STANDARDIZATION AND PHARMACOLOGICAL SCREENING OF *NAAGA SANGU PARPAM*

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CONTENTS

Introduction

Siddha system of medicine is an integrated part of Indian system, which is a very potent and unique system in existence and practiced in India for thousands of years and above. It is an earliest medical science that stress on positive health, a harmonious blending of physical, mental, social, moral and spiritual welfare of individuals. The *Siddha* system has developed a rich treasure of medicinal knowledge that includes the use of herbs, metals and minerals. It is a traditional system of medicine which is gradually evolved along with the Dravidian's culture and hence this system is also known as Dravidian system of medicine. *Siddha* system also deals with the concept of salvation in life. The aim of *Siddha* medicine is to make the body perfect, imperishable and to promote longevity.

The exponents of *Siddha* system of medicine are called *Siddhars*. They are the super human beings with high culture and intellectual abilities. It is considered that Siddha medicine was created by Lord Siva and he is the first *Siddhar*. There were 18 important *Siddhars* in olden days and they developed this system of medicine. Hence, it is called *Siddha* medicine. *Siddhars* were spiritual adepts who possessed the *Ashta siddhis* or the eight supernatural powers. They practiced intense yogic practices, including years of periodic fasting and meditation and were believed to have achieved supernatural powers and gained the supreme wisdom and overall immortality. Through this spiritually attained supreme knowledge, they wrote scriptures on all aspects of life, from arts to science and truth of life to miracle cure for diseases.

Siddhars not only focused on the diseased state and treatment, they also enlightened the system through preventive measures. They were of the concept that a healthy soul can only be developed through a healthy body. So they developed methods and medications that are believed to strengthen their physical body and thereby their souls. *Siddhars* propose that the state of salvation can be achieved through the use of natural *Siddha* medicines and meditation. For the healthy life, *Siddhars* have mentioned daily and seasonal regimen including dietary habits and also insisted some code of ethics. The contribution of *Siddhars* towards *Siddha* literature with its boundless therapeutics and wonderful pharmaceutical preparation of medicine is acclaimed pre-eminent even in this 21st century and worthy of its remarkable results.

Siddha system is mainly based on ***Anda Pinda Thathuvam*** that means the relationship between the universe and human body. It was quoted by the *Siddhar Sattamuni* as,

“அண்டத்தில் உள்ளதே பிண்டம்

பிண்டத்தில் உள்ளதே அண்டம்

அண்டமும் பிண்டமும் ஒன்றே

அறிந்துதான் பார்க்கும் போதே”

- சட்டமுனி ஞானம்

These two are interlinked through the five basic principles which are known as “***Pancha bootham****”*. This system believes that all the objects in the universe are made up of five basic elements which are:

1. Earth
2. Water
3. Fire
4. Air
5. Sky

The food we consume and the medicines we take are also composed of these five elements. The amount and proportion of these elements in the medicines vary. It is their preponderance that is responsible for the medicinal actions and therapeutic results.

The structural aspect of the human body is said to be “***Udal Thathukkal*** *”* (i.e. the physical component of the human body) which consists of seven elements: first is *Saram* (Plasma) responsible for growth, development and nourishment; second is *Senneer* (Blood) responsible for nourishing muscles, imparting colour and improving intellect; the third is *Oon* (Muscle) responsible for shape of the body; fourth is *Kollzuppu* (Fatty tissue) responsible for oil balance and lubricating joints; fifth is *Enbu* (Bone) responsible for body structure and posture and movement; sixth is *Moolai* (Nerve) responsible for strength and the last is *Sukilam* (Semen) responsible for reproduction.

The functional units of the human body are said to be “***Uyir Thathukkal****”* (i.e. *Vatham*, *Pitham* and *Kabham).* They are considered as three pillars of health and support, the structure and functions of the body. They are involved in regulating all the functions of the body and maintain the balance in the physical, emotional and mental spheres. These *Uyir*

thathukkal co-exist in all the cells of the body. They function in a harmonious manner to create a balance.

Vatham is formed by *Vayu* (Air) and *Agayam* (Space). It controls the sensory and motor functions of the nervous system such as sensation and movement. *Pitham* is formed by *Thee* (Fire). It controls the metabolic processes in the body such as digestion and assimilation and helps to maintain the warmth of the body. *Kabham* is formed by *Neer* (Water) and *Munn* (Earth) and controls stability. The equilibrium of humors is considered as health and its disturbance or imbalance leads to a diseased state.

The factors assumed to affect this equilibrium are environment, climatic conditions, diet, physical activities and stress. The food, which is the basic building material for the human body, gets processed into these body tissues, humors and waste products to determine the balance of the *Uyir thathukkal* in the body. *Siddha* medicine works by revitalizing and rejuvenating the dysfunctional organs to maintain the ratio of *vatham, pitham* and *kabham*, thereby providing a healthy state of equilibrium in the body.

“அனிலபித்தத் தொந்தமலாது மூலம் வராது”

According to *Siddhar Thaeraiyar* derangement of *vatham* and *pitham* causes *moolam* (Hemorrhoids). In *Siddha* system of medicine *Siddhars* who are spiritual scientists have explained briefly about the Hemorrhoids (21 types) and numerous number of medicine preparations to cure the disease. In modern term, they are the varicosities of the veins of the hemorroidal plexus.

