

# Pharmacognosy and Analytical Specification of *Kadarpaasi Chooranam* - A Novel Siddha Drug

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**Abstract Background:** Algae are an excellent wellspring of biologically active secondary metabolites and have been shown to exhibit a wide range of therapeutic properties. Several Asian cultures have a strong tradition of utilizing various seaweeds extensively in cooking as well as in herbal medicines preparations. Their use in traditional medicine has been reported since time immemorial. **Aims and Objectives:** To evaluate the analytical specification, proximate analysis, and trace elemental analysis of *Kadarpassi Chooranam*. **Methods:** *Halimeda gracilis* (green alga) was collected from the Mandapam region and identified by a marine biologist. *Halimeda gracilis* was made into chooranam, and it was purified by using the 'Avi Enthiram' method and analytical specification, Proximate analysis, and trace elemental analysis were performed. **Results:** *Kadarpassi Chooranam* was standardized as per PLIM guidelines testing protocol, the Heavy metals were found within the normal range, Aflatoxin, Pesticide residues, Organochlorine, Organophosphorus, and Synthetic pyrethroids were found to be below the limit of quantitation (BLQ) and the microbiological test showed free of harmful pathogen. Trace elemental analysis and proximate analysis of *Kadarpassi Chooranam* showed significant presence, which can be used as health supplements. **Conclusion:** *Kadarpassi Chooranam* contains the permissible level of heavy metals, toxins and is free of harmful pathogens. The chemical constituents of the chooranam were characteristically studied, which showed that they contain organic compounds, which may

have various pharmacological activities. So, *Kadarpassi Chooranam* is the first of its kind in the medicinal field. Future studies (in vitro and in vivo) will explore the potential medicinal properties.

**Keywords** Marine Seaweeds, Chooranam, Analytical Specification, Proximate Analysis, Trace Elemental Analysis, Phytochemical Analysis

## 1. Introduction

An archaeological evidence indicates that seaweeds have been included as a traditional medicine in India 300 BC [1]. Around 7000, marine natural products have been isolated and 25% of them are from marine macroalgae [2]. Seaweeds (or) Marine macroalgae are non-flowering plants occurring in the sea, estuaries, and backwater. For centuries, many of the seaweed secondary metabolites have been utilized for traditional medicines due to their therapeutic potentials [3]. They are the most abundant in shallow rocky coastal areas, especially they are exposed at low tide to coastal people around the world [4]. Biological compounds extracted from seaweed families are Chlorophyceae (green algae), Phaeophyceae (brown algae), Rhodophyceae (red algae), and Cyanophyceae (Blue-green algae) [5]. From the literature, the edible

seaweeds provide a significant amount of proteins, vitamins, and minerals, which are essential nutrients for human [6]. Being excellent sources of amino acids, carbohydrates, lipids, growth hormones, micro and macro-elements including iodine, many seaweed species are utilized as food throughout Asia and the Pacific region [7]. It has been reported to incorporate secondary metabolites which include glycosides, saponins, tannins, steroids, and related active metabolites, and have been broadly utilized in the pharmaceutical industry [8]. Recent research has proved that compounds originating from marine algae exhibit various biological activities. Therefore, there is a new trend to isolate novel bioactive compounds from seaweed [9,10]. So, the present study uses seaweeds as Siddha preparation, which is one of the Indian traditional medicines. Kadarpasi chooranam was evaluated for the analytical specification and characterization was performed. *Halimeda gracilis* belongs to:

Kingdom	Plantae
Subkingdom	Viridiaeplantae
Phylum	Chlorophyta
Class	Ulvophyceae
Order	Bryopsidales
Family	Halimedaecae
Genus	<i>Halimeda</i>
Species	<i>gracilis</i>

## 2. Description about *Halimeda gracilis*

The plant is green in color but slightly calcified. Thalli are mostly large, densely intricate populations forming a thick cushion on the substratum. The colour becomes whitish from the green after drying. The basal portion is not seen branching and branches arise from the segment. Segments are flattened up to 5 cm broad, subterete below, cuneate to subreniform in shape some cylindrical segments distributed in different branches, the upper margin of the segments slightly undulate or entire generally trilobed [11].

