

Standardization of the Herbomineral formulation of Kodi Pavala Chunnam- A potential tool for quality assurance.

¹Divya Bharathi. V, ²Tamilarasan.G, ³Dr.K.Nalina Saraswathi,M.D(S),
⁴Dr.M.D.Saravana Devi, M.D (S)

^{1,2}PG Scholar, ³Lecturer, ⁴Professor, Head of the Department
Post Graduate Department of Gunapadam (Pharmacology)
Govt. Siddha Medical College
Chennai, TN, India

Abstract- Kodi Pavala Chunnam, a herbo mineral preparation, is a crucial part of the treatment for Eraippu (Bronchial Asthma) which is mentioned in Siddha literature. The preparation of Kodi Pavala Chunnam was done in accordance with Siddha literature, Noigaluku Siddha parigaaram part 2, and the medicines required for experts authentication. The prepared drug was assessed in accordance with PLIM guidelines and subjected to physical-chemical, biochemical, microbial load, heavy metal, and phytochemical testing. The drug's safety and purity are revealed by the physicochemical analysis. Chemical constituents were present, according to an HPTLC analysis. According to the findings, the drug had no microbial contamination. Pesticide and heavy metal residues were found at levels below the quantification threshold. The findings of the above mentioned studies indicated that the drug has potent active components that may be used to treat bronchial asthma.

Keywords: Bronchial asthma, Eraippu, Kodi Pavala Chunnam, standardization, Kodi pavalam, PLIM guidelines.

INTRODUCTION

The Universe is made up of five basic elements (Panchabootha's). The relationship between Panchabootha's and the human body is the same as the universe. In Panchabootha's, water and air are crucial for meeting a person's survival needs. Air is necessary for breathing for both humans and other living things. Breaths are inhaled and exhaled in respiratory cycles during physiological respiration. To breathe, air must be inhaled through the nose, passed through the trachea, bronchi, and lungs, and then exhaled once more [1]. One respiratory condition that affects the lungs' airways is asthma. The Global Asthma report 2022, shows prevalence about 35 million people are suffer from asthma in India [2]. People with asthma are compelled to take medications for the rest of their lives, though the condition gets worse. Bronchodilators and steroidal drugs are commonly used for asthma in many years. Asthma is treated with a variety of herbal and mineral medications according to Siddha medicine. One of the marine products among them is coral, which is mentioned in classical Siddha literature as a remedy for asthma. Coral acts as a bronchodilator and has more potent chemical components. Using contemporary methods, this article demonstrates the standardization of Kodi Pavala Chunnam.

MATERIALS AND METHOD

The test substance, Kodi Pavala Chunnam, is an herbo-mineral combination that is listed as treating bronchial asthma in the Siddha text "Noigaluku Siddha Parigaaram-part 2" [3]. Table 1 contains a list of Kodi Pavala Chunnam's ingredients.

Table-1: Ingredients of Kodi Pavala Chunnam

S.N O	INGREDIEN TS	BOTANIC AL NAME	PAR T USE D	QUANTI TY
1.	Pavalam (coral)	Corallium rubrum	Whol e part	35 gm
2.	Adathodai ilai	Justica adathoda	Leav es	Q.S
3.	Lemon	Citrus limon	fruit	Q.S
4.	cow's milk	Bas taurus milk	milk	Q.S

The Drug Materials Are Collected

The Ramasamy Chettiyar shop in Parry's Corner, Chennai, Tamil Nadu, is where the raw drug Pavalam (Corallium rubrum) was purchased. Cow's milk (Bos taurus Lactus), Adathodai (Justica adathoda), and Elumitchai (Citrus Limon) were procured from Ayanavaram, Chennai, and Tamil Nadu.

Drug's Identification and Authentication

Botanists and experts from the PG Gunapadam (pharmacology) Department of the Govt Siddha Medical College in Arumbakkam, Chennai, identified and validated all of the drugs. The specimens of Citrus limon, Justicia adathoda, Bas taurus milk, and Corallium rubrum were all tagged. The labeled specimens are kept in the PG Gunapadam lab for future use.