Hemorrhoids (piles) are dilated veins within the anal canal in the sub epithelial region formed by radicals of the superior, middle and inferior rectal veins similar to varicose veins. In their normal state, they are cushions that help with stool control. They become a disease when swollen or inflamed. Hemorrhoids have a number of causes, although often the cause is unknown. They may result from straining during bowel movements or from the increased pressure on these veins during pregnancy. Hemorrhoids may be located inside the rectum (internal hemorrhoids) or they may develop under the skin around the anus (external hemorrhoids).

Worldwide, the overall prevalence of hemorrhoids in the general population is estimated to be 4.4%. In India the prevalence of hemorrhoids is very high. Country dig up

second place in prevalence rate. Males and females are both affected with about equal frequency. According to WHO, 40% of the people in the world suffer from hemorrhoids. In India approximately 80% of the sufferers are in the age group of 21-50 years.

The *Siddha* system of medicine uses a fascinating combination of herbs, minerals and metals to promote good health and longevity. Before preparing medicines, *Siddhars* laid a great emphasis in purification of raw drugs. More than 80% of the *Siddha* medicines are formulated by herbal products, but in certain life threatening disease and in many chronic diseases, *Siddhars* enumerated some herbo-metal, and herbo-mineral.

Naaga Sangu Parpam is one of the herbo-metal formulation mentioned in ancient *Siddha* literature *Kannusamy paramparai vaithiyam* which is prepared from *Naagam* (Zinc), *Sangu* (Conch shell), *Uthamani* (*Pergularia daemia*) indicated for *Moolam* (Hemorrhoids), *Powthiram* (Fistula in ano), *Vellai* (Leucorrhoea), *Vettai* (Venereal disease). The toxicity studies were carried out previously [(1)].

In acute toxicity study (OECD Guidelines 423), Wistar albino rats of both sex were treated with various dose level of *Naaga Sangu Parpam* at 50 mg/kg/bw, 100 mg/kg/bw, 300 mg/kg/bw, 500 mg/kg/bw, 1000 mg/kg/bw and 2000 mg/kg/bw, showed normal behavioral changes. When *Naaga Sangu Parpam* was given at the dose level of 4000 mg/kg/bw, mild writhing and resulted in death of an animal. So the maximum tolerated test dose was up to 2000mg/kg/bw. After 14 days of acute oral toxicity study, no animal death was recorded and no pathological alterations were detected in the surviving animals. Pathological examinations of the organs on gross macroscopic basis indicated that there were no detected abnormalities[(1)].

In Repeated dose 28-days oral toxicity study (OECD Guidelines- 407), *Naaga Sangu Parpam* was administered orally to Wistar albino rats of both sex at the dose level of 200mg/kg/bw (low dose), 400mg/kg/bw (mid dose) and 800mg/kg/bw (high dose). The results revealed that all the animals treated with *Naaga Sangu Parpam* at the dose level of 200mg/kg/bw (low dose) were normal and did not show any significant toxic signs in hematological parameters and histopathological slides of various organs. The mid dose (400mg/kg/bw) and high dose (800mg/kg/bw) group animals, showed the following changes in spleen (mild congestion), lung (interstitial pneumonitis), stomach (mild ulcer), liver (mild congestion). Thus the study concluded that low dose (200mg/kg/bw) of *Naaga Sangu Parpam* (which is near the therapeutic dose mentioned in *Kannusamy paramparai vaithiyam*) is the

maximum safe dose which showed no mortality or toxicity when prepared as per the *Siddha* literature [(1)].

When traditional literatures were reviewed, it revealed that *Naagam* has astringent and styptic properties, *Sangu* has astringent and anodyne properties and the research articles revealed that the individual ingredients of *Naaga Sangu Parpam* possess Styptic, Anti-inflammatory and Analgesic activities but as a finished product no pharmacological activities has been carried out for this formulation.

Many research workers have conducted a number of pharmacological and toxicological experiments for various *Siddha* formulations which revealed that the toxicity of the crude drug is quite different from that of the finished *Siddha* formulations. Hence the researcher selected the drug *Naaga Sangu Parpam* to standardize and evaluate the pharmacological activities such as Styptic activity, Anti- inflammatory activity and Analgesic activity.

Aim

To Standardize and evaluate the Pharmacological activities of the test drug "***Naaga Sangu Parpam***'' in animal models.

Objectives

The following methodology was adopted to Standardize and evaluate the Pharmacological activities of the test drug.

- Review of various information (*Siddha* and Modern) relevant to the study.
- Preparation of the drug as per classical *Siddha* literature.
- Analytical study of the prepared drug
  - Physicochemical and phytochemical analysis
  - Chemical analysis to evaluate acidic and basic radicals.
  - Heavy metal analysis (ICP-OES)
  - Scanning Electron Microscopy with EDAX
  - Fourier Transform Infrared
  - X- Ray Diffraction
  - Thermogravimetric analysis

- Evaluation of pharmacological activities in animal models.
  - Styptic activity - Tail cutting method
  - Analgesic activity - Eddy's Hot plate method
  - Anti-inflammatory activity - Carrageenan induced paw oedema method

Standard Operating Procedure of *Naaga Sangu Parpam* [2]

Drug selection

The drug ***Naaga Sangu Parpam***, is a herbo-metal *preparation* (*Parpam* = one of the *32* types of *internal* medicine), mentioned in *Siddha* text *Kannusamy paramparai vaithiyam*, pg.no: 414, indicated for *Moolam* (Hemorrhoids), *Powthiram* (Fistula in ano), *Vellai* (Leucorrhoea) and *Vettai* (Venereal disease).

