 150 mL of sterile distilled water at room temperature for 24 h, manually crushed, and filtered through sterile gauze followed by Whatman No. 1 filter paper. The filtrate was stored at 4 °C.

The filtrate was evaporated at 40 °C to obtain a dry extract. The extraction yield (%) was calculated as:

$$\text{Yield (\%)} = \frac{\text{Dry extract weight}}{\text{Raw material weight}} \times 100$$

The obtained yield was 4.8% w/w.

Phytochemical screening

Qualitative tests were performed to detect:

Alkaloids: Dragendorff's test

Phenolics: Ferric chloride test

Flavonoids: Shinoda test

Tannins: Gelatin test

Saponins: Froth test

Glycosides: Keller–Killiani test

Terpenoids: Salkowski test

Selected microorganism

The tested organisms were *S. pneumoniae* (ATCC 12386), *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), MRSA (clinical isolate), and *C. albicans* (ATCC 10231).

Antimicrobial assay

Well diffusion was carried out using Mueller-Hinton agar for bacteria and Sabouraud agar for yeast. Wells (6 mm in diameter) were loaded with 10 µL of aqueous extract, amoxicillin (10 µg/mL), fluconazole (25 µg/mL), or sterile saline (negative control). Plates were incubated at 37 °C for 18–24 h, and inhibition zones were recorded in millimeters. All experiments were performed in triplicate.

Statistical analysis

Results are expressed as mean ± SD from three independent experiments.

Results

Phytochemical profile

The extract contained alkaloids, flavonoids, tannins, saponins, glycosides, and phenolic compounds; terpenoids were not detected.

Antimicrobial action

Table 1 summarizes the inhibition zones and the Bar chart representation of zone of inhibition. The extract inhibited all bacterial strains, including MRSA, but did not show activity against *C. albicans*.

Table 1: Summarized inhibition zones

Microorganism	Extract	Positive control	Negative control
S. pneumoniae	12.4 ± 0.5	28.1 ± 0.7	0
E. coli	10.2 ± 0.4	22.6 ± 0.5	0
S. aureus	14.3 ± 0.6	30.4 ± 0.6	0
MRSA	11.8 ± 0.5	0	0
C. albicans	0	19.7 ± 0.5	0

Discussion

The cold aqueous extract of *A. galanga* exhibited significant antibacterial activity, especially against *S. aureus* and *S. pneumoniae*, corroborating its traditional use for skin infections. Activity against MRSA is noteworthy, considering the clinical challenges posed by antibiotic resistance. Polar phytochemicals such as flavonoids, phenolics, and saponins likely account for the antibacterial effect[11–14].

The absence of antifungal activity may be due to the lack of non-polar terpenoids, which are more effectively extracted with organic solvents. Although inhibition zones were smaller than those produced by amoxicillin, the results indicate that traditional cold-water macerations preserve bioactive compounds, supporting ethnomedical practices.

Future studies should:

Determine minimum inhibitory (MIC) and bactericidal concentrations (MBC).

Isolate and identify active constituents using HPLC or GC-MS.

Assess safety profiles in vitro and in vivo.

Develop standardized formulations suitable for clinical use.

Conclusion

The cold aqueous extract of *A. galanga* rhizome displays broad antibacterial activity, including against MRSA, validating its traditional use for infected skin conditions. Further pharmacological and phytochemical studies are recommended.

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Standardization of *Keezhanelli chooranam* (KNC): A Single-Herbal Siddha Formulation

Soruban T.[1*] and Sathiyaseelan V.[2]

