

## SKM VAIDHYA AMIRTHAM

News Letter of SKM in Siddha, Ayurveda and Unani

Vol: 1 Issue: 4 **OCTOBER - DECEMBER 2022** 



## Effective Remedy for **CONSTIPATION!**





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### When you should wake up?



## ब्राह्ममुहूर्तउत्तिष्ठेत्स्वस्थोरक्षार्थमायुषः।।

Ayurveda recommends waking up early in the morning. According to ayurveda, you should wake up 96 minutes (around 1.5 hours) before sunrise. It is called Brahma Muhurta in ayurveda and yoga science. This is the perfect time for waking up for a healthy person.

(Ref: Ashtanga Hridayam Sutrasthana 2/1)

Articles are invited in Slddha, Ayurveda and Unani fields about clinical experience, rare medicinal preparations, successful treatments, Herbal informations and AYUSH

Seminathony and Property And Information Seminathony and Information Semination Sem Foods for our "SKM Vaidhya Amirtham" News letter which has around 10000 copies of circulation.

Please send your Articles/Suggestions to:

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# <u>Phytochemical Standardization of Serankottainei</u> (a Siddha drug from milk extract of SemecarpusAnacardium nuts) and its in-vitro antitubercular activity against H37Rv strain

#### INTRODUCTION

Siddha system of Medicine is one of the ancient traditional medical systems in India. The classical Siddha literatures describe lakhs of herbal formulations for the management of human and animal diseases. Serankottainei(SN) is a popular Siddha drug, used in the treatment of lung infections including tuberculosis, rheumatoid arthritis, degenerative osteoarthritis, cancers, skin diseases and neurological pain. In this preparation, Semecarpusanacardiumnut was boiled in cow milk and cow ghee, thus also called as milk extract of Semecarpusanacardium. Earlier studies have proven the protective role of SN on cell membranes in aflatoxin b1 induced hepatocellular carcinoma bearing rats.] In Chronic myeloid leukemia mice model, anticancer activity of SN was comparable to standard drug imatinib. The beneficial role of SN in rheumatoid arthritis rat model was proven in earlier studies. SN has anticancer activity against hepatocarcinoma, antioxidant activityin animal models. Thirty days administration of SN to rats did not show toxicity, thus SN is considered as safe. The phytochemical standardization and antitubercular activity of SN have not been done so far. This study was aimed to standardize the Serankottaineiand to screen its antitubercular activity against H37Rv strain.



#### MATERIALS AND METHODS

#### Chemicals

Serankottaineiwas procured from SKM Siddha and Ayurveda Co India Ltd, Erode, Tamil Nadu. Mycobacterium tuberculosis (H37Rv strain, No-27294) was purchased from ATCC, USA and maintained in the microbiology lab. All other chemicals used in the study were of analytical quality purchased from the local vendor.

#### Seperation of unsaponifiablematter

Weighed 100 g of the serankottaineiwas taken in a 2 litre round bottomed flask. Added 1 litre of alcoholic KOH in to the sample. Boiled gently but steadily under reflux condenser for one hour. The condenser was washed with 10 ml of ethyl alcohol and the mixture was collected and transferred to a separating funnel. The transfer was completed by washing the sample with ethyl alcohol and cold water. Altogether, 1 litre of water was added to the separating funnel followed by an addition of 250 ml of petroleum ether. The stopper was inserted and shaken vigorously for 1 min and allowed it to settle until both the layers were clear. The lower layer containing the soap solution was transferred to another separating funnel and repeated the ether extraction six times more using 250 ml of petroleum ether for each extraction.

All the extracts were collected in a separating funnel. The combined extracts were washed in the funnel 3 times with 100 ml of aqueous alcohol and shaken vigorously, drawing off the alcohol-water layer after each washing. The ether layer was again washed repeatedly with 100 ml of water until the water no longer turns pink on addition of a few drops of phenolphthalein indicator solution. The ether layer was transferred to a tarred flask containing few pieces of pumice stone and evaporated to dryness on a water bath. Placed the flask in an air oven at 85°C for about 1 h toremove the last traces of ether. Ten ml of acetone was added and evaporated to dryness on a water bath. Cooled in a desicator to remove last traces of moisture and then weighed. Percentage of unsaponifiable matter determined with respect to weight of sample taken.