Ingredients

1. Purified *Naagam (Zinc)* - *1 palam* (35gm)
2. Purified *Sangu (Conch shell)* - *1 palam* (35 gm)
3. *Uthamani* leaf juice *(Pergularia daemia. Linn)* - 350ml

Collection of the Plant materials

Naagam and *Sangu* were procured from a well reputed country shop in Parrys, Chennai. *Uthamani* was freshly collected from Tambaram sanatorium.

Naagam and Sangu were purified and the medicine was prepared in the *Gunapadam* laboratory of National Institute of Siddha.

Identification and Authentication of the drug

1. *Sangu* was authenticated by Dr.Rajkumar Rajan, Scientist D, Marine Biology Regional Centre, Zoological Survey of India, Chennai.
2. Metal drug was authenticated by Dr.M.Suresh Gandhi, Department of Geology, University of Madras, Chennai.
3. The Herbal drug was Identified and authenticated by Dr.D.Aravind M.D(s), Botanist, National Institute of Siddha, Tambaram Sanatorium, Chennai.

Purification process

Naagam

- The ghee of south Indian Mahua (*Madhuca longifolia*) – *Illuppai* ghee was taken in a mud pot.
- Two pieces of *Ammonium chloride* (*Navaacharam*) were placed in a pot in such a way that half of the portion of the pieces were immersed in the ghee on opposite direction.
- The zinc melted in an iron pot was poured twenty-one times to the ghee of south Indian Mahua and washed.

Sangu

- Equal quantities of lime stones and fuller's earth was taken and mixed with 8 parts of purified water and the clear filtrate was obtained.
- Conch shell was allowed to boil in the above said filtrate for 1hr 30 mins.

Uthamani

- Before extracting the juice, the leaf of *Pergularia daemia* was washed lightly in a cold running water to remove any soil, dust, bugs or other foreign material.
- The leaf was drained thoroughly on absorbent towel.

Method of Preparation

- Purified *Naagam* was taken in an iron pan and subjected to excessive heat, till *Naagam* reached its melting consistency.
- Conch powder was poured over the melting *Naagam* and the mixture was stirred well till it reached the powder form.
- The powder was grounded with *Uthamanai* leaf juice for 12 hours and made into small cakes and subjected to calcination process with cow dung cakes.
- Finally, the *Parpam* was collected and stored in an air tight glass container.

Labelling

Name of the preparation	:	*Naaga Sangu Parpam*
Date of preparation	:	12/10/2017
Dose	:	1 *kundri* (130mg), bd
Adjuvant/Vehicle	:	Cow's ghee
Indications	:	*Moolam* (Hemorrhoids), *Powthiram* (Fistula in ano), *Vellai* (Leucorrhoea), *Vettai* (Venereal disease)
Date of expiry	:	100 years from the date of manufacture

Therapeutic administration of drug

Form of medicine	-	*Parpam*
Route of administration	-	Oral
Dose	-	130mg twice daily
Vehicle	-	Ghee

INGREDIENTS OF *NAAGA SANGU PARPAM*

Naagam

Before purification

During purification

After purification

Sangu

Before purification

During purification

After purification

Uthamani

Naaga Sangu Parpam preparation

Grinding with *Uthamani* leaf juice ***Villai***

Seelai ***Pudam* with cow dung**

Naaga Sangu Parpam

Velipparuththi [3]

Synonyms : *Uthamani*

Uthamamaakani

Uthamakannigai

Botanical name : *Pergularia daemia* (Forssk.) Chiov.

English name : Dog's bane white low plant

Family : Asclepiadaceae

Source

It is a shrub found throughout India. It is generally found twining to the branches of the tree and to the hedges in backyard. Most commonly it is seen over the fences and hence its *Tamil* name is *Velipparuththi*.

Parts used

Leaf, Root

Organoleptic characters

- Taste - Bitter
- Nature - Hot
- Division - Hot

Action

- Expectorant
- Anthelmintic
- Emetic

UTHAMANI

“வேலிப்பருத்தி முழு தாலாற் பற்றாதுவேலிப்பரு”

Intake of decoction prepared from the root, vine, leaf and latex of the plant for 48 days will cure diathesis due to vitiation of *Vathapitha* humors caused by morbid affection of cold.

General characters

ஆலித் தெழுந்தநோய் அத்தனை யுந்தீருமே
வேலிப் பருத்தியதின் மெல்இலையால் - வேலொத்துக்
கண்டிக்கும் வாதங் கடுஞ்சன்னி தோடமும்போம்
உண்டிக்கும் வாசனையாம் ஓது.

- அகத்தியர் குணவாகடம்

உத்தா மணியிலையால் உள்வயிற்றுக் குன்மமொடு
குத்தாம் வலியுங் குளிரும்போம் - பற்றி
இசிக்கும் வலியிரைப்பும் எத்தடிப்பும் ஏகும்
புசிக்குமதி மாந்தமும்போம் பார்.

- அகத்தியர் குணவாகடம்

Uses

It is used in the treatment of *Vatha* diseases like piercing and boring pain in the course of nerves, swelling, tremulousness, epilepsy and *Kabha* diseases like asthma, cold and cough. It improves appetite.

Medicinal uses

- Decoction of the leaves are given to children as an anthelmintic.
- 16 ml of leaf juice can be given to treat Bronchial asthma. Powdered leaves in doses of 5 to 10 grains are also good expectorant.
- Mix slaked lime with fresh juice of *Pergularia daemia* leaves and apply it externally for oedema of the limbs.
- Leaves are made into paste for using as an external application for fissure.

