

Standardization of Siddha herbal drug formulation-Adathoda Rasayanam for the Management of Soolikanam (Childhood asthma)

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Abstract

Review Article

Siddha system of medicine is one of the oldest and well documented Indian traditional system of medicine which has its origin in Southern part of India and Srilanka. Siddha system of medicine is capable of treating all types of diseases even some emergency cares. This trial medicine Adathodai Rasayanam is the sathric preparation. The aim of the present study is to investigate the phytochemical and phytochemical analysis of the trial drug Adathodai Rasayanam. Preliminary phytochemical analysis, HPTLC analysis and physicochemical analysis such as ash values, extract values and loss on drying were determined. Phytochemical analysis gave positive test for alkaloids, steroids, triterpenoids, phenol, tannin saponin and sugar. The HPTLC finger printing analysis of Adathodai Rasayanam has shown two prominent peaks which indicates the presence of two versatile phytochemicals. Physicochemical parameters such as the total ash value was found to be 0.3167 ± 0.105 , acid insoluble ash was found to be 0.1067 ± 0.05033 and Loss on drying at 105°C was found to be 6.5 ± 2.666 . The water soluble extractive and alcohol soluble extractive was found to be 19.4 ± 5.051 and 12.07 ± 1.102 .

Keywords: Siddha, Adathodai Rasayanam, ash value, disease.

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INTRODUCTION

The period of Siddha tradition was prevedic and it is unique for its own specialization such as treatments for fetus, children, women, pregnancy care, skin, varmam etc... Siddha system categories Kanam, a respiratory disease into 24 types. In this 24 types Soolikanam nearly correlates with the symptoms of childhood asthma. Soolikanam has been described in the Siddha literature as the disease frequently occurring in children. According to the Siddha text 'Kuzhandhai Maruthuvam (Balavagadam)' it is characterized by prolonged expiration and expiratory wheeze, prolonged cough, inflammatory changes due to chronic persistent cough and breathing difficulty, nasal congestion, abdominal bloating and discomfort due to mouth breathing [1]. Though there are effective drugs to treat the childhood asthma they are not entirely free of other adverse effects. So, there is a need to develop more safer drugs in future. Although different pharmaceutical companies have introduced a number of new

antibacterial medicines, but resistance to these agents has also increased and it became a worldwide problem today. So, there is a need to explore alternative medicine to manage bronchial asthma. Siddha system of medicine having good number of formulations to control asthmatic symptoms and generally the medicines are based on pure herbals. The trial drug Adathodai Rasayanam comprises of 12 herbal ingredients like Justicia adathoda, Solanum trilobatum, Solanum xanthocarpum, Anacyclus pyrethrum, Piper longum, Glycyrrhiza glabra, Terminalia chebula, Zingiber officinale, Mucuna pruriens, Alpinia galanga, Alpinia officinarum and Scindapsus officinalis. The herbal ingredients of Adathodai Rasayanam possess immunomodulatory, anti-bacterial and anti-viral property which have the tendency to cure respiratory disorders. Standardisation is the process of implementing and developing technical standards. Standardisation of herbal formulations is essential in order to assess the quality of drugs [2]. The present study is to investigate the physicochemical,

phytochemical and HPTLC analysis of the trial drug Adathodai Rasayanam a shasthric preparation which is mentioned in Siddha text Chikkicha ratna deepam.

Ingredients of Adathodai Rasayanam

1. Adathodai (*Justicia adathoda*) - 105ml
2. Thoodhuvalai (*Solanum trilobatum*) - 105 ml
3. Kandakathiri (*Solanum xanthocarpum*) - 105 ml
4. Karuppu poonai kanchori (*Mucuna pruriens*) -105 ml
5. Thippili (*Piper longum*) - 6.96 gm
6. Aanaithippili (*Scindapsus officinalis*) - 6.96 gm
7. Kadukkai (*Terminalia chebula*) - 6.96 gm
8. Adhimadhuram (*Glycyrrhiza glabra*) - 6.96 gm
9. Chitharathai (*Alpinia officinarum*) - 6.96 gm
10. Perarathai (*Alpinia galanga*) - 6.96 gm
11. Chukku (*Zingiber officinale*) - 6.96 gm
12. Agragaram (*Anacyclus pyrethrum*) - 6.96 gm
13. Honey

Collection of Raw Drugs

The Drugs were purchased from Ramasamy chettiyar raw drugs store at Parris, Chennai.