Abstract

Standardization of herbal formulations is essential to ensure the quality, safety, and efficacy of traditional medicines. This study aimed to establish standardization parameters for *Keezhanelli Chooranam* (KNC), a Siddha formulation prepared from *Phyllanthus amarus*, traditionally used in the management of *Madhumegam* (diabetes mellitus). KNC was prepared according to classical Siddha methods and analyzed using modern techniques in accordance with AYUSH guidelines. Physico-chemical evaluation revealed a brownish, moderately fine powder with characteristic odour, particle size of 94.67 ± 25.5 µm, pH 6.6, and good solubility in water, ethanol, and DMSO. Loss on drying (5.467 ± 0.30%), total ash (0.31 ± 0.026%), extractive values, and absence of acid-insoluble ash were within acceptable limits. ICP-OES analysis confirmed the absence of toxic heavy metals. HPTLC profiling showed multiple phytochemical peaks with Rf values ranging from 0.01 to 0.66. Microbial studies confirmed sterility of the formulation. The results indicate that KNC meets standard quality and safety requirements, supporting its suitability for further pharmacological and clinical evaluation.

Keywords: *Keezhanelli Chooranam*, *Keezhanelli*, *Phyllanthus amarus,* Standardization, Siddha Medicine

Introduction

Sri Lanka has a rich heritage of traditional systems of medicine, including Ayurveda, Siddha, Unani and Desiya Chikitsa[1]. These systems have made significant contributions to the healthcare of humankind. However, a major limitation of these systems is the lack of standardized quality control parameters[2]. As the global demand for herbal medicinal products continues to grow rapidly, there is an urgent need to establish reliable quality control measures for herbal medicines originating from these traditional systems[3].

Standardization is essential to ensure the quality, purity, safety, and efficacy of herbal formulations[4,5]. *Keezhanelli chooranam* (KNC) is a traditional Siddha formulation prepared from *Keezhanelli* (*Phyllanthus amarus*) and this formulation is documented in the Siddha Pharmacopoeia of India[6].

Siddha literature highlights its potential for treating *Madhumegam* (Diabetes mellitus), *Kannoikal* (eye diseases), *Mega noi* (sexually transmitted diseases) and *Mega Pun* (wounds associated with sexually transmitted diseases)[7]. Despite its potential therapeutic uses, there is no documented evidence regarding the standardization of *Keezhanelli Chooranam*. Therefore, this study was undertaken to establish a contemporary approach to the standardization of this novel Siddha formulation, as per AYUSH guidelines, to ensure consistent quality and control over the manufacturing process. Therefore, this study was carried out to prepare *Keezhanelli chooranam* as per the standard operating procedure and also, to evaluate the standardization parameters according to the AYUSH guidelines.

[1]*Post Graduate Institute of Indigenous Medicine, University of Colombo, Sri Lanka.*
[2]*Faculty of Siddha Medicine, University of Jaffna, Sri Lanka.*

Correspondence: Soruban T., Post Graduate Institute of Indigenous Medicine, University of Colombo, Sri Lanka.
Email: sorruthiru@gmail.com

Study methodology

Preparation of the drug

Raw material collection

The *Keezhanelli* (*Phyllanthus amarus*) plant was collected from vegetable gardens in the Palali area of the Jaffna District. The whole plant of *Phyllanthus amarus* was collected during the months of January - February 2024, corresponding to its optimal vegetative growth phase

Identification and Authentication

The identification and authentication of the plant material were carried out by the National Herbarium, Department of National Botanical Gardens, Peradeniya (Ref. No: 2024/388).

Preparation Method of Keezhanelli chooranam

The whole plant of *Phyllanthus amarus* was collected, cleaned (purified and washed), and then dried under sunlight for several weeks. The dried raw material was weighed, finely powdered, and sieved through a fine mesh (80 - 100 mesh). The formulation was prepared at the Siddha Drug Production Unit, Kaithady Siddha Teaching Hospital.

Organoleptic evaluation

Description

The KNC was placed on a watch glass for macroscopic examination of its morphology and texture[4,6].

Color

The KNC was placed on a watch glass against a white background under tube light for its color observation[4,6].

Odor

The KNC was smelled twice with a two-minute interval between assessments to minimize olfactory adaptation[4,6].

Particle size

Particle size determination was carried out using the optical microscopic method. Samples were dissolved in sterile distilled water (approximately 1/100 dilution). Diluted samples were mounted on a slide and fixed on the stage at the appropriate location. Light microscopic images were drawn with a scale micrometer to determine the average particle size[8].