Purification of Medications

Purification was carried out in accordance with Indian material medicine [4]. After placing the coral in the mud pot, lime juice was gradually added until the coral was completely submerged. Then the mouth of the mud pot was closed with the other proper mud plate. The coral was removed from the mud pot, washed in hot water, and allowed to dry thoroughly before being placed in an airtight container. This was left alone for 24 hours.

Preparation of Kodi Pavala Chunnam

35 grams of purified coral were taken, covered in a paste made from Justicia adathoda leaves, and allowed to dry before being burned in a pit with 600 cakes of cow dung. After being cooled, the product obtained was taken into a pot and some were soaked with ½ liter cow's milk and kept aside. The following day, it was taken, ground with Justicia adathoda leaf juice, formed into pellets, allowed to dry, and then burned in a pit alongside 400 cakes of cow dung. The final product that Chunnam had obtained was then grounded and taken, and it was stored in an airtight glass container.

Indication

According to the available research, 2 to 4 grains (130-260 mg) should be administered twice daily with cow's ghee or honey to treat bronchial asthma, cough, and tuberculosis.

Qualitative Analysis Investigation

According to the PLIM (Pharmacopoeial Laboratory for Indian Medicines) guidelines, the analysis of the trial drug KPC was assessed. The Noble Research Institute in Perambur, Chennai performed Physico-chemical, phytochemical, biochemical, heavy metal, TLC&HPTLC, and sterility analyses as well as pathogen tests, pesticide residue analyses, and Aflotoxins assays.

Properties for Organoleptic

The test medication's Organoleptic properties were evaluated. 1 gm of the test drug's color, aroma, taste, texture, particle size, and other characteristics were assessed with the naked eye in broad daylight. The outcome was recorded

Physico-chemical Evaluation [5], [6]

- ❖ Physicochemical Analysis was done
- ❖ Finding total ash was done
- ❖ Determination of acid-insoluble ash was done
- ❖ Determination of water soluble ash was done
- ❖ Acid soluble extractive was done
- ❖ Water-soluble determination extractive was done.
- ❖ pH determination was also done
- ❖ solubility test was done
- ❖ Particle size determination was done

Phytochemical Analysis was done for the following tests [7]

- ❖ Alkaloids analysis – was performed with Dragendroff's Test
- ❖ Coumarins
- ❖ Saponin--Foam's Test
- ❖ Tannins-FeCl₃ Test
- ❖ Glycosides - Borntrager's Test
- ❖ Flavonoids- Alkaline reagent Test
- ❖ Phenol's - Gelatin test
- ❖ Steroids
- ❖ Triterpenoids -Burchard test
- ❖ Cyanins
 - A. Anthocyanin
 - B. Betacyanin
- ❖ Carbohydrates - Benedict's test
- ❖ Proteins -Biuret Test
- ❖ TLC [8]
- ❖ HPTLC was done [9]
- ❖ Chromatogram Development was done
- ❖ Scanning was done
- ❖ Biochemical analysis was done [10]
- ❖ Heavy Metal Analysis By AAS Standard was done [11]

Standard preparation

- As & Hg- 100 ppm sample in 1mol/L H Cl

- Cd & Pb- 100 ppm sample in 1mol/L HNO₃
 - ❖ Test for Sterility was done by Pour Plate Method ^[12]
 - ❖ Specific Pathogen ^[13]

Methodology for Specific Pathogen

Table 2: A description of a particular media and its acronym

Organism	Abbreviation	Medium
Escherichia coli	EC	EMB Agar [Eosin Methylene Agar]
Salmonella species	SA	Deoxycholate Agar
Staphylococcus aureus	ST	Mannitol salt Agar
Pseudomonas aeruginosa	PS	Cetrimide Agar

- ❖ Pesticide Residue was done ^{[14], [15]}
- ❖ A test for Aflotoxins was done ^[8]

RESULTS

Organoleptic characters

The drug Kodi Pavala Chunnam is a fine powder that has a bitter taste and an odorless, whitish color. Figure 1 depicts the test drug KPC. Table 3 presents the results in tabular form.