Method of Purification [3]

The ingredients of Adathodai Rasayanam were purified and prepared in Gunapadam lab, National Institute of Siddha, Tambaram Sanatorium, Chennai.

Adathodai

The plant was purified by cleaning it with clean cloth. Then the petiole and veins of leaves were removed.

Thoodhuvalai

The plant was purified by cleaning it with clean cloth and then the petiole and veins of leaves were removed.

Kandankathiri

The plant was purified by cleaning it with clean cloth. Then the petiole and veins of leaves were removed.

Karuppu Poonai Kanchori

The plant was purified by cleaning it with clean cloth. Then the petiole and veins of leaves were removed.

Thippili: The drug was purified by soaking it in lemon juice and dried.

Aanai thippili: The drug was purified by soaking it in vinegar for 3 hours and then dried.

Kadukkai: Kadukkai was purified by soaking it in rice water. Then the seed was removed and dried.

Adhimadhuram:

The drug was washed with clean water, the outer skin is removed. Then it was cut into small pieces and dried.

Arathai: The outer layer was removed. Then it was cut into small pieces and dried.

Chukku: The drug was soaked in limestone water. Then the outer layer was removed and dried.

Aragaram: The outer layer was removed.

Method of Preparation [4]

All the above ingredients were purified. Juice (saaru) was taken from the above first four ingredients (Adathoda, Thoodhuvalai, Kandakathiri and Karuppu poonai kanchori). Honey with the equal quantity of plant juice (saaru) was mixed. Then it was kept in stove with mild flame. The other ingredients were fine powdered and mixed with the above mixture. Then it was slowly stirred till it comes to rasayanam texture (veralalunthum patham). Then it was preserved in dried glass container.

Dosage

3-4 years - 170mg (tds), After food.

5-7 years - 500mg (tds), After food.

Duration of Treatment: 45 Days.

Physicochemical Analysis [5, 6]



Sample description

State	Semisolid
Nature	Dense Semisolid
Odor	Characteristic
Touch	Greasy
Flow Property	Non-free flowing
Appearance	Dark Brownish black

Solubility Profile of Adathodai Rasayanam

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

Percentage Loss on Drying

Test drug was accurately weighed in evaporating dish. The sample was dried at 105°C for 5 hours and then weighed.

Determination of Total Ash

Test drug was accurately weighed in silica dish and incinerated at the furnace a temperature 400 °C until it turns white in color which indicates absence of carbon. Percentage of total ash will be calculated with reference to the weight of air-dried drug.

Determination of Acid Insoluble Ash

The ash obtained by total ash test will be boiled with 25 ml of dilute hydrochloric acid for 6mins. Then the insoluble matter is collected in crucible and will be washed with hot water and ignited to constant weight. Percentage of acid insoluble ash will be calculated with reference to the weight of air-dried ash.

Determination of Alcohol Soluble Extractive

Test sample was macerated with 100 ml of Alcohol in a closed flask for twenty-four hours, shaking frequently during six hours and allowing it to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat-bottomed shallow dish, and dry at 105°C, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

Determination of Water-Soluble Extractive

Test sample was macerated with 100 ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowing it to stand and for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat-bottomed shallow dish, and dry at 105°C, to constant weight and weigh. Calculate the percentage of water-soluble extractive with reference to the air-dried drug.

Final Test report

S.no	Parameter	Mean (n=3) SD
1.	Loss on Drying at 105 °C (%)	6.5 ± 2.666
2.	Total Ash (%)	0.3167 ± 0.105
3.	Acid insoluble Ash (%)	0.1067 ± 0.05033
4.	Water soluble Extractive (%)	19.4 ± 5.051
5.	Alcohol Soluble Extractive (%)	12.07 ± 1.102

Phytochemical Analysis [7]**Test for alkaloids:**

Mayer's Test: To the test sample, 2ml of Mayer's reagent was added, a dull white precipitate revealed the presence of alkaloids.