Physicochemical analysis

Determination of moisture content (Loss on drying)

Accurately weigh 4 g of the drug. Place it in a pre-weighed beaker and dry at 105º C constantly for 5 hours. Now weigh the drug. Continue drying and weighing every hour. This process has to be continued until the two corresponding weights don't exceed 0.25 percent. Constant weight is said to be obtained when drying and cooling for 30 minutes in a desiccator didn't show a difference more than 0.01 g[4,6].

Calculation:

$$\text{Percentage of loss on drying at } 105\,^{\circ}\text{C} = \frac{\text{Loss in weight of the sample}}{\text{Weight of the sample taken}} \times 100$$

Determination of total ash

Incinerate about 3 g accurately weighed of the ground drug in a pre-weighed silica dish. Calculate the percentage of ash with reference to the air dried drug[4,6].

Calculation:

$$\text{Percentage of total ash} = \frac{\text{Weight of ash}}{\text{Weight of the sample taken}} \times 100$$

Determination of water soluble ash

Boil the ash obtained from above test for few minutes with 25 ml of distilled water; repeat the process for one more time. Filter the insoluble matter on an ash less filter paper and ignite in a silica crucible to constant weight. Calculate the percentage of water soluble ash with reference to the air dried drug[4,6].

Calculation:

$$\text{Percentage of water soluble ash} = \frac{\text{Weight of the total ash - weight of water insoluble residue}}{\text{Weight of the sample}} \times 100$$

Determination of acid insoluble ash

Boil the ash obtained from the above test for 5 min with 25 ml of dilute hydrochloric acid. Filter the insoluble matter on an ash less filter paper, wash with hot water and ignite in a silica crucible to constant weight. Calculate the percentage of acid insoluble ash[4,6].

Calculation

Percentage of Acid insoluble ash =

$$\frac{\text{Weight of the acid insoluble residue}}{\text{Weight of the sample}} \times 100$$

Determination of alcohol soluble extractive

About 5 g of the sample was macerated with 100 ml of alcohol in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing it to stand for the remaining 18 hours. Filter rapidly, avoiding solvent loss. Evaporate 25 ml of the filtrate to dryness in a tared flat-bottomed dish, dry at 105°C to constant weight, and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug[4,6].

Determination of water-soluble extractive

About 5 g of the sample was macerated with 100 ml of chloroform-water in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing it to stand for the remaining 18 hours. Filter rapidly, avoiding solvent loss. Evaporate 25 ml of the filtrate to dryness in a tared flat-bottomed dish, dry at 105°C to constant weight, and weigh. Calculate the percentage of water-soluble extractive with reference to the air-dried drug[4,6].

Determination of pH

10 grams of sample was weighed and mixed with 90 mL of distilled water. The mixture was stirred for three hours of time using rotary shaker. Then the pH was measured using pH meter[4,6].

HPTLC analysis

High-Performance Thin Layer Chromatography (HPTLC) was carried out using a CAMAG HPTLC Scanner III. The stationary phase consisted of Merck Silica Gel 60 F_{254} plates, while the mobile phase comprised chloroform: *n*-butanol : methanol : water : acetic acid (4:1:1:0.5:0.5). The sample was dissolved in methanol**,** and 10 µL of the solution was applied to the plate. The developed chromatogram was scanned at 254 nm and 365 nm wavelengths to detect and compare the phytochemical constituents present in the formulation[9].

Heavy metal analysis

The heavy metal content of *Keezhanelli chooranam* (KNC) was analyzed using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) with a Perkin-Elmer 5300 DV instrument. This method was employed to quantitatively determine the presence of heavy metallic elements and to ensure the formulation complied with safety standards[10].

Microbiological tests

Sterility test (Pour Plate Method)

The sterility of KNC was evaluated using the pour plate method. The sample was inoculated into sterile Petri dishes, and 15 mL of molten agar (maintained at 45°C) was poured and gently swirled to ensure even mixing. After solidification for about 10 minutes, the plates were inverted and incubated at 37°C for 24 - 48 hours. For fungal observation, the incubation period was extended to 72 hours. The number of microbial colonies formed was counted and expressed as colony-forming units (CFU) to assess microbial contamination[4,6].