## 3. Materials and Methods

### 3.1. Collection and Preparations

The fresh marine green alga *Halimeda gracilis* was collected from Rameswaram coastal area, Tamil Nadu, India and was brought to the research laboratory by keeping them in waterproof bags with seawater. After that seaweed (*Halimeda gracilis* Harvey ex J. Agardh 1887) authentication done by a marine biologist, herbarium, and museum specimens were prepared for the repository. The alga was thoroughly washed with seawater to remove

epiphytes, followed by tap water to remove the salts and other extraneous materials. The alga was shade dried and prepared Kadarpasi Chooranam (KPC) by using a standard protocol and purified as per the methods mentioned in the Siddha literature [12].

### 3.2. Purification of Chooranam

The purification was done by using Avi enthiram method.

#### Avi Enthiram method:

The prepared chooranam was baked in a mud pot with water and milk. The milk was reduced and it was ensured that the vapor is not lost. Subsequently, the baked chooranam was sun-dried and finely sieved in a fine cotton cloth [12].

### 3.3. Organoleptic Evaluation

The organoleptic characters of the Kadarpasi Chooranam (KPC) were assessed based on the method described by Siddiqui et.al, method. As per Indian Pharmacopoeia assessed the color, odor, and taste [13,14].

### 3.4. Physicochemical Evaluation: (Table 1) [13,14]

Percentage Loss on Drying:

10gm of KPC was accurately weighed in dissipating dish and it was air-dried at 105°C for 5 hours, afterward weighed

Determination of Total ash:

3gm of KPC in silica dish and burned at 400°C until it becomes white color, which indicates the absence of carbon percentage of total ash was determined with references to the weight of the air-dried drug.

$$\text{Total Ash} = \frac{\text{Weight of Ash}}{\text{Weight of the crude drug taken}} \times 100$$

Determination of Acid-Insoluble Ash:

The obtained ash was boiled with 25ml of Dilute hydrochloric acid for 6 minutes. Then the insoluble matter was collected in a crucible and washed in hot water and ignited to a constant weight. The percentage of acid-insoluble ash is calculated as the weight of air-dried ash.

$$\text{Acid - Insoluble Ash} = \frac{\text{Weight of Ash}}{\text{Weight of crude drug taken}} \times 100$$

Determination of water-soluble ash:

The obtained ash was boiled with 25ml of water for 5 minutes. The insoluble matter was collected in a crucible

and washed with boiling water and ignited for 15 minutes at a temperature not exceeding 450°C. The weight of the insoluble matter was subtracted from the weight of the ash, the difference in weight represents the water-soluble ash.

$$\text{Water - Insoluble Ash} \\ = \frac{\text{Weight of Ash}}{\text{Weight of crude drug taken}} \times 100$$

Determination of pH:

5gm of KPC was dissolved in 25ml of distilled water and filtered. The resultant solution is permitted to stand for 30 minutes afterward subject to pH evaluation.

Determination of water-soluble extractive value:

5gm of KPC was taken in 250 ml of iodine flask. Add 100ml of water and keep it in a shaker for around 6 hours and it is left to stand for the whole night, and then it is filtered with 4 size filter paper. Take 10 ml from that filtrate into a 250 ml weighed beaker. It is kept in an oven at 110°C for one hour and then it is cooled and weighed. Percentage of water-soluble extractive

$$= \frac{W2 - W1g}{\text{Sample Weight}} \times 100/10 \times 100$$

W1 = Beaker Weight

W2 = Dried Sample weight

Determination of Alcohol soluble extractive value:

2.5 grams of KPC were taken in 250 ml of iodine flask. Add 50ml of ethanol and then keep it in a shaker for about 6 hours and it is left to stand for the whole night, and then filtered with 4 size filter paper. Take 10ml from that filtrate into a 250ml weighed beaker. It was kept in an oven at 110°C for one hour and then it is cooled and weighed.