Figure 1. Test drug KPC

Table 3. Organoleptic characters of KPC

S.NO	PARAMATER	RESULT
1.	State	Solid
2.	Nature	Very fine
3.	Odour	None
4.	Touch	Soft
5.	Flow property	Free flowing

6.	colour	Whitish
7.	Taste	Bitter

Results of Physicochemical parameters

Table 4. Results of physicochemical analysis of KPC

S.No	Parameter	Mean (n=3)SD
1.	Loss on Drying at 105°C (%)	0.8667 ± 0.6041
2.	Total Ash (%)	50.67 ± 9.603
3.	Acid insoluble Ash (%)	0.64 ± 0.12
4.	Water soluble Extractive (%)	3.1 ± 1.024
5.	Alcohol soluble Extractive (%)	0.2533 ± 0.0432
6.	pH	9
7.	Particle size	71.87 ± 19.46 μm

Particle size determination

Microscopic observation of the particle (average) size was found to be 150.7 ± 60.92 μm. The sample has particles with the size range of lowest 70 μm to most elevated 215 μm.

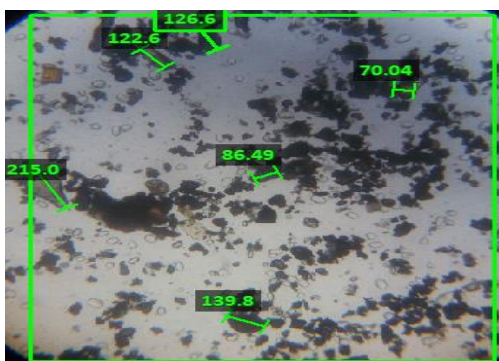


Figure no.2 Particle size microscopic observation for the sample KPC

Table no.5 results of solubility profile of KPC

S.No	Solvent Used	Solubility / Dispersibility
1	Chloroform	In Soluble
2	Ethanol	Soluble
3	Water	Soluble
4	Ethyl acetate	In Soluble
5	Hexane	In Soluble
6	DMSO	Soluble

Qualitative phytochemical screening of KPC

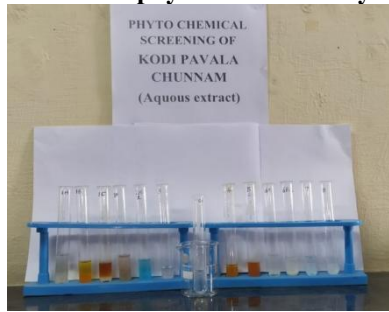
The qualitative phytochemical analysis results indicate that the drug KPC shows the presence of alkaloids, saponins, tannins, phenols and flavonoids. The outcomes were displayed in a table,

Table 6. Result of qualitative Phytochemicals screening

S.No	Phytochemicals	Test name	Aqueous extract

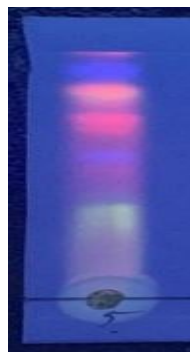
1.	Alkaloids	Dragendroff's test	Positive
2.	Saponins	Foam test	Positive
3.	Tannins	Ferric chloride test	Positive
4.	Phenols	Gelatin test	Positive
5.	Flavonoids	Alkaline reagent Test	Positive