#### Preliminary phytochemical screening

The preliminary phytochemical screening was done according to the standard procedure. The brief of the procedure are as follows;

#### Tests for alkaloids

**Dragendroff's test:** To a few mg of extract dissolved in alcohol, a few drops of acetic acid and Dragendroff's reagent were added and shaken well. An orange red precipitate formed indicates the presence of alkaloids.

**Wagners's test:** To a few mg of extract dissolved in acetic acid, a few drops of Wagner's reagent was added. A reddish brown precipitate formed indicates the presence of alkaloids.

**Mayer's test:** To a few mg of extract dissolved in acetic acid, a few drops of Mayer's reagent was added. A dull white precipitate formed indicates the presence of alkaloids.

**Hager's test:** To a few mg of extract dissolved in acetic acid, 3 ml of Hager's reagent was added, the formation of yellow precipitate indicates the presence of alkaloids.



#### Tests for carbohydrates

**Molisch's test:** To the extract, 1 ml of  $\alpha$ -naphthol solution and conc. sulphuric acid were added along the sides of test tube. Violet colour formed at the junction of the two liquids indicates the

**Fehling's test:** A few mg of extract was mixed with equal quantities of Fehling's solution A and B. The mixture was warmed on a water bath. The formation of a brick red precipitate indicates the presence of carbohydrates.

**Benedict's test:** To 5 ml of Benedict's reagent, a few mg of extract was added, and boiled for two minutes and cooled. Formation of a red precipitate indicates the presence of carbohydrates.

# SERANKOTTAI NEI CAPSULES Grantinanicas, quoi teologidate titoretà in dorprin

#### **Test for steroids**

**Libermann-Burchard test:** To the extract was dissolved in chloroform, 1 ml of acetic acid and 1 ml of acetic anhydride were added, then heated on a water bath and cooled. Fewdrops of conc. Sulphuric acid were added along the sides of the test tube. Appearance of bluish green colour indicates the presence of steroids.

**Salkowski test:** The extract was dissolved in chloroform and equal volume of conc. Sulphuric acid was added. Formation of bluish red to cherry red colour in chloroform layer and green fluorescence in the acid layer indicates the presence of steroids.

#### **Test for saponins**

To a few mg of extract, distilled water was added and shaken. Stable froth formation indicates the presence of saponin.

#### **Test for tannins**

To the extract, a few drops of dilute solution of ferric chloride was added, formation of dark blue colour shows the presence of tannins.

#### Test for flavonoids

**Shinoda's test:** To the extract in alcohol, a few magnesium turnings and few drops of conc. hydrochloric acid were added and heated on a water bath. Formation

#### Test for phenol

To the extract in alcohol, added two drops of alcoholic ferric chloride. Formation of blue to blue black indicates the presence of phenol.

#### **Test for coumarins**

To the extract in alcohol, a few drops of 2 N sodium hydroxide solution was added. Dark yellow colour formation indicates the presence of coumarins.

#### **Test for triterpenoids**

The extract was warmed with tin bits and few drops of thionyl chloride. Formation of pink colour indicates the presence of triterpenoids.

#### Test for carboxylic acid

Extract dissolved in water is treated with sodium bicarbonate. Brisk effervescence indicates the presence of carboxylic acid.

#### **Test for resin**

Few mg of the sample was mixed with water and acetone. Turbidity indicates the presence of turbidity.

#### Test for quinine

A few mg of alcohol extract was treated with 0.5% of sodium hydroxide. Deep coloration like pink, purple or red indicates the presence of quinine.

#### High Performance Thin Layer Chromatography

Unsaponifiable matter of Serankottaineiwas dissolved in 10 ml of chloroform and 3 and 6  $\mu$ l of the above sample was applied on to a pre-coated silica plate to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in toluene: ethyl acetate (9.0:1.0). The developed plates were visualized in UV 254, 366, 540 (White light) and scanned under UV, 366 nm, 540 nm and 620 nm post derivatization. Rf, colour of the spots and densitometric scan were recorded.