Percentage of Alcohol soluble extractive

$$= \frac{W2 - W1g}{\text{Sample Weight}} \times 50/10 \times 100$$

W1 = Beaker Weight

W2 = Dried Sample weight

Particle Size:

Molecule size assurance was done by optical microscopic technique. In which the test was disintegrated in the sterile refined water (application 1/100th

weakening). The debilitated model was mounted on the slide and fixed with the phase of the fitting area. Light microscopic pictures were attracted with scale micrometer to show up at the normal molecule size. At least 30 perceptions were made to learn the mean normal molecule size of the sample [15].

3.5. Proximate analysis

Proximate parameters (moisture content, protein, ash value, Food lipid, Acid insoluble ash, and nitrogen-free extract) of *Halimeda gracilis* Chooranam were determined using the Association of Official Analytical Chemists (AOAC, 2000) method [16] (Table 2).

### 3.6. Analytical specifications of Chooranam

Analytical specifications were processed as per the guidelines of Protocol for testing of Ayurvedic, Siddha, and Unani medicines. Such as 1) Loss on drying at 105°C, 2) Total ash, 3) Acid insoluble ash, 4) pH, 5) Total soild, 6) Test for heavy metals: Lead, Cadmium, Mercury, Arsenic, 7) Test for Aflatoxins (B1, B2G1, G2), 8) Microbial contamination: Total bacterial and fungal count, 9) Test for specific pathogen: E.Coli, Salmonella SPS, S.aureus, Pseudomonas aeruginosa 10)Pesticide residue: Organochlorine pesticides, Organophosphorus pesticides were performed with kadirpassi chooranam [17] (Table 3-8).

### 3.7. ICP-MS Analysis

Kadirpassi Chooranam was carried out for elemental analysis by the Inductively Coupled Plasma-Mass Spectrometry method for measuring the levels of different elements like cadmium, calcium, cobalt, copper, iron, lead, manganese, magnesium, nitrogen, phosphorus, potassium, sodium, and zinc [18,19] (Table 9).

## 4. Results and Discussion

### Organoleptic characters

Colour: Ash color

Odor: No odour (typical)

Taste: Slightly saltish.

Particle size: 52.98 µm

**Table 1.** Physicochemical analysis

SNO	Parameter	Before Chooranam purified	After Chooranam purified	Reference of test methods
1.	Description	Cream-colored fine powder	Ash color coarse powder	IP Vol-I, 1996, p7
2.	pH (1%w/v solution)	8.21	8.24	IP Vol-I, 2014, p169
3.	Total Solids	1.04 %w/w	1.08%w/w	IP Vol-I, 2014, p277
4.	Total Ash	56.8 %w/w	79.24%w/w	IP Vol-I, 2014, p98
5.	Acid Insoluble ash	2.39 %w/w	3.06	IP Vol-I, 2014, p98
6.	Water soluble ash	21.8 %w/w	28.9%w/w	IP Vol-I, 2014, p98
7.	Loss on Drying at 105 °C	0.509 %w/w	3.50%w/w	IP Vol-I, 2014, p162
8.	Water Soluble Extractive (WSE)	6.64 %w/w	9.10%w/w	IP Vol I, 2014, P-27
9.	Alcohol Soluble Extractive (ASE)	1.74 %w/w	8.47%w/w	IP Vol I, 2014, P-27

**Table 2.** Proximate analysis

S.NO	Proximate analysis	Range	Range
1.	Moisture content	In Percentage	5.32
2.	Crude Protein content	% on Dry Matter Basis	5.24
3.	Crude Fiber content		10.61
4.	Fat content		1.87
5.	Total ash content		64.09
6.	Acid insoluble ash		29.36
7.	Nitrogen free extract level		18.19

**Table 3.** Test for heavy metals

Sl.No	Metals	Method of Testing	Units of Measurement	Results	Specifications as per Ayush Normal Range
1.	Lead	BVILCH/INS/SOP-053 by ICP OES	mg/kg	0.86	10ppm
2.	Cadmium		mg/kg	ND (DL:0.01)	0.3ppm
3.	Mercury		mg/kg	ND (DL:0.01)	1ppm
4.	Arsenic		mg/kg	2.90	3.0ppm