Test for coumarins:

To the test sample, 1 ml of 10% sodium hydroxide was added. The presence of coumarins is indicated by the formation of yellow color.

Test for saponins:

To the test sample, 5 ml of water was added and the tube was shaken vigorously. Copious lather formation indicates the presence of Saponins.

Test for tannins:

To the test sample, ferric chloride was added, formation of a dark blue or greenish black color showed the presence of tannins.

Test for glycosides- Borntrager's Test

Test drug is hydrolysed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate is subjected to the following tests. To 2 ml of filtered hydrolysate, 3 ml of chloroform is added and shaken, chloroform layer is separated and 10% ammonia solution is added to it. Pink colour indicates presence of glycosides.

Test for flavonoids:

To the test sample about 5 ml of dilute ammonia solution were been added followed by addition of few drops of conc. Sulfuric acid. Appearance of yellow color indicates the presence of Flavonoids.

Test for phenols:

Lead acetate test: To the test sample; 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicated the presence of phenolic compounds.

Test for steroids:

To the test sample, 2ml of chloroform was added with few drops of conc. Sulphuric acid (3ml), and shaken well. The upper layer in the test tube was turns into red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of steroids.

Triterpenoids

Liebermann–Burchard test: To the chloroform solution, few drops of acetic anhydride was added then mixed well. 1 ml concentrated sulphuric acid was added from the sides of the test tube, appearance of red ring indicates the presence of triterpenoids.

Test for Cyanins- Anthocyanin:

To the test sample, 1 ml of 2N sodium hydroxide was added and heated for 5 min at 100°C.

Formation of bluish green colour indicates the presence of anthocyanin.

Test for Carbohydrates - Benedict's test

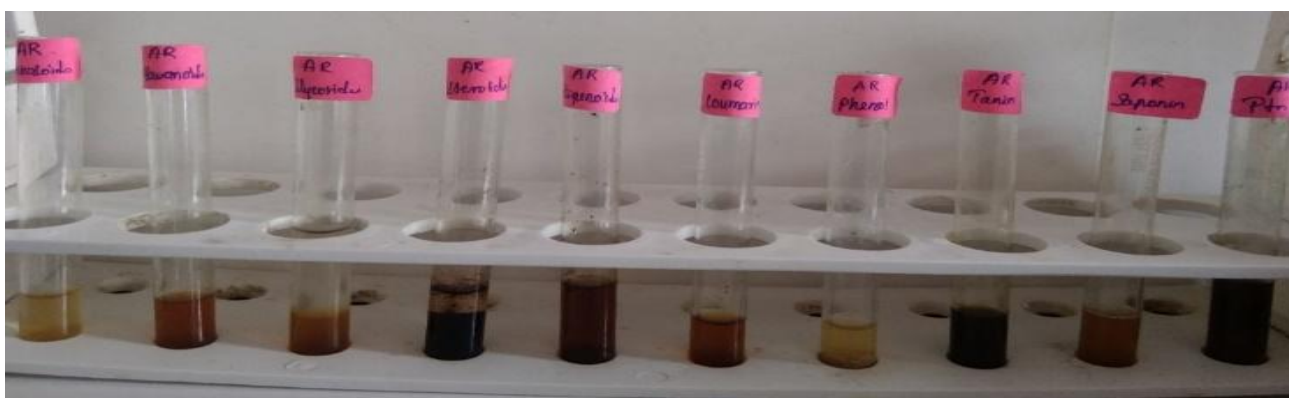
To the test sample about 0.5 ml of Benedict's reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic coloured precipitate indicates the presence of sugar.