Figure.3 Results of phytochemical analysis of KPC

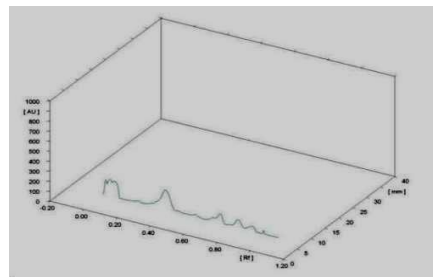


HPTLC Analysis of KPC

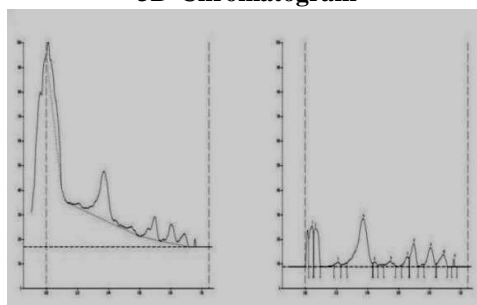
Eleven significant peaks were found in the sample's HPTLC analysis, indicating that there were eleven different phytochemicals present. Rf values at the peaks range from 0.02 to 0.94.



**Fig 4.TLC Chromatogram of KPC
TLC visualization of KPC at 366 nm**



3D-Chromatogram



**Fig 6.HPTLC fingerprinting
Table 7.HPTLC Peak Table**

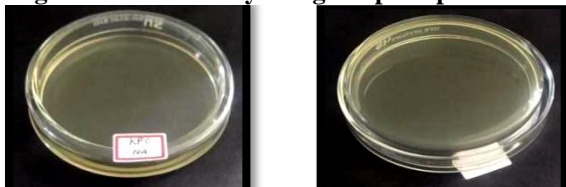
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.02	115.7	0.04	167.8	19.05	0.05	139.4	2359.5	12.82
2	0.05	139.7	0.06	158.5	18.00	0.10	3.6	2737.7	14.87
3	0.18	8.0	0.21	19.2	2.18	0.23	8.9	315.6	1.71
4	0.27	15.9	0.37	196.0	22.25	0.43	13.2	7109.1	38.61
5	0.44	13.6	0.44	19.0	2.16	0.47	3.9	247.1	1.34
6	0.51	9.0	0.55	24.2	2.75	0.58	2.2	535.1	2.91
7	0.62	5.6	0.66	40.3	4.57	0.67	35.9	621.6	3.38

AAS's Heavy Metal Analysis:

According to the current investigation, the KPC contained no traces of heavy metals like arsenic, mercury, lead, or cadmium. Table 8 contains a summary of the heavy metals analysis findings.

Table 8: Results of Heavy Metal Analysis of KPC

Name of the Heavy Metal	Absorption Max A max	Result Analysis	Maximum Limit
Mercury	253.7 nm	BDL	1ppm
Lead	217.0nm	BDL	10 ppm
Arsenic	193.7 nm	BDL	3 ppm
Cadmium	228.8 nm	BDL	0.3 ppm

BDL - Below Detection Limit**Fig.7 Test for sterility using the pour plate method****Observation**

Nothing grew after the incubation period. So it demonstrates the absence of a particular pathogen.

Result

There was no growth or colonies on any plate where the KPC was inoculated.

Table 9.Result of sterility test of KPC

Test	Result	Specification	As per AYUSH/WHO
Total Bacterial Count	Absent	NMT 105CFU/g	As per AYUSHspecification

Total Fungal Count	Absent	NMT 103CFU/g	
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Results for specific Pathogen

E. coli, Salmonella, Staphylococcus aureus, and Pseudomonas aeruginosa were specifically screened for in test drug KPC, but no traces of any of the four pathogens were found. The findings were listed in Table 10 for the culture plates in Figures 5, 6, 7, and 8.

Table no: 10 Results of test specific pathogen

Organism	Specificatio n	Result	Method
E-coli	Absent	Absent	As per AYUSH Specificat ion
Salmonella	Absent	Absent	
Staphylococ us Aureus	Absent	Absent	
Pseudomonas Aeruginosa	Absent	Absent	