**Table 4.** Test for Aflatoxins

SI. No	Aflatoxin	Method of Testing	Units of Measurement	Results
1.	Aflatoxin B1	AOAC 2008.02	µg/kg	BLQ (LOQ:0.5)
2.	Aflatoxin B2		µg/kg	BLQ (LOQ:0.5)
3.	Aflatoxin G1		µg/kg	BLQ (LOQ:0.5)
4.	Aflatoxin G2		µg/kg	BLQ (LOQ:0.5)

**Table 5.** Test for microbial contamination

SI. No	Microbiological Test	Method of Testing	Units of Measurement	Results	Specifications as per Ayush Normal Range
1.	Total Plate count	API-II	CFU/g	111000	NMT105 CFU/g
2.	Yeast & Mould		CFU/g	<10	NMT103 CFU/g
3.	E.coli		Per g	Absent	Absent
4.	Salmonella		Per g	Absent	Absent
5.	Staphylococcus aureus		Per g	Absent	Absent
6.	Pseudomonas aeruginosa		Per g	Absent	Absent

**Table 6.** Organochlorine test analysis

Sl. No	Organochlorine	Method of Testing	Units of Measurement	Results
1.	Aldrin	BVILCH/INS/SOP-091	mg/kg	BLQ (LOQ:0.01)
2.	Chlordane		mg/kg	BLQ (LOQ:0.01)
3.	Chlorothalonil		mg/kg	BLQ (LOQ:0.01)
4.	DDT		mg/kg	BLQ (LOQ:0.01)
5.	Dicofol		mg/kg	BLQ (LOQ:0.01)
6.	Dieldrin		mg/kg	BLQ (LOQ:0.01)
7.	Endosulphan		mg/kg	BLQ (LOQ:0.01)
8.	Endrin		mg/kg	BLQ (LOQ:0.01)
9.	HCH		mg/kg	BLQ (LOQ:0.01)
10.	Heptachlor		mg/kg	BLQ (LOQ:0.01)
11.	Lindane		mg/kg	BLQ (LOQ:0.01)

**Table 7.** Synthetic Pyrethroids analysis test

Sl. No	Synthetic Pyrethroids	Method of Testing	Units of Measurement	Results
1.	Allethrin and Bioallethrin	BVILCH/INS/SOP-091	mg/kg	BLQ (LOQ:0.01)
2.	Bifenthrin		mg/kg	BLQ (LOQ:0.01)
3.	Cyfluthrin		mg/kg	BLQ (LOQ:0.01)
4.	Cypermethrin		mg/kg	BLQ (LOQ:0.01)
5.	Deltamethrin		mg/kg	BLQ (LOQ:0.01)
6.	Etofenprox		mg/kg	BLQ (LOQ:0.01)
7.	Fenopropathrin		mg/kg	BLQ (LOQ:0.01)
8.	Fenvalerate		mg/kg	BLQ (LOQ:0.01)
9.	Lamda-Cyhalothrin		mg/kg	BLQ (LOQ:0.01)
10.	Permethrin		mg/kg	BLQ (LOQ:0.01)
11.	Tau-Fluvalinate		mg/kg	BLQ (LOQ:0.01)
12.	Transfluthrin		mg/kg	BLQ (LOQ:0.01)