- Leaf juice can be given alone or with honey to treat dysmenorrhoea or it can be given as an adjuvant to other medicines given for the same problem.
- The combination of 5 drops of leaf juice, 5 drops of honey, 12 mg of *Thambira Chenduram* and 12 mg of *Kasturi* are used for treating bronchial asthma.
- Oils made from the *Pergularia deamia* are used as an internal medicine in treating arthritis and uterine problems.
- *Pavala parpam* and *Aamaiottu parpam* made with the help of *Pergularia deamia* leaf juice will be more alkaline in nature.
- The powder of root bark with the adjuvant of milk is also used as a purgative in rheumatic cases.

Pergularia daemia - Velipparuththi

Synonyms

- *Pergularia extensa*
- *Daemia extensa*
- *Asclepias daemi* [4]

Botanical Classification [5]

Kingdom	:	Plantae
Sub kingdom	:	Tracheobionta
Super division	:	Spermatophyta
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Sub class	:	Asteridae
Order	:	Gentianales
Family	:	Asclepiadaceae
Genus	:	Pergularia L.
Species	:	*P. daemia* (Forssk.) Chiov.

Vernacular names [6]

Tamil	:	*Vaelipparuththi, Uththaamani*
English	:	Pergularia extensa
Hindi	:	Uttaran
Telugu	:	Juttu paku

Description

A slender foul smelling perennial milky twining herb with hispid stems. The leaves are oppositely placed, thin, broadly ovate, heart-shaped or nearly circular, hairless above, velvety beneath. The leaves are foul-smelling when bruised and stems are hairy with much milky juice.

The leaves show antibacterial activity against *Micro- coccus pyogenes var. aureus* and *Escherichia coli*. Stems yield a fiber said to be strong and useful as a substitute for flax in fishing lines [(7).]

Habitat

Throughout the hotter parts of India, up to 900 m.

Propagation

Seeds, Vegetative method.

Parts used

Whole plant

Phytochemicals [(6, 8)]

Plant	-	Lupeol, sterol, hentriacontane, α- amyrin, β-amyrin, β-sitosterol, glycoside uzarigenin.
Root	-	β-sitosterol and its glucoside, α – amyrin and its acetates, Calcitin.
Seeds	-	Calctin, Calotropin and Calotropagenin.
Stems	-	Uzarigenin Coroglaucigenin.

RESEARCH REVIEW

[A] Phytochemical analysis of *Pergularia daemia* for its bioactive components through gas chromatographic mass spectrometry [9]

S. No	Name of the compound	Compound nature	Therapeutic use
1	Benzoic acid, 2-hydroxy-methyl ester	Benzoic acid compound	Antimicrobial
2	2-methoxy-4-vinylphenol	Phenolic compound	Antimicrobial, Analgesic, Anti-oxidant, Anti-inflammatory
3	Phthalic acid, di- (1- hexan-5-yl) ester	Organic acid ester	Anti-fungal
4	1-(+)-Ascorbic acid 2,6-dihexadecanoate	Fatty acid	Antioxidant, anti-bacterial anti-inflammatory, enhances sperm motility and anti-nociceptive.
5	Methyl (z)-5, 11,14,17-cicosateraenoate	Fatty acid ester	Anti-bacterial, to treat dysentery and diarrhoea

[B] Acute oral toxicity study of *Pergularia daemia*

The fresh leaves of *Pergularia daemia* (Forsk) were collected from the area of railway station near to Yeola. The acute oral toxicity of leaf extract of *Pergularia daemia* (Forssk) was determined by using Swiss albino mice of either sex weighing between 25 ± 02 gm maintained under standard condition.

The animals were fasted for 3 hrs prior to the experiment. Animals were administered with the single dose of either petroleum ether or methanol leaf extract of *Pergularia daemia* (Forsk) and observed for its mortality up to 48 hrs. Both petroleum ether and methanol extracts did not produce any sign and symptoms of toxicity till oral dose 2000mg/kg. Hence the extract was used in the range of 100-300mg/kg orally assuming that LD_{50} dose is 2000mg/kg [(10)]

Pharmacological activities

[A] Assessment of antioxidant potential and acute toxicity studies of whole plant extract of *Pergularia daemia* (forsk)

Matured *P. daemia* (Forsk.) plant was collected from river banks of Pudukkottai District, Tamil Nadu, India. Naturally the plant has powerful antioxidants including polyphenols, flavonoids, steroids and terpenoids. The aim of this study is to evaluate the *in vitro* antioxidant potential and to determine the median lethal dose (LD_{50}) of crude ethyl acetate and methanol extracts of *Pergularia daemia*. The study revealed that *Pergularia daemia* possess effective scavenging activity against 2, 2' azino bis (3 ethylbenzothiazoline 6 sulfonic acid (ABTS*), nitric oxide and reducing power radicals at different concentrations (100, 200, 300, 400 & 500 µg/mL) of ethyl acetate and methanol extracts of *Pergularia daemia*.

Acute toxicity study revealed that the extracts showed no signs of toxicity up to a dose level of 2500 mg/kg b.wt and in vitro study revealed that the methanolic extract exert higher antioxidant activity at 400 µg/mL than ethyl acetate extract of *Pergularia daemia* [(11)].