Fig 8.Culture plate with E-coli (EC) specific medium

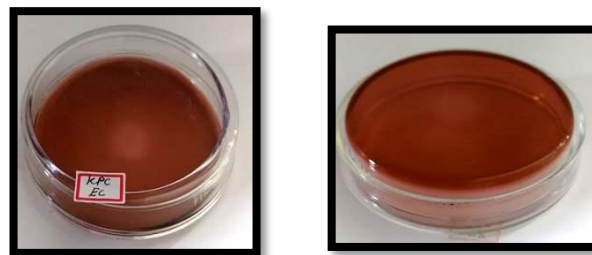


Fig9.Culture plate with Salmonella (SA) specific medium

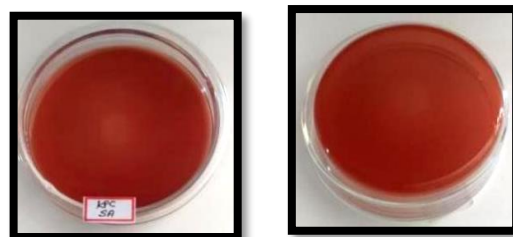


Fig 10 .Culture plate with Staphylococcus Aureus (ST) specific medium

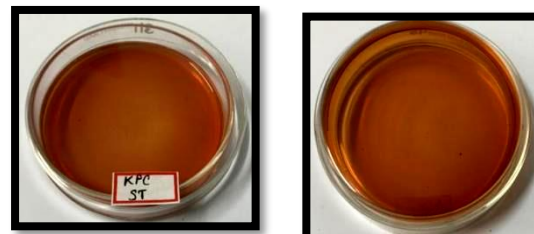
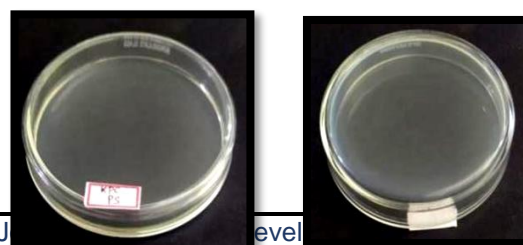


Fig 11 .Culture plate with Pseudomonas Aeruginosa (PS) specific medium



Results for pesticide Residue

According to a pesticide residue analysis, KPC is completely free of Pyrethroids, Organochlorine, and Organophosphorous residues. Table 11 contains the observed pesticide analysis results.

Table. 11 Test results for pesticides of the sample KPC

Pesticide Residue	Sample KPC	AYUSH Limit (mg/kg)
I. Organo Chlorine Pesticides		
Alpha BHC	BQL	0.1mg/kg
Beta BHC	BQL	0.1mg/kg
Gamma BHC	BQL	0.1mg/kg
Delta BHC	BQL	0.1mg/kg
DDT	BQL	1mg/kg
Endosulphan	BQL	3mg/kg
II.Organo Phosphorus Pesticides		
Malathion	BQL	1mg/kg
Chlorpyriphos	BQL	0.2 mg/kg
Dichlorovos	BQL	1mg/kg
III. Organocarbamates		
Carbofuran	BQL	0.1mg/kg
III.Pyrethroid		
Cypermethrin	BQL	1mg/kg

BQL-BelowQuantificationLimit.

Result for Aflatoxins by TLC (B1, B2, G1, G2)

When KPC was loaded on TLC plates and the results were compared to the standards, no spots were found, proving that KPC was free of Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, and Aflatoxin G2.

Table 12.Aflatoxins results of the sample kpc

Aflot oxins	Sample KPC	AYUSH Specification limit
B1	Not Detected- Absent	0.5ppm

B2	Not Detected- Absent	0.1 ppm
G1	Not Detected- Absent	0.5 ppm
G2	Not Detected - Absent	0.1 ppm

Results for Acid and Basic radicals

Results for Acid Radicals

According to the biochemical analysis, KPC contains carbonates, sulfates, and phosphates. Table 13 contains a tally of these.

Specific Radicals	Test Report
Test for carbonates	Positive
Test for Sulfates	Positive
Test for nitrates	Positive

Results for Basic Radicals

The biochemical analysis of the KPC reveals the absence of essential radicals.