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	ATRAMORES		
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(Vagners test	Mooden brown preophate	Modeln Snows preoprises	
Mayers leat	Dull white preciptate	Dut white precipitate	4.
Hagers test	Veltow precipitate Silventes	Yerow precipitate	
Liebernarn-badues fect	Bush green	Bush green	
Sakuwak tesi	otherty ned open in observations sayer and broads green fluorescence in acre sayer	sherry ned coner in oncondition layer and bruken green fluorescence in acid layer	
	Cartiohydrate		
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Wat Fact,	Cart blue or green or brown Flavonceps	Teles ole	+
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	Amino acids		
Nintegative reagent.	Purple polar	Coorney solution	- 1

254 nm	366 nm	Post derivatisation
	0.21 (FL. blue)	
	-	0.33 (D. purple)
	0.44 (FL. blue)	
9	0.80 (FD. blue)	
0.90 (L. green)	2	0.90 (D. purple)
	9	190000000000000000000000000000000000000

able 3: Minimum inhibitory concentration (MIC) o tandard drugs and <i>Serankottal nel</i> against H37Rv train		
Drug name	Minimum inhibitory concentration (MIC)	
Pyrazinamide	3.125 µg/ml	
Ciprofloxacin	3.125 µg/ml	
Streptomycin	6.25 µg/ml	
Serankottai nei	1.6 µg/mi	



#### In-vitro anti tubercular activity

The anti-mycobacterial activity of Unsaponifiable matter of Serankottaineiwas assessed against Mycobacterium tuberculosis (H37Rv strain from ATCC, No-27294) using microplate Alamar Blue assay (MABA). This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method. Briefly, 200 µl of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of test drug were made directly on plate. The final drug concentrations tested were 100 to 0.8 µg/ml. Plates were covered and sealed with parafilm and incubated at 37°C for five days. Pyrazinamide, streptomycin, ciprofloxacin and serankottaineiwere added in different concentrations. After this time, 25 µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The Minimum Inhibitory Concentration (MIC) was defined as lowest drug concentration which prevented the color change from blue to pink.

#### **RESULTS**

#### Preliminary phytochemical tests

We obtained 8% w/w USM from *serankottainei*, which showed presence of alkaloid, steroid, terpenoid and phenol (Table 1).

#### **High Performance Thin Layer Chromatography**

On photo documentation at 254 nm, 366 nm and under white light (post derivatization with vanillin sulphuric acid), *serankottainei* showed one spot (Rf0.90), three spots (Rf0.21, 0.44, 0.80) and two spots (Rf0.33, 0.90) respectively (Figure 1, Table 2).

On densitometric scan at 254 nm, *serankottainei* showed 13 peaks; peak with Rf0.76 and 0.89 being the major spots contributing to 29.38% and 19.72% area (Figure 2). At 366 nm, drug showed 5 peaks; peaks with Rf0.02, 0.33 and 0.05 being the major spots of 39.27%, 35.16% and 15.08% area (Figure 3). At 620 nm, drug showed 7 peaks; peak withRf0.88, 0.27 and 0.01 being the major peak with 35.19%, 28.71% and 23.78% area (Figure 4). Thus, this protocol could be useful for fingerprinting the *serankottainei*.

#### In-vitro anti tubercular activity

Different doses of USM of *serankottainei* were screened for *in-vitro* antitubercular activity against H37Rv strain using Alamar Blue Dye method.

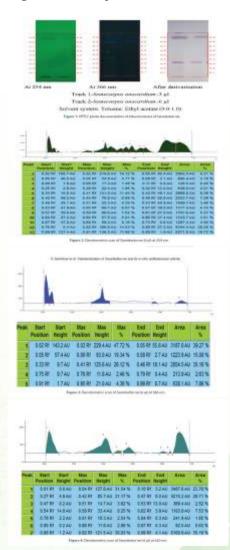
The Minimum Inhibitory Concentration (MIC) of pyrazinamide, streptomycin, ciprofloxacin was 3.125, 6.25 and 3.125  $\mu$ g/ml respectively. Whereas, the MIC of serankottaineiwas 1.6  $\mu$ g/ml, which was almost 25 to 50% of standard drugs. (Figure 5 and Table 3).