*****is a chemical compound which is frequently used in food industry to determine the antioxidant capacity

[B] Evaluation of analgesic activity of leaf extract of *Pergularia daemia* (forsk) in experimental animals

(i) Hot plate method

The fresh leaves of *Pergularia daemia* (Forsk) were collected from the area of railway station near to Yeola. The analgesic effect was studied using digital hot plate (Columbus-USA) instrument wherein the reaction time (paw licking, jumping or any other sign of discomfort) was recorded 60 minutes after administration of respective drugs as mentioned below on 1st (acute model) 11th and 21st day (chronic model). The temperature of the plate was maintained at 55°C ± 01° C. A cut off reaction time of 30 seconds was chosen in order to avoid injury. *Pentazocin* (30 mg/kg/s.c.) was used as a reference standard and it was given only on 1st, 11th and 21st day. The results showed that PEPD (Petroleum ether extract of *Pergularia daemia*) 300 mg/kg and MPD (Methanol extract of *Pergularia daemia*) 300 mg/kg possess significant and equipotent analgesic activity and are less as compared to the reference standard. The potency was found to be same on 11th and 21st day [(10)].

(ii) Tail immersion method

The fresh leaves of *Pergularia daemia* (Forsk) were collected from the area of railway station near to Yeola. The analgesic effect was studied using Tail immersion model (Digital Water Bath- V J India) wherein the reaction time i.e. time taken for flicking of tail out of water was recorded 60 minutes after administration of respective drugs as mentioned below on 1st (acute model) 11th and 21st day (Chronic Model). The temperature of the water was maintained at 55°C ± 01° C.

A cut off reaction time of 30 seconds was chosen in order to avoid injury. Pentazocin (30 mg/kg/s.c.) was used as a reference standard and it was given only on 1st, 11th and 21st day. The results showed that PEPD 300 mg/kg and MPD 100 and 300mg/kg possess significant analgesic activity. Wherein both the doses of MPD (Methanol extract of *Pergularia daemia*) are equipotent and more significant as compared to the PEPD (Petroleum ether extract of *Pergularia daemia*) 300 mg/kg dose. The methanol extract of leaf of *Pergularia daemia* (Forsk) is more potent than petroleum ether extract of leaf of *Pergularia daemia* (Forsk) [(10)].

[C] Anti-inflammatory activity of *Pergularia daemia* (forsk)

The plant materials were collected from Virudhunagar District, Tamilnadu, India. Albino rats of Wister strain (150-200 g) of either sex were selected. The animals were divided into 4 groups. Acute inflammation was produced by sub plantar injection of 0.1 ml of 1% suspension of carrageenan with normal saline, in the right hind paw of the rats. One hour after oral administration of the drug. The paw oedema was measured plethysmometrically at 0 and 3 hours after the carrageenan injection.

Group 1 received normal saline (10 ml/kg/orally) served as normal control. Group II received Diclofenac sodium (10 ml/kg-IP, Served as standard control). Group III treated with petroleum ether extract of *Pergularia daemia* (100 mg/kg/orally) suspended with 1% CMC. Group IV treated with chloroform extract of *Pergularia daemia* (100 mg/kg/orally) suspended with 1% CMC.

In this acute inflammation model, chloroform extract and petroleum ether extract of *Pergularia daemia* (100 mg/kg) and the standard drugs produced significant inhibition of paw oedema as compared to the control. All the extracts were found to be less effective than diclofenac sodium. Thus the results were found to be highly significant ($p<0.01$) in comparison to the control [(12)].

[D] Analgesic activity of *Pergularia daemia* (forsk)

The plant materials were collected from Virudhunagar District, Tamilnadu, India. The analgesic activity of the extracts was screened by employing tail flick method. Rats of either sex weighing between 150-200 gm taken in 4 groups of each 6 animals. Aspirin (200 mg/kg) was used as a standard drug for comparison of analgesic activity. Tail flick response was evoked by placing rat tail over a wire heated electrically. The intensity of heat was adjusted so that the base line tail flick latency averaged 3-4 s in all the animals. Cut off period of 15 s was observed to prevent the damage to the tail. The chloroform extract of *Pergularia daemia* exhibited significant analgesic activity than petroleum extract [(12)].

Other pharmacological activities of *Pergularia daemia*

- Hepatoprotective [13]
- Antifertility [14]
- Antidiabetic [15]
- Antipyretic [16]

Dried leaf used as an emetic [17], anti-rheumatic [18], dysmenorrheal [19, 20], healing cuts and wounds [21].

Entire plant used as an emmenagogue [22], emetic [23, 24], expectorant [23, 24, 25], antivenin[26] and used to treat post-partum hemorrhage [27] .

Medicinal uses [6]

- The plant extract is used for uterine and menstrual troubles and to facilitate parturition. It has a stimulating action on uterine and other involuntary muscles, stimulating that of pituitrin in many ways.
- Traditionally it has been used as a laxative, expectorant, besides treatment of infantile diarrhea, malarial intermittent fevers, toothaches and colds [16].
- The leaves are fried in castor oil and fomenting will cure join pain, hip pain and screwing pain.
- For one sided headache apply 2 or 3 drops of the leaf juice externally. For right side headache, apply into left ear and vice- versa.
- Apply the fresh juice of the leaves of *Pergularia daemia* over the parts where there is itching, swelling and places where there is bite of an unknown creature.
- Dry and pulverize the root of *Pergularia daemia*. Give about a pinch or two of this powder with milk to children. This will induce loose motion and problems arising due to wind humor will be cured. All the worms from the stomach will also be expelled.
- To the fresh juice of the leaves of *Pergularia daemia* add powdered dried ginger and asafoetida. Boil to consistency. Applying this paste, reduces swelling due to wind humor and also the associated pain. This paste can be applied with advantage if elephantiasis is in the initial stage. In about a month and half all the swellings will disappear.