DISCUSSION

Kodi Pavala Chunnam is a fine powder, free-flowing, whitish in color, and odorless. The indirect indicator of moisture content was the 0.12% loss on drying, which indicated a good shelf life and stability. The purity of Kodi Pavala Chunnam is indicated by its total ash value, which is 46% and represents the minerals present. The amount of siliceous matter in the drug is indicated by the acid insoluble ash value, highlighting the siliceous matter which is 0.4% in this drug, indicating good quality. KPC's water soluble ash value is 5%, which shows that the medication was prepared correctly and to detect the drug exhausted by water or not [16]. The diffusion capacity, which was found to be 3.2%, is indirectly indicated by the extractive that is water soluble. Extractives that dissolve in alcohol are useful for both quality and purity. KPC's extractive value that is soluble in alcohol in this case is 0.2%. The outcome showed that the drug is of good quality and purity, and it shows that the raw drug KPC is free of adulteration. The pH of KPC is 9 (weekly alkaline). This drug has very good oral bioavailability. KPC is highly soluble in water, ethanol, DMSO and insoluble in chloroform, ethyl acetate and hexane. Solubility impacts the bioavailability and pharmacological efficacy of a drug [17]. Phytochemicals, a naturally occurring bioactive compound found in plants, are effective in treating diseases like asthma. The therapeutic efficacy of the drug Kodi Pavala Chunnam will be improved by the presence of phytochemicals like alkaloids, flavonoids, phenol, tannins, and saponins. Because of their anti-inflammatory and anti-histamine compounds, such as alkaloids, flavonoids, tannins, and phenols, plants are a natural source of antioxidants and potent herbal medicines. These compounds improve performance by inhibiting the release of histamine, the COX pathway, and cytokine mediators [18, 19, 20, 21]. The chloroform extract's HPTLC fingerprint scan revealed four significant peaks that corresponded to its eleven variable photo components. The Rf values ranged from 0.02 to 0.94. Carbonates, sulfates, and nitrates are detected by the acid radical test. In particular, carbonate derivatives are used to treat asthma patients and suppress coughs [22-23]. Through the sulfation of numerous endogenous and exogenous compounds, sulfates play a significant role in biological processes such as biosynthesis and detoxification. As the bronchial muscles are relaxed and the airways are expanded, more air can enter and exit the lungs [24]. Nitrates are rapidly absorbed from the skin, GI tract, and mucous membranes and they have antibacterial and relaxing effects on the bronchial muscles [25, 26]. Basic radical revealed the absence of essential radical and other heavy metals were absent. Lead, arsenic, cadmium, and mercury concentrations were all well below the WHO-recommended limits of 10, 3, and 1 ppm, respectively. Escherichia coli, Salmonella, Pseudomonas aeruginosa, Staphylococcus aureus, and the absence of total bacterial and fungal count were not present in the drug, which may indicate that it is of high quality and safe for treating asthma. Organo phosphorus, organo carbamates, organo chloride and pyrethroid pesticides were not present in the drug KPC. Therefore, this medication is non-toxic and non bio-accumulative. The Aflatoxin B1, B2, G1, and G2 assay results revealed that there were no spots in the TLC plates with loaded test samples of KPC compared to the standard, indicating that the drug is non-toxic, decontaminated, and does not act as a carcinogen. KPC, a medication that can be taken internally to treat asthma, is therefore a safer option.

CONCLUSION

Even though many of the drugs mentioned in Siddha literature have been proven to be successful in treating asthma, they lack scientific validation and standardization. Based on physicochemical, phytochemical, and biochemical analyses the herbo-mineral formulation Kodi Pavala Chunnam will benefit greatly from standardization and quality control assessment tools such as

screening, sterility testing, and tests for specific pathogens, Aflatoxins, and pesticide residue. Increased polar secondary metabolites like alkaloids, flavonoids, phenol, tannins, and saponins were found through phytochemical analysis. This establishes that the above trial drug Kodi Pavala Chunnam was safe to use as internal medicine to treat asthma. Pharmacological studies also determine the drug's therapeutic value.

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Conflicts of interest

The authors state that there are no conflicts of interest.

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