Proteins (Biuret Test)

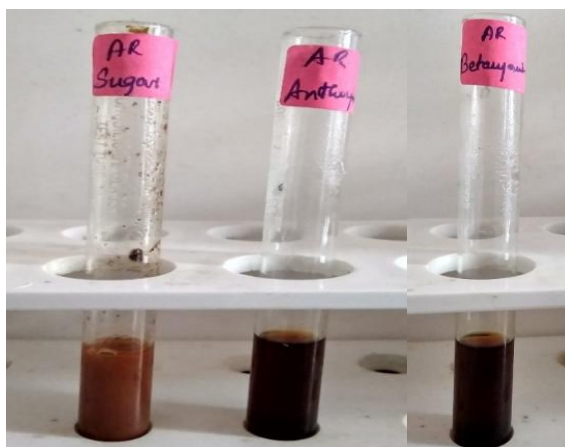
To extracts 1% solution of copper sulphate was added followed by 5% solution of sodium hydroxide, formation of violet purple colour indicates the presence of proteins.

Result of phytochemical analysis

The phytochemical analysis showed the presence of alkaloids, triterpenoids, steroids, phenol, tannin, protein, saponin, sugar and anthocyanin.



Qualitative Phytochemical Investigation



Anthocyanin / Betacyanin and carbohydrates

TLC AND HPTLC ANALYSIS [8, 9]

TLC Visualization of AR - TLC plate visualization at 366 nm



3D View of Chromatogram

Test sample was subjected to thin layer chromatography (TLC) as per conventional one-dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Micro pipette was used to spot the sample for TLC applied sample volume 10-micro litre by using pipette at distance of 1 cm at 5 tracks. In the twin trough chamber with the specified solvent system. After that run plates are dried and was observed using visible light Short-wave UV light 254nm and light long-wave UV light 365.

High Performance Thin Layer Chromatography Analysis of Adathodai Rasayanam

HPTLC method is a modern sophisticated and automated separation technique derived from TLC. Pre-coated HPTLC graded plates and auto sampler was used to achieve precision, sensitive, significant separation both qualitatively and quantitatively. High performance thin layer chromatography (HPTLC) is a valuable quality assessment tool for the evaluation of botanical materials efficiently and cost effectively. HPTLC method offers high degree of selectivity, sensitivity and rapidity combined with single-step sample preparation. Thus, this method can be conveniently adopted for routine quality control analysis. It provides chromatographic fingerprint of phytochemicals which is suitable for confirming the identity and purity of phytotherapeutics.

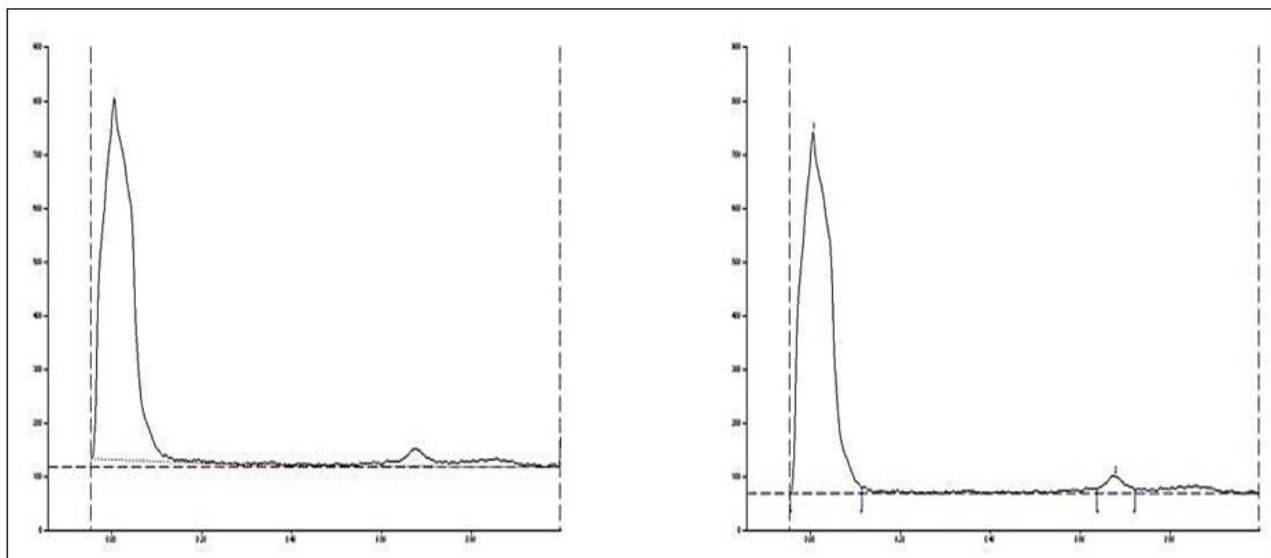
Chromatogram Development

It was carried out in CAMAG Twin Trough chambers. Sample elution was carried out according to

the adsorption capability of the component to be analyzed. After elution, plates were taken out of the chamber and dried.