- Grind 5 grams of the fresh root of *Pergularia daemia* in milk and filter. Give this only in the morning for 3 days. This will remove poison due to insect bite, eczema and colic pain.
- Soak sufficient amount of pepper in the juice of *Pergularia daemia* leaves. Keep under the sun and dry. Repeat the process for seven times adding fresh juice each time. Once thoroughly dried, pulverize the pepper. Give about half a gram of this powder either with milk or honey. This will cure many of the children diseases like indigestion, vomiting, zymotic condition, fever and sudden chillness of limbs.
- Take a handful of the leaves of *Pergularia daemia*, *Phyla nodiflora* (*Poduthalai*), *Morinda corcia* (*Nunaa*) and *Vitex negundo* (*Nochchi*) and extract juice. Giving 10ml of the juice will cure acute infectious disease attended with phlegm.
- Fresh leaves made into pulp are applied to carbuncles and their juice is used in the treatment of catarrhal affections

Sangu (28)

Zoological Name : Turbinella pyrum

English Name : Sacred Chank, Conch shell.

Synonyms

Nandhu, Naagu, Vandu, Sangam, Kodu, Varanam, Devathatham, Kambu, Suthi, Idampuri, Valai, Vellai.

“சங்கினுடபேர்தனையே சாற்றக் கேளு
தவள மாந்திசனாதம் வளையமாடும்
வங்கினிட வலம்புரியாமிடம் புரியுமாகும்
வாருதியி னாதந்தான் சின்னமாகும்
நங்கினிட சங்காகும் பணிலம் பாணி
நலமான சலம்புரியும் பட்சிகம்பு
சங்கினிட நீங்காத வோசை யோனாம்
சலஞ்சலமாஞ் சங்கினிட நாம மாமே.”

- போகர் நிகண்டு – 1200

Thavalam, Thidainatham, Valai, Varuthinatham, Valampuri, Idampuri, Chinnam, Panilam, Salampuri, Patchi, Kambu, Salanjalam, Neengatha vosaiyon.

Occurrence

Conch is available in plenty in sea shores of Indian ocean. Although there are many varieties in conches, *oodhusangu* variety (blowing conch) above is used for medicinal purposes.

Sangu is one among the 5 important substances found in the sea (*Kadalpadu Dhiraviyam*). They are *Sangu* (Conch), *Muthu* (Pearl), *Pavalam* (Coral), *Uppu* (Salt), *Okkolai* (Amber).

“இப்பி ஆயிரஞ் சூழ்ந்தது ஒரு இடம்புரியென்றும்

இடம்புரி ஆயிரஞ் சூழ்ந்தது ஒரு வலம்புரியென்றும்

வலம்புரி ஆயிரஞ் சூழ்ந்தது ஒரு சலஞ்சலம் என்றும்

சலஞ்சலம் ஆயிரஞ் சூழ்ந்தது ஒரு பாஞ்சசன்னியம்”

- போகர் நிகண்டு – 1200

- If the conch (*sangu*) surrounded by 1000 *cippi* (oyster) it is called *idampuri.*
- The conch (*sangu*) surrounded by 1000 *idampuri* is called *valampuri.*
- The conch *(sangu*) surrounded by 1000 *valampuri* is called *panilam.*
- The conch (*sangu*) surrounded by 1000 *panilam* is called *paanch sanniyam*.

Properties and uses

The conch has got body strengthening, deflatulent, appetite stimulant, bitter and mucolytic properties.

General characters

“கசிவா மிரத்த பித்தங் கண்ணோய்க ளேகும்

பசியாறும் வாதம் பறக்கு – மிசிவுடனே

தங்கு முளைவிரணந் தாளகலு மேவெள்ளைச்

சங்கமது வுண்டாயிற்றான்.”

Sangu is useful in the treatment of Hypertension, eye diseases, epilepsy and derangement of *vatham* humor. It stimulates appetite.

Medicinal uses

- ❖ *Sangu Parpam* when given with plant juice of *Tabernaemontana divaricate* (*Nanthiyavattam*) cures ulcer, anaemia, ascites, venereal diseases, piles, gonorrhoea, enlargement of spleen and tuberculosis.
- ❖ *Sangu Parpam* when given with honey cures piles, anaemia, mania and excess saliva.

- *Sangu Parpam* prepared by *Daemia extensa* (*Uthamani*) is used to treat cough, piles, enlarged tonsils, stomach disease, *gunmam*, *vayu* and chest pain.
- *Siddhar Theraiyar* also reiterates that tuberculosis and *kapha* diseases are cured with conch shell.
- There is also a practice to prescribe *Sangu Parpam* with crushed snail in a conch for curing tuberculosis.
- *Sangu Parpam* prepared by *Phyllanthus niruri* (*Keezhanelli*) cures chest pain, dysuria, leucoderma, heart attack and burning sensation in chest.
- *Sangu Chenduram* when given with lemon juice cures white leprosy.
- *Sangu Chenduram* when given with jaggery it treats ulcer associated with diabetes.
- The conch is rubbed with breast milk or with *Murraya koenigii* (*Karivepilai*) and applied over the pimples, acne and boils of the eye.
- *Sangu* is one of the major ingredient in *Vellai mathirai* which is used to treat eye related problems.

Sangu - *Turbinella pyrum* [29]

Zoological Name : *Turbinella pyrum*, Lam.

Commercial Name : Sacred Chank, Conch shell.

Synonyms : Xachus pyrum

Gastropoda

Scientific Classification

Kingdom	:	Animalia
Phylum	:	Mollusca
Class	:	Gastropoda
Order	:	Neogastropoda
Superfamily	:	Muricoidea
Family	:	Turbinellidae
Sub family	:	Turbinellinae
Genus	:	Turbinella
Species	:	*T.pyrum*

Vernacular Name

Tamil	:	*Sanka, Sangu*
Eng	:	Conch; Conch shell
San	:	Shankha
Bengal	:	Sankh

Source

Indian ocean coasts

External appearance

A porcelaneous shell is of an oblong or conical form. The oblong form is bulged in the middle and tapering at each end. The conical variety is peculiar. The upper portion is like cork screw, twisted and tapering at the end. The base is broad. The interior is hollow.