Test for specific pathogens

Specific pathogenic contamination was assessed by inoculating the sample into selective media using the pour plate method. The following media were used: Eosin Methylene Blue (EMB) agar for *Escherichia coli***,** Deoxycholate Citrate (DCC) agar for *Salmonella* species, Mannitol Salt Agar for *Staphylococcus aureus***,** and Cetrimide agar for *Pseudomonas aeruginosa***.** The inoculated plates were incubated at 37°C for 24 - 72 hours, and the pathogens were identified based on their characteristic colony color and morphological features specific to each differential medium[4,6].

Development steps of KNC

Figure 1 and 2 shows the Fresh raw materials and dried and purified raw materials of *Phyllanthus amarus* which was used to prepare *Keezhanelli chooranam.*

Fig.1: Fresh raw materials of *Phyllanthus amarus*

Fig.2: Dried and purified raw materials of *Phyllanthus amarus*

Organoleptic evaluation

Table 1 summarizes the organoleptic characteristics of the *Keezhanelli Chooranam* was a brownish, moderately fine powder with a characteristic odour and soft consistency.

Table 1: Organoleptic character of the KNC

Parameters	Interpretation
State	Solid
Nature	Moderately fine
Odor	Characteristic
Touch / Consistency	Soft
Flow Property	Non Free flowing
Appearance	Brownish

Particle size

Microscopic observation of the particle size analysis reveals that the average particle size of the KNC was found to be 94.67 ± 25.5 µm further the sample has particle with the size range of lowest 56.20 µm to highest 100.8 µm (Figure 3).

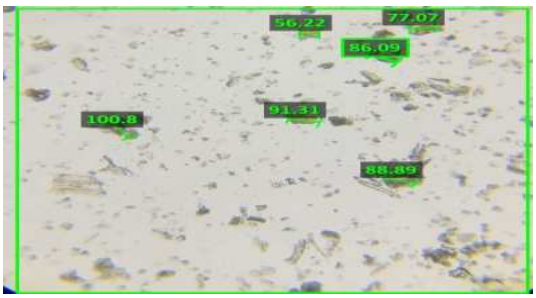


Fig.3: Microscopic observation of particle size for the sample KNC

Solubility of the KNC

The *Keezhanelli chooranam* (KNC) exhibited solubility in ethanol, water, and dimethyl sulfoxide (DMSO), indicating the presence of both polar and semi-polar phytoconstituents. The solubility in ethanol and DMSO suggests that KNC contains compounds such as alkaloids, flavonoids, and phenolic constituents, while its partial solubility in water indicates the presence of hydrophilic components like glycosides and carbohydrates. This broad solubility profile implies that KNC possesses a diverse range of bioactive constituents, contributing to its potential pharmacological activities.

Physicochemical analysis of the KNC

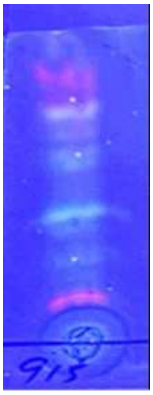
According to Table 3, the physicochemical analysis of *Keezhanelli chooranam* (KNC) showed acceptable quality, with low moisture content, minimal ash values, high water-soluble extractives, moderate alcohol-soluble extractives, and a near-neutral pH indicating good formulation stability and biological compatibility.

HPTLC analysis

As shown in Table 4 and figure 4 and 5, presence of ten prominent peaks corresponds to the presence of versatile phytocomponents present with in it. The major Rf value of the peaks ranges from 0.01 to 0.66.