#### **DISCUSSION**

The preliminary analyses of chemical composition are one of the best methods to analyse quality of herbal formulations. The testsshowed presence of alkaloid, phenol, steroid and terpenoid. The HPTLC finger printprofile of *serankottainei* was been obtained with suitable solvent system. Based on our study, HPTLC fingerprinting is an effective technique of screening the same herbal formulation for authenticity and quality.

Previous study on water extract of *Semicarpusanacardium* has shown promising antitubercular activity. Our study also showed that the milk extract of *Semicarpusanacardium* (*serankottainei*) has the potent antitubercular activity. Thus, there is rationale for using *serankottainei* in the treatment of tuberculosis by Siddha physicians.

*Serankottaine*i has shown promising antitubercular activity in in-vitro study. Further studies should be focused on isolation of compound responsible for antitubercular activity and its molecular mechanism.



3.12



#### <u>In Effect of Poorna Chandrodayam Chendooram (metallic drug)</u> <u>on liver function, kidney function and lipid profile parameters of rats</u>

#### INTRODUCTION

Indian alchemy is one of the disciplines in which Parpam, Chendooram and Chunnam were first described as intriguing formulations of metals and minerals such as gold, silver, copper, iron, zinc, mercury, and so forth, apparently associated with organic macromolecules derived from the herbal juices by alchemic processes making these biologically assimilable. (Savrikar 2004). Minerals are combined with herbs that assist the assimilation and delivery of the ingredients to the human body (Suoboda 1998). Theseherbomineral medicine are prepared by repeated incineration of metals or their salts (preferably oxides) with medicinal herbs or their extracts so as to eliminate their harmful effects and are taken along with honey, milk, butter, or ghee (a preparation from milk) (Patel., 1986). Most of the medicines are mixture of compounds and because of its synergistic action; toxicity is being diminished, thereby increasing bioavailability through the cells of the body. Treating the minerals with herbal juices may lead to reduction in particulate size even up to nano levels (less than 100 nm) enable increased potency. Poornachandrodayamchendooram is a well-known, mercurial preparation with gold and sulphur (Thiagarajan., 1992) widely used for many ailments like tuberculosis, jaundice, fever, rat bite, cancerous ulcer, sprue and male sterility. (Muthaliar, 1987) Hibiscus and Aloe juice is added for titration. (Mahdihassan 1985) These drugs are mostly a mixture of compounds and because of its synergistic action and purification process (Austin, 2002) toxicity is being diminished. (Hardy et al., 1995), thereby increasing bioavailability through the cells of our body. (Sudha et al., 2009) These drugs are known to be effective even in low concentration. (Kumar et al., 2006) The phytochemical studies of this drug Poornachandrodayamchendooram has shown to contain flavonoids, phenols, and Vitamin C (Muthukumaran and Hazeena Begum., 2014), but a clear picture of its toxicokinetics is still obscure. The present study was aimed at evaluating the Liver, kidney and Lipid profile of Normal and PCC treated in experimental animal model.



#### MATERIALS AND METHODS SELECTION OF ANIMAL

Healthy and pure strain Male Wistar rats, Rattusnorvegicus, ranging from the body weight of 120-150 g were procured from the Venkateshwara Enterprises, Bangalore and maintained in the Central Animal House, Department of Siddha Medicine, Tamil University, and Thanjavur. Experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Tamil University, Thanjavur. The animals were maintained on standard diet (Kamadhenu Agencies, Bangalore) and water was given ad libitum.

#### DRUG PREPARATION

The (Poorna Chandrodayam Chendooram drug obtained from the SKM Siddha and Ayurvedic Medicine's India Private Limited, Saminathapuram, mudakurichi, Erode- 638104. Tamilnadu, India. The drug (PoornaChandrodayamChendooram) is not soluble in water therefore a suspension of gum acacia is made for oral administration. The 10 gm. of gum acacia dissolved in 100 ml of distilled water by gradual trituration in a mortar. Then well prepared solution was taken and added Poornachandrodayamchendooram at the dose of 3 mg/ml/100 gm.

#### **EXPERIMENTAL DESIGN**

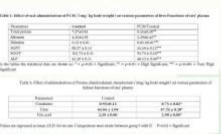
After acclimatization, the rats were divided into 2 groups, each having 8 rats.