The surface is hard of a dull white colour. The upper surface is highly tuberculate, the under surface is shining, very brittle and translucent, chiefly formed of calcium carbonate.

Male chank measures about 57 – 60 mm in its diameter and female chank about 58 – 60mm. So usually the chanks measuring minimum 64 mm are only picked.

Valampuri Chank

Chanks are characterized by large shells with fine texture and are highly valued. Normally, the chank shells are formed in a dextral spiral.

Occasionally shells with a sinistral spiral are also formed. This peculiar type of chank is called as *"Valampuri chank"*. These have a high value and very rarely caught, almost one in a lakh.

Actions [28]

- Expectorant
- Carminative
- Digestive
- Astringent
- Stomachic
- Febrifuge
- Anodyne

RESEARCH REVIEW

Toxicological studies

[A] Acute toxicity study of *Sangu Parpam*

(i) Swiss strain albino mice of either sex (20- 25 gm) were selected. *Sangu Parpam* was given to the mice in the dose level of 1 gm/kg, 2.5 gm/kg and 5 gm/kg. Mice were observed initially for 4 hrs for behavioral changes, if any. Mice administered above mentioned drugs did not show any abnormal behavior and no mortality was observed during 72 hrs in any experimental group. Hence the drug is safe up to 5000 mg/kg dose in albino mice [(31)]

(ii) Wistar albino rat of both sexes of weight 80 – 120 gms were selected. 30 rats were divided into 6 groups; each group consists of 5 rats. One group is kept as control, by giving water alone. Group II, III, IV, V, VI were administrated with *Sangu Parpam* in the dose level of 100 mg, 200 mg, 400mg, 800mg, 1600mg / animal respectively. It was found that the drug *Sangu Parpam* did not produce any mortality even up to 1600 mg / animal. So, it is inferred that the drug is found to be safe up to 1600 mg / animal in Wistar albino rat [(30)].

[B] Sub chronic toxicity study of *Sangu Parpam*

Charles Foster albino rats of either sex was selected. *Sangu Parpam* was given at the dose level of 15 and 50 mg/kg, nearly 3 and 10 times higher than recommended dose respectively, were administered orally for 30 consecutive days.

Body weight of rats, was monitored periodically on 0, 10^{th}, 20^{th} and 30^{th} day, while feed and water consumption were monitored daily throughout the study. On day 31^{st} blood was collected by cardiac puncture, for performing hematological and biochemical tests. After collection of blood, animals were sacrificed for isolation of liver, stomach and kidney to observe histopathological changes, if any.

The levels of glucose, cholesterol, alkaline phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, blood urea nitrogen, creatinine, total protein and albumin were not altered compared to control rats. Serum electrolyte concentration viz. sodium, potassium, chloride and calcium showed no significant change, when compared to the control rats. The levels of haemoglobin and WBC count were not changed significantly. There was no sign of hemopoetic, hepatic or renal toxicity.

Histopathological observation of control and drug treated rats indicate absence of any gross pathological lesion in liver, stomach and kidney. All animals were found healthy during entire study period and devoid of deleterious effects. Thus it clearly showed the non-toxic nature and high safety profile of *Sangu Parpam* [31]

[C] Chronic toxicity study of *Sangu Parpam*

Wistar albino rat of both sexes of weight 80 – 120 gms were selected. 15 albino rats were divided into 3 groups, each group consisting of 5 rats. Group I - control, Group II and Group III receiving 100mg / 100gm body weight of animal and 200mg / 100gm body weight of the animal respectively. The drug was administered orally for about 90 days. These two doses (100mg and 200 mg) were selected from the acute toxicity study (dose levels were 100 mg, 200 mg, 400mg, 800mg, 1600mg / animal respectively). These doses did not have any acute toxicity effect and presumed to be safe for long term administration in animals.

The drug does not show any mortality in this study, but it produces significant histopathological changes as congested sinusoids in liver, inflammatory infiltration in kidney at the dose of 200mg/ 100gm body weight of animals. But there are no remarkable pathological changes at the dose of 100 mg / 100 gm body weight of animal. Thus the drug produces mild toxic effect on long term administration.

The dose administered for chronic toxicity studies in rats are relatively very high when compared to human dose level. The aim of giving such a high dose is to find out the type of toxicity produced by it.

Moreover, all these changes are warning about the adverse effect of *Sangu Parpam* in long term administration at clinical side. So the prescribed dose to the patients as said in the *siddha* literature is safe and recommended [30].

Pharmacological activities

[A] Acute anti-inflammatory activity

Nine healthy albino rats weighing 100 – 150 gm were taken and divided into 3 groups each consisting of 3 rats. First group was kept as control by giving distilled water of 2ml/100gm body weight. The second group was given Ibuprofen at a dose of 10mg /100gm body weight. The third group received the test drug of 1ml /100gm body weight (25 mg of *Sangu Parpam* was dissolved in 10ml of honey and 10ml of water). Before administration of test drug, the hind-paw volume of all rats were measured. This was done by dipping the hind-paw up to the tibio–tarsal junction in a Mercury Plethysmograph. The method is more suitable for studying the anti-inflammatory activity in acute inflammation. *Sangu Parpam* possesses 38.3% acute anti-inflammatory effect in albino rats [(32)].