Table 3: Physicochemical analysis of the KNC

Parameter	Mean (n=3) SD	Interpretation
Loss on Drying at 105 °C (%)	5.467 ± 0.30	Acceptable; ideally <10% to prevent microbial growth
Total Ash (%)	0.31 ± 0.026	Low, indicates minimal inorganic or adulterant content.
Acid insoluble Ash (%)	0 ± 0	Ideal; confirms absence of siliceous (gritty) impurities.
Water soluble Extractive (%)	9.467 ± 0.40	High; reflects rich water-soluble phytochemicals
Alcohol Soluble Extractive (%)	6.1 ± 0.2	Moderate; suggests presence of non-polar actives like resins.
pH	6.6	Near-neutral; suitable for biological compatibility and formulation stability.



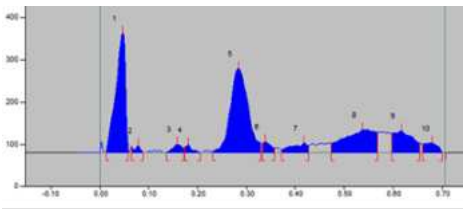


Fig.5: HPTLC fingerprinting of KNC

Fig.4: TLC plate under visible light and 366 nm

Table 4: HPTLC peak table of KNC

Peak No	Start R_f value	Max R_f value	Max %	Area	Area %
1	0.01	0.05	39.64	4247.6	26.72
2	0.06	0.08	2.37	166.3	1.05
3	0.14	0.16	2.75	288.8	1.82
4	0.17	0.18	2.54	188.7	1.19
5	**0.23**	**0.26**	**27.84**	**5466.2**	**34.38**
6	0.33	0.34	3.65	345.8	2.17
7	0.37	0,42	3.15	564.7	3.55
8	0.47	0.54	7.67	2612.0	16.43
9	0.60	0.62	7.21	1537.1	9.67
10	0.66	0.68	3.18	482.1	3.03

Heavy metals

Heavy metals analysis of KNC through the ICP OES is shown in Table 5.

Table 5: Heavy metals analysis of KNC through the ICP OES

Elements	Requirement	KNC
Arsenic	3ppm	BDL
Mercury	1ppm	BDL
Cadmium	0.3ppm	BDL
Lead	10ppm	BDL

The heavy metals analysis showed (Table 5) the presence of mercury, lead, arsenic and cadmium below the limit of quantification.

Elements analysis of KNC through the ICP OES is shown in Table 6.

Table 6: Elements analysis of KNC through the ICP OES

Element	Concentration (mg/L)
Carbon	175.150
Calcium	1.163
Copper	BDL
Iron	BDL
Potassium	21.110
Magnesium	1.321
Sodium	71.390
Phosphorus	121.300
Sulphur	1.101
Zinc	1.280

The elemental analysis of *Keezhanelli chooranam* (KNC) revealed that toxic heavy metals such as arsenic (As), cadmium (Cd), copper (Cu), iron (Fe), mercury (Hg), and lead (Pb) were not detected (below detectable limits), indicating that the formulation is free from heavy metal contamination and safe for therapeutic use. The sample showed a high carbon content, suggesting a strong organic composition and the presence of various carbon-based bioactive compounds. Calcium (Ca) and magnesium (Mg) were present in small quantities, both of which are essential minerals that play vital roles in physiological functions. Potassium (K) content was moderately high, contributing to cellular activity and maintaining electrolyte balance. Sodium (Na) levels were found to be elevated, which may influence osmotic balance and could be relevant for the formulation's stability. Phosphorus (P) was present in high concentration, possibly indicating the existence of phospholipids or other energy-related compounds, while sulfur (S) was detected in trace amounts, suggesting the presence of sulfur-containing amino acids or secondary metabolites.

Microbiological Tests

Sterility test results of KNC is shown in Figure 6 and 7 and Table 7.