**Table 8.** Test for Pesticide Residues:

Sl. No	Organophosphorus	Method of Testing	Units of Measurement	Results
1.	4-Bromo-2-chlorophenol	BVILCH/INS/SOP-091	mg/kg	BLQ (LOQ :0.01)
2.	Acephate		mg/kg	BLQ (LOQ:0.01)
3.	Chlorfenvinphos		mg/kg	BLQ (LOQ:0.01)
4.	Chlorpyrifos		mg/kg	BLQ (LOQ:0.01)
5.	Chlorpyrifos-methyl		mg/kg	BLQ (LOQ:0.01)
6.	Diazinon		mg/kg	BLQ (LOQ:0.01)
7.	Dichlorvos		mg/kg	BLQ (LOQ:0.01)
8.	Dimethoate		mg/kg	BLQ (LOQ:0.01)
9.	Edifenphos		mg/kg	BLQ (LOQ:0.01)
10.	Ethion		mg/kg	BLQ (LOQ:0.01)
11.	Etrimphos		mg/kg	BLQ (LOQ:0.01)
12.	Fenitrothion		mg/kg	BLQ (LOQ:0.01)
13.	Fenthion		mg/kg	BLQ (LOQ:0.01)
14.	Iprobenphos		mg/kg	BLQ (LOQ:0.01)
15.	Malathion		mg/kg	BLQ (LOQ:0.01)

Table 8. Continued

Sl. No	Organophosphorus	Method of Testing	Units of Measurement	Results
16.	Methamidophos	BVILCH/INS/SOP-091	mg/kg	BLQ (LOQ:0.01)
17.	Monocrotophos		mg/kg	BLQ (LOQ:0.01)
18.	Omethoate		mg/kg	BLQ (LOQ:0.01)
19.	Oxydemeton-methyl		mg/kg	BLQ (LOQ:0.01)
20.	Parathion ethyl		mg/kg	BLQ (LOQ:0.01)
21.	Parathion methyl		mg/kg	BLQ (LOQ:0.01)
22.	Phenthoate		mg/kg	BLQ (LOQ:0.01)
23.	Phorate		mg/kg	BLQ (LOQ:0.01)
24.	Phosalone		mg/kg	BLQ (LOQ:0.01)
25.	Phosphamidon		mg/kg	BLQ (LOQ:0.01)
26.	Profenophos		mg/kg	BLQ (LOQ:0.01)
27.	Primiphos-methyl		mg/kg	BLQ (LOQ:0.01)
28.	Propetamphos		mg/kg	BLQ (LOQ:0.01)
29.	Quinalphos		mg/kg	BLQ (LOQ:0.01)
30.	Temephos		mg/kg	BLQ (LOQ:0.01)
31.	Thiometon		mg/kg	BLQ (LOQ:0.01)
32.	Triazophos		mg/kg	BLQ (LOQ:0.01)

Table 9. ICP-MS Analysis

S. No	Parameters	Unit	Result	FDA Approved Require
1.	Cadmium	mg/kg	BDL (DL:0.1)	Nil
2.	Calcium	mg/kg	128700	1000mg
3.	Cobalt	mg/kg	0.98	1.5micro gram
4.	Copper	mg/kg	1.54	2mg
5.	Iron	mg/kg	719	18mg
6.	Lead	mg/kg	BDL (DL:0.1)	Nil
7.	Manganese	mg/kg	25.87	2mg
8.	Magnesium	mg/kg	1484	400mg
9.	Nitrogen	mg/kg	6066	Rich in Protein
10.	Phosphorus	mg/kg	12500	2mg
11.	Potassium	mg/kg	1286	3,500mg
12.	Sodium	mg/kg	8823	2,400mg
13.	Zinc	mg/kg	4.43	15mg

## Discussion

Marine seaweeds are ecologically significant and have been utilized as food and drugs from days of yore. Today different types of marine green growth give food as well as produce separates for commercial uses like medicines, pharmaceuticals, animal feed, nutraceuticals, cosmetics care products, and hydroponics reasons. It also generated an enormous measure of interest in the pharmaceutical industry as a new wellspring of bioactive compounds with

tremendous restorative potential [20]. It is vital to set down standardization and quality control parameters for plants under investigation [21]. Pharmacognostical parameters for easily recognizable pieces of proof like leaf constituents, microscopy, and physicochemical examination are a few of the basic protocols for the standardization of herbals [22]. The information obtained from the analytical specification, trace elemental analysis and proximate analysis will reveal helpful findings of the nature of the drug. The total ash value and the extractive

value will be helpful in the identification and validation of the plant material [23,24].