Scanning

Plates were scanned under UV at 366nm. The data obtained from scanning were brought into integration through CAMAG software. chromatographic finger print was developed for the detection of phytoconstituents present in each sample and their respective rf values were tabulated.



HPTLC finger printing of Sample Adathodai Rasayanam

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.05	1.7	0.00	672.2	95.33	0.11	11.0	34565.9	96.58
2	0.64	9.1	0.68	32.9	4.67	0.72	8.2	1223.8	3.42

Peak table

Report

HPTLC finger printing analysis of the sample reveals the presence of two prominent peaks corresponds to presence of two versatile phytocomponents present within it. Rf value of the peaks ranges from 0.05 to 0.64. Further the peak occupies the major percentage of area of 96.58 which denotes the abundant existence of such compound.

Biochemical analysis of Adathodai Rasayanam

Biochemical analysis of Adathodai Rasayanam was done at the Biochemistry lab at National Institute of Siddha, Chennai by the method of Kolkate.

Preparation of Extract

5gm of sample was taken in a 250ml clean beaker and added with 50ml of distilled water. Then it was boiled well for about 10 minutes. Then it was cooled and filtered in a 100ml volumetric flask and made up to 100ml with distilled water. This preparation

was used for the qualitative analysis of acidic/basic radicals and biochemical constituents in it.

Procedure:

Test for Silicate: A 2ml of the sample was shaken well with distilled water.

Action of Heat: A 2ml of the sample was taken in a dry test tube and heated gently at first and then strong.

Ash Test: A filter paper was soaked into a mixture of extract and dil. cobalt nitrate solution and introduced into the Bunsen flame and ignited.

Test for Acid Radicals

Test for Sulphate: 2ml of the above prepared extract was taken in a test tube to this added 2ml of 4% dil. ammonium oxalate solution

Test for chloride: 2ml of the above prepared extract was added with 2ml of dil. HCl until the effervescence ceases off.

Test for Phosphate: 2ml of the extract were treated with 2ml of dil. ammonium molybdate solution and 2ml of con. HNO_3 .

Test for carbonate: 2ml of the extract was treated with 2ml of dil. magnesium sulphate solution.

Test for Nitrate: 1gm of the extract was heated with copper turning and concentrated H_2SO_4 and viewed the test tube vertically down.

Test for Basic radicals

Test for lead: 2ml of the extract was added with 2ml of dil. potassium iodine solution.

Test for copper:

One pinch (25mg) of extract was made into paste with con. HCl in a watch glass and introduced into the non-luminous part of the flame.

Test for Aluminium: To the 2ml of extract dil. sodium hydroxide was added in 5 drops to excess.

Test for Iron:

a. To the 2ml of extract 2ml of dil. ammonium solution was added.

b. To the 2ml of extract 2ml of thiocyanate solution and 2ml of con HNO_3 is added.

Test for Zinc: To 2ml of the extract dil. sodium hydroxide solution was added in 5 drops to excess and dil. ammonium chloride was added.

Test for Calcium: To 2ml of the extract with 2ml of 4% dil. ammonium oxalate solution was added.

Test for Magnesium: To 2ml of extract dil. sodium hydroxide solution was added in drops to excess.

Test for Ammonium: To 2ml of extract 1 ml of Nessler's reagent and excess of dil. sodium hydroxide solution were added.

Test for Potassium:

A pinch (25mg) of extract was treated of with 2ml of dil. sodium nitrite solution and then treated with 2ml of dil. cobalt nitrate in 30% dil. glacial acetic acid.