Sterility test by pour plate method

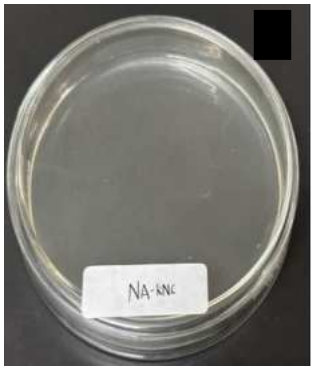


Fig.6: Control

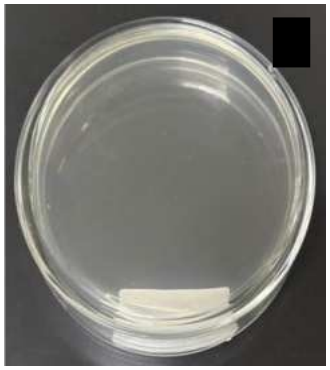


Fig.7: KNC

Table 7: Sterility test results of KNC

Test	Result	Specification
Total Bacterial Count	Absent	NMT 10^5CFU/g
Total Fungal Count	Absent	NMT 10^3CFU/g

Test for specific pathogens

Results of the Specific pathogens results of KNC is shown in Table 8.

Table 8: Specific pathogens result of KNC

Organism	Specification	Result
E-coli	Absent	Absent
Salmonella	Absent	Absent
Staphylococcus aureus	Absent	Absent
Pseudomonas aeruginosa	Absent	Absent

Culture plates showing the presence or absence of specific pathogens: *Escherichia coli* (EC) (Figure 8), *Staphylococcus aureus* (ST) (Figure 9), *Pseudomonas aeruginosa* (PS) (Figure 10), and *Salmonella* (SA) (Figure 11). Plates without and with the respective selective media.

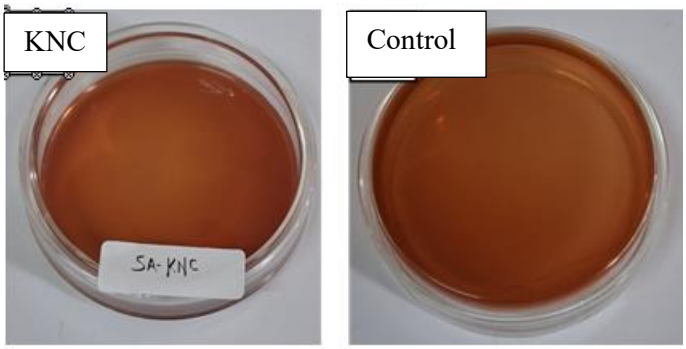


Fig.8: *Escherichia coli*

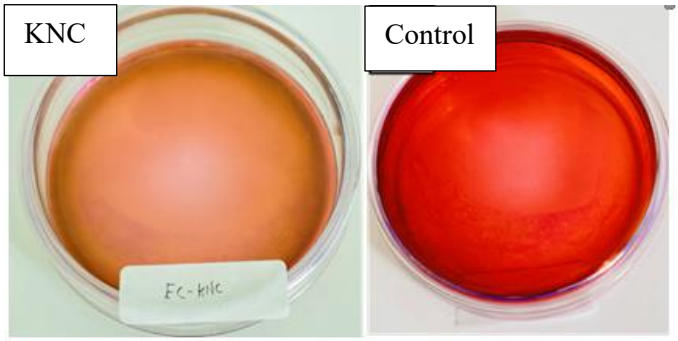


Fig.9: *Escherichia coli*

Fig.10: *Staphylococcus aureus*

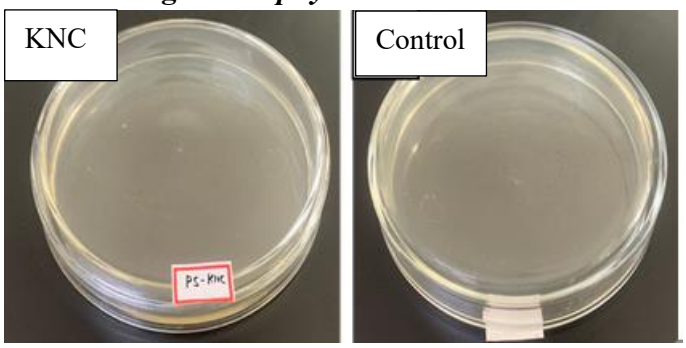


Fig.11: *Salmonella*

Discussion

The present study focused on the standardization and quality evaluation of *Keezhanelli chooranam* (KNC), a traditional Siddha formulation prepared from *Phyllanthus amarus*. The comprehensive analysis included organoleptic, physicochemical,

phytochemical, elemental, and microbiological parameters, all of which play a vital role in establishing the quality, purity, and safety of Siddha formulations as per AYUSH guidelines.