**Group I:** Untreated control were received water only.

**Group II:** Young rats were treated with Poornachandrodaya chendooram 4 (3.0 mg / kg body wt. calculated from human dose) with oney for 7 weeks (orally administered).

#### BLOOD SAMPLES COLLECTION AND PREPARATION OF PLASMA

Blood samples were collected from post vena cava and transferred into heparinised tubes immediately. Blood was then centrifuged at 4,000 g for 10 min using bench top centrifuge to remove red blood cells and recover plasma. Plasma samples were separated and were collected using dry Pasteur pipette and stored in the refrigerator for analyses. All analyses were completed within 24 h of sample collection.



#### RESULT AND DISCUSSION

#### LIVER FUNCTION

Proteins are important organic substances required by an organism in the tissue building, the cellular organelles repair and also cellular metabolism (Yeragiet al., 2000). Albumin constitutes a major antioxidant defense against oxidizing agents (Halliwell et al 1998). Bilirubin estimation is reliably sensitive in the diagnosis of hepatic disease (Harper., 1991), because bilirubin is a by-product of the breakdown of hemoglobin. The determination of the pathophysiological enzymes like SGOT and SGPT is a common mean of detecting the liver status.



Alterations in SGOT and SGPT values are reported in hepatic disease or damage. SGOT, SGPT and bilirubinare the bio-markers for liver functions (Martin et al., 1981; Ronald and koretz, 1992; Mazumder, 1999). Alkaline phosphatase is amembrane bound enzyme and its inactivation leads to membrane damage of hepatic cells (Flora et al., 1994).

Increased Alkaline phosphatase is responsible for intra-and extra-hepatic disease. Table. 4. presents the effect of PCC on serum, serum protein, albumin bilirubin, SGPT, SGOT and ALP in control and experimental group of rats. It shows the slightly increased level of serum total protein, albumin, and level of serum PCC treated rats. The increase level was 35.24% for total protein, 17% for albumin and 48 % for bilirubin. But, the SGPT,SGOT and ALP levels were decreased. The decrease level was 25 % for SGPT,24 % for SGOT and 32% for ALP in serum PCC treated rats when compared to normal rats These proteins are important liver function marker.

#### KIDNEY FUNCTION

Kidneys are the chief organs for the excretion of wastes. Besides their excretory function, kidney function in a significant manner in the maintenance of internal environment of the body. The damaged kidneys cause an elevated Urea because the kidneys are less able to clear urea from the blood stream. Urea measures the amount of urea nitrogen, a waste product of protein metabolism in the blood. It is also useful to detect the function of kidney tissue. Urea is typically measured to assess kidney function (Mitchell et al., 1972). Creatinine is also used to measure the filtration rate of the kidney. It is the indicators for the function of kidney (Gyton, 1991). Uric acid is a major contributor to total radical trapping capacity (TRAP) (Kharb and Singh 2004) Table 2. represents the effect of PCM on kidney Creatinine, Urea and Uric acid levels in control and Drug treated rats. Creatinine and Urea levels were significantly decreased PCC treatment the decrease levels were 21% for creatinine and 27% for urea when compared to control rats. But the Uric acid levels were significantly increased 12% in PCM treatment. Creatinine and urea content, major kidney function parameter, in the male plasma was decreased significantly but the content of uric acid were slightly changed in significant manner. This reduced creatinine and urea level might have results from the decreased synthesis or increased functional capacity of tubular excretion (Mitchell et al., 1972; Zilvaet al., 1991) There are significant changes in serum urea, creatinine and uric acid. Yet these values were proving the safety of the drug. There is an increase in uric acid levels which aids to the safety of the drug. Renal function test credits the safety of the drug. PCC did not accumulate in renal tissues which could be evidently seen by the urea creatinine and uric acid in serum.