Test for Sodium: 2 pinches (50mg) of the extract was made into paste by using HCl and introduced into the blue flame of Bunsen burner.

Test for Mercury: 2ml of the extract was treated with 2ml of dil. sodium hydroxide solution.

Test for Arsenic: 2ml of the extract was treated with 2ml of dil. sodium hydroxide solution

Miscellaneous

Test for Reducing Sugar

5ml of Benedict's qualitative solution was taken in a test tube and allowed to boil for 2 minutes and added 8 to 10 drops of the extract was added and again boiled for 2 minutes. The colour changes were noted.

Test for Starch: 2ml of extract was treated with weak dil. Iodine solution.

Test for the Alkaloids:

a) 2ml of the extract was treated with 2ml of dil. potassium Iodide solution.

b) 2ml of the extract was treated with 2ml of dil. picric acid.

c) 2ml of the extract was treated with 2ml of dil. phosphotungstic acid.

Test for Tannic Acid: 2ml of extract was treated with 2ml of dil. ferric chloride solution.

Test for Unsaturated Compound: To the 2ml of extract 2ml of dil. Potassium permanganate solution was added.

Test for Amino Acid: 2 drops of the extract were placed on a filter paper and dried well. 20ml of Burette reagent is added.

Test for Type of Compound: 2ml of the extract was treated with 2 ml of dil. ferric chloride solution

DISCUSSION

The ash value represents the purity of the drugs. The total ash includes both physiological ash and non-physiological ash. The physiological ash is derived from the organic matter and non-physiological ash is the residue of the extraneous matters like sand / soil, inorganic materials. The non-physiological ash is represented by acid insoluble ash. The total ash in Adathodai Rasayanam was found to be 0.3167 ± 0.105 and acid insoluble ash was 0.1067 ± 0.05033 . The both ash values were under limits. The minimal level of acid insoluble ash shows the less inorganic residue and purity of the drug Adathodai Rasayanam. The water-soluble extract of Adathodai Rasayanam was found to be 19.4 ± 5.051 and alcohol soluble extract was found to be 12.07 ± 1.102 . In this trial drug water soluble extract was found to be more than alcohol soluble extract. This increased water soluble extract may produce immediate drug action. The phytochemical analysis of Adathodai Rasayanam revealed the presence of certain phytochemicals in it. They are alkaloids, steroids, triterpenoids, phenol, tannin, saponin and sugar. Biochemical analysis of Adathoda Rasayanam revealed the presence of sulphate, phosphate, carbonate, iron, calcium, arsenic, reducing sugars, alkaloids and tannic acid. The data obtained from the scanning through CAMAG software revealed the results of HPTLC

analysis. The HPTLC finger printing analysis of Adathodai Rasayanam has shown two prominent peaks which indicates the presence of two versatile phytocomponents in it. Peak1 occupies the major percentage of area of 96.58 which denotes the abundant existence of such compound.

CONCLUSION

The above analysis showed the sample description, solubility profile, ash value, water soluble extractive, alcohol soluble extractive, phytochemicals and certain phytocomponents. As the water-soluble extractive was found to be more than alcohol soluble extract which may produce immediate drug action. Saponin rich herbs when consumed orally tend to cause an increase in the production of mucus in the lungs as well as coughing. This effect can be helpful for the patients with coughs particularly dry cough. All saponins tend to be immuno- modulatory [10]. Triterpenoids exert a plethora of biological activities including suppression of inflammation. This activity of Triterpenoids may reduce the chronic inflammation of the lungs i.e. bronchial asthma. Tannin possess styptic and astringent properties which generally treat respiratory infection like pharyngitis and tonsillitis. Phenol is used as an analgesic or anaesthetic in the products which treat pharyngitis. Sulphate aids in the detoxification of drugs, food additives and toxic metals. Phosphate is necessary for glycolysis and oxidative phosphorylation resulting in energy from the formation of ATP from ADP. Iron (heme) part of hemoglobin is important for transport of oxygen from lungs to different tissues. Hence, by the presence of above activities Adathodai Rasayanam is expected to treat respiratory infections including bronchial asthma.

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