Natural medications ought to be liberated from an unfamiliar issue like different pieces of a similar plant, moulds (or) insects including their excreta, visible contaminants like sand and stones, noxious and destructive substance residues. Fundamentally, the measure of foreign matter in herbal products is within the standard endorsed by the pharmacopeia monograph. On the other hand, organoleptic properties are the parts of food or different substances like senses, taste, smell, and touch. Powder microscopy is utilized to contemplate the particular minuscule characters of restorative plants utilizing distinctive staining reagents. These investigations give a reasonable demonstrative apparatus to the standardization and identification of debasements [25].

The contaminations of heavy metals in plants could foster genuine medical issues because there is a thin focus range between the inadequacy and poisonousness levels of the substantial metals in humans [26]. WHO has accentuated different standard scientific methods for the investigation of harmful weighty metals in plant items to find out their security [27]. Aflatoxins, the secondary metabolites produced by the *Aspergillus* species defile an assortment of horticultural and food items. Aflatoxins are characterized into various subtypes. However, the main ones are B1, B2, G1, and G2. These mycotoxins are perceived to be hepatotoxins and cancer-causing agents in people. WHO asks the levels of Aflatoxins to be diminished as low as sensibly feasible [28]. Defilement of medicinal plants with pesticide buildups additionally presents critical wellbeing hazards that incorporate carcinogenesis. Consequently, it is imperative to foster a compelling technique for the identification of these Chooranam. The presence of microbial contaminants in herbal products can diminish or even inactivate the therapeutic activity of the products and it has the potential to adversely affect patients taking these medicines. Subsequently, makers ought to guarantee the most reduced conceivable degree of microorganisms in the crude materials, completed dose structures, and the bundling segments to keep up the suitable quality, security, and adequacy of the regular items [25].

The investigated medicinal plants in this study are a source of the essential trace elements calcium, cobalt, copper, iron, manganese, magnesium, nitrogen, potassium, sodium, zinc, and phosphorus. Essential trace elements are important for human health [29]. Previous studies have been conducted to determine the levels of essential trace elements in medicinal plants. In some of the countries, the element contents were already determined [30]. Kadarpassi Chooranam reported the concentrations of essential elements, namely, calcium, cobalt, copper, iron, manganese, magnesium, nitrogen, potassium, sodium, zinc, and phosphorus. Our study also reported the concentrations of these elements. Calcium 128.7g, cobalt

0.98mg, copper 1.54mg, iron 719mg, manganese 25.87mg, magnesium 1.4gm, nitrogen 60.6gm, potassium 1.2gm, sodium 8.8gm, zinc 4.43mg, and phosphorus 125gm levels were found in our study; Lead and Cadmium are BLQ were observed in our study. Iron was reported in high levels in all tested medicinal plants in this study as well as in ours. These outcomes showed that Kadarpassi chooranam regardless of their seaweeds is a wellspring of comparative high groupings of micronutrients like iron, Zinc, Calcium, and cobalt. Nitrogen showed high concentrations, which is one of the primary nutrients critical for the survival of all living organisms.

Proximate analysis showed KPC has a high measure of ash due to the high salinity of the Gulf of Mannar and determined the fundamental minerals. The majority of green algae have shown a high amount of fiber indicates the hot ecological conditions increase the opportunities for photosynthesis. The fluctuations in the biochemical composition compared with other countries might be due to the environmental conditions to contributing the growth; physical, chemical, the nutritional composition of Seaweed species. Nonetheless, the chemical composition became higher in its contents and it represents that are nutritionally beneficial [31].

## 5. Conclusion

Siddha specifications for kadarpassi chooranam were examined to affirm the proper processing of the raw drug to the final drug. The molecule size analysis revealed that the chooranam fulfills the fineness. The presence of macromolecule distinguished in the chooranam certainly plays an important role in increasing the efficiency and in making these drugs biologically assimilable. The pragmatic clinical application of the drug may be due to the presence of various elements. Data obtained through Physico-chemical evaluation might be useful for further standardization of Kadarpassi chooranam.

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