#### LIPID PROFILE

Atherogenicity with subsequent cardiovascular manifestations is one of the major causes of death and morbidity in the world (Raju and Binda, 2005). The important lipids whose elevations are implicated in these disease conditions are cholesterol and triacylglycerols. Lipids are transported as lipid-protein complexes called lipoprotiens, which are classified based on their density and charges. The High-density Lipoprotein cholesterol (HDL) transports lipids out of blood cells to the liver, while the Low Density Lipoproteins cholesterol (LDL) mobilizes lipids against the cells and blood vessels. Triacylglycerols have been found to be elevated along with total cholesterol elevation. Therefore, elevated low-density cholesterol, triacylglycerols and total cholesterol with reduced HDL will enhance the development of atherosclerosis and related cerebrovascular disorders (Nwanjo, 2004). Figure 1. represents the level of lipid profile in PCM treated rats & Normal control rats. The value of TG, TC and LDL were significantly decreased. The decrease levels were 32.23 % for Triglycerides, 27.58 % for Cholesterol and 19.29 % for Low density lipoprotein in PCC treatment than normal control. But the HDL level was significantly increased. The increase level was 44 % in PCM treatment than normal control rat's serum. The plants constituents (Lee et al., 2000) reduced TG level and it could be suggested that PCM increased lipase activity which hydrolyzed TG. Among the lipids, increased blood level of TC and LDL as well as lowered level of HDL has been identified as contributors in the development of hyperlipidemia (Ross, 1999) which is the consequences of, in majority of the cases, diabetes mellitus (Pushparajet al., 2000; Pepatoet al., 2003; Sharma et al., 1983). The elevation of lipid components is a risk factor for coronary heart disease (Mironovaet al., 2000). PCM may act as inhibitor for enzyme such as hydroxyl-methylglutaryl-CoA reductase, which is the keyenzyme in de novo cholesterol biosynthesis as has been suggested for some plants earlier (Gebhardt and Beck, 1996; Eidiet al., 2006).

This reduction could be beneficial in improving lipid metabolism and complications in diabetes (Cho et al., 2002) Abnormalities in serum lipids are associated with diabetes (Virella-Lopes and Virella, 2003; NCEP, 2002).

#### **CONCLUSION**

Interestingly, it is seen that PCC has steady decreased levels of urea, creatinine, SGOT, SGPT, ALP levels which reveal that fact that this drug may also be useful treatment of hepatic disorders and renal diseases. The myth that heavy metal cause toxicity is broken out in this study when the drug is properly prepared and given safe dosage during the duration of treatment. It is confirmed that Metal base drug PCC is a safe and effective drug. It is evident that the trial drug eliminates the toxic substances from the body and enhances the longevity of life.



## Our presence in 9th Ayurveda Congress - GOA

The SKM Siddha and Ayurveda company make her presence in the 9th Ayurveda congress (Internationa Arogya Expo - 2022) held in Goa on Dec-2022.

























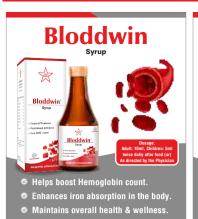
## Abhyanga (Massage)

अभ्यङ्गं आचरेतिनत्यं, सजराश्रमवातह दृष्टिप्रसादपुष्टिआयु: सुस्वप्नसुत्वक्दार्ढ्यकृत् शिर: श्रवणपादेषुतंविशेषनशीलयेत वर्ज्योअभ्यंग: कफग्रस्थकृतसंशुद्धिअजिणिभि



Abhyanga means massage. It should be done daily, morning. It delays ageing, relieves tiredness and excess of Vata (aches and pains). It improves vision, nourishes body tissues, prolongs age, induces good sleep and improves skin tone and complexion. Massage should be specially done on ears, head and legs. Massage should be avoided when there is increase of Kapha in the body, soon after Shodhana (Panchakarma procedure) and during indigestion.

## **SKM NEW PRODUCTS**





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- Stimulates appetite & improves digestion.



- Treats viral fever.
- Quick relief from respiratory problems.





Effective for Cervical spondylosis





- Relieves all types of viral fever.
- Best for body pain due to fever.
- Very effective for Headache





- Provides relief from sprains & strains





- Alleviates allergic respiratory disorders.
- Reduces bronchial mucosal irritation



- Beneficial in treating congestion of lungs
- Relaxes respiratory tract & reduces wheezing
- Reduces shortness of breath



- Best remedy for Indigestion
- Regulates bowel movements

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