The organoleptic evaluation of KNC revealed a brownish, moderately fine powder with a characteristic odour and soft consistency, aligning with the expected physical features of a finely prepared *Chooranam*. The observed organoleptic characteristics, including colour, texture, taste, and fine powder nature, indicate the genuineness, purity, and overall quality of the test drug KNC[11]. The particle size of 94.67 ± 25.5 µm confirmed the moderate fineness of the formulation. Particle size influences solubility, processing properties, bioavailability, product uniformity, stability, and therapeutic efficiency[12]. The solubility profile in ethanol, water, and DMSO indicated the presence of both polar and semi-polar phytoconstituents, suggesting a diverse range of bioactive compounds such as alkaloids, flavonoids, glycosides, and phenolics[13,14]. Drugs that are poorly soluble in water often require higher doses to attain therapeutic plasma concentrations after intake[15].

Physicochemical parameters serve as essential quality indicators for herbal formulations[4]. The loss on drying value (5.467 ± 0.30%) was within the acceptable limit (<10%), confirming the minimal moisture content and reduced risk of microbial growth[4]. The total ash (0.31 ± 0.026%) and absence of acid-insoluble ash indicated low inorganic contamination and absence of earthy or siliceous materials, reflecting purity of the sample[4]. The extractive values further highlighted the presence of both water-soluble and alcohol-soluble constituents, which are indicative of the phytochemical richness of *Phyllanthus amarus*. The near-neutral pH (6.6) supports formulation stability and biological compatibility, making it suitable for internal use[16,17].

The HPTLC profile revealed ten prominent peaks with Rf values ranging from 0.01 to 0.66, confirming the presence of multiple phytoconstituents[18,19,20]. This chromatographic fingerprint can serve as a reference standard for future identification and quality control of KNC batches. Similar multi-component patterns have been reported in other standardized Siddha formulations, supporting the reliability of HPTLC as a powerful tool for phytochemical profiling.

Heavy metal analysis through ICP-OES demonstrated that toxic metals such as mercury, lead, cadmium, and arsenic were below detectable limits, ensuring the safety of the formulation. The presence of essential elements like calcium, magnesium, potassium, and phosphorus reflects the mineral richness of the formulation, which may contribute to its therapeutic efficacy. These findings are consistent with similar studies on Siddha formulations that emphasize the importance of ensuring heavy metal-free preparations for patient safety[4,21,22].

Microbiological evaluation confirmed the absence of total bacterial and fungal counts, as well as specific pathogens such as *E. coli*, *Salmonella*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. This indicates the formulation's sterility and compliance with AYUSH standards for microbial safety in herbal products[4].

Overall, the study successfully established a comprehensive standardization profile for *Keezhanelli chooranam*. The results confirm that the formulation meets the acceptable quality limits and is safe for therapeutic application. The combined application of traditional Siddha preparation techniques and modern analytical methods ensures reproducibility, authenticity, and quality assurance. Further studies, including pharmacological and clinical evaluations, are recommended to validate its therapeutic efficacy and to support its inclusion in evidence-based Siddha pharmacopeial standards.

Conclusion

The present study successfully established comprehensive standardization parameters for *Keezhanelli chooranam* (KNC), a classical single-herb Siddha formulation. All evaluated physicochemical, phytochemical, elemental, and microbiological parameters were found to be within prescribed AYUSH limits, confirming the formulation's quality, purity, and safety. The generated HPTLC fingerprint can serve as a reference

standard for future batch-to-batch quality control. These findings support the scientific validation of KNC and provide a foundation for further pharmacological and clinical investigations to substantiate its therapeutic efficacy.

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