[B] Chronic anti-inflammatory activity by cotton pellet granuloma method

Nine healthy albino rats were taken which weighs about 100-150 gm and divided into three groups each consisting of 3 rats. First group was kept as control by giving distilled water of 2ml/100gm body weight. To the second group, the standard drug (Ibuprofen) of 20mg/100gm body weight and to the third group, the test drug *Sangu Parpam* 1ml/100gm of body weight (25 mg of *Sangu Parpam* was dissolved in 10ml of honey and 10ml of water) was given. After 7 days of drug administration, the animals were anaesthetized The weight of cotton pellets in each rat was weighed. From this value, the chronic anti–inflammatory action was calculated and compared. This method is more suitable for studying chronic anti– inflammatory action. *Sangu Parpam* possesses 27.8 % chronic Anti-inflammatory effect in rats [(32)].

[C] Analgesic activity

Three groups of rats on either sex were selected and divided into three groups consisting of three rats each. First group was given 2ml of water and kept as control. Second group was administered with pethedine (1mg/ kg body weight) intra-peritoneally. The test drug *Sangu Parpam* at the dose of 1ml/100gm body wt (25 mg of *Sangu Parpam* was dissolved in 10ml of honey and 10ml of water), was administered to the third group. The test drug *Sangu Parpam* has got mild Analgesic action [(32)].

[D] Anti-microbial activity

Sangu Parpam has mild inhibitory action against E.Coli, Klebsiella, Proteus, Pseudomonas and Staphylococci in 30 mg/10 ml, 40mg/10 ml and 50 mg/10 ml concentrations [30].

[E] Anti-ulcer activity

Male wistar rats of weight (175± 5gm) were selected. Animals of control group received saline (5 ml/kg) and test groups received *Sangu Parpam* (25 mg/kg and 50 mg/kg) for 6 days. From day 6, the animals received saline or test drug, 2 hrs prior to the administration of indomethacin (20 mg/kg, orally). Overnight fasted animals were sacrificed by cervical dislocation 3 hrs after the last dose of ulcerogen. The stomach was incised along the greater curvature and examined for ulcers. The response of the *Parpam* on ulcer index, lipid peroxidation (thiobarbituric acid reacting substances TBARS) in gastric tissue and serum calcium was determined. *Sangu Parpam* caused significant reduction in ulcer index ($P< 0.001$) in both the indomethacin and cold restraint models [33].

Test for Conch [30]

- Take well water in a vessel.
- Immerse Conch in it for 5 days.
- After 5 days if the colour of the Conch remains same it is original.
- If the conch turns into black colour and crack appears on it, that conch is not original.

Medicinal uses [34]

- ❖ Conch shell is used in treating dyspepsia, digestion impairment, malabsorption syndrome hepatomegaly.
- ❖ Wrinkles on skin can be reduced by rubbing with a Conch on face and neck after bath. Glow of skin will increase naturally.
- ❖ Dark Circles under eyes can be cured by gently rubbing with Conch for 5 minutes per day before sleep.
- ❖ Store some water in a conch overnight and next morning massage your skin with this water. This cures many skin diseases, rashes, allergies etc.

- *Sangu Parpam* is used for treating diarrhoea (loose stools), acne, pimples, liver enlargement (hepatomegaly), splenomegaly, abdominal pain, indigestion, loss of appetite, heartburn, acid reflux, abdominal distension and irritable bowel syndrome.
- *Sangu Parpam* is used for ear ache, ulcer and eye troubles and is indicated internally in case of dyspepsia, gonorrhoea, colic dysentery, jaundice, tympanitis and flatulence.
- It is given for shooting pain and inflammatory condition in the joints.

Naagam [28]

As the metal gives hissing noise like snake while melting, it is called in the name of Cobra – *Naagam*.

Synonyms

Seeral, Porumal, Pongal, Iraichal, Aimbugai, Sorum, Vasuki, Vennagam, Thaambirathin vedhai, Vathathiriku uyir.

Source

- Zinc is available in nature mixed with other substances. After separation, it gains shiny, whitish blue in colour in the form of solid.
- Zinc can be made as a rod or as thin sheets. It dissolves in acid.
- When it melts with copper, it converts into brass.

Antagonistic substances to zinc

Egg (*Andam*), opium (*Papaver somniferum*), ferrous sulphate (*Annabedhi*), asphalt (*Kalluppu*), sea shell, plumbi oxidum (*Singi*), gold, tin (*Thara*), crab shell, pepper (*Piper nigrum*), glass gale (*Valayaluppu*), mercuric chloride (*Veeram*), potassium nitrate (*Vediyuppu*), borax (*Vengaaram*), silver and arsenic tri- oxide.

Agonist substances to zinc

Mica (*Appiragam*), iron, sulphur (*Gandhi*), magnet (*Kaantham*), lead (*Kaareeyam*), arsenic pentasulphide (*Gowri*), ammonium chloride (*Navacharam*), bitumen (*Sila saththu*), mercury (*Sootham*), copper and detoxified copper oxide (*Mayura cembu*).

Actions

- Astringent
- Coagulant
- Tonic
- It controls hemorrhage.

General characters

மேகங் கிளர்பேதி வெட்டையழலைத் தணிக்கும்

வேகங் கிராணி விலங்குங்காண் - போகாப்

பரியமுளைப் புண்ணைப் பயித்தியத்தைப் போக்கும்

அரியதுத்த நாக மது.

Zinc is useful in the treatment of Venereal disease (*Megam*), Diarrhoea, Chronic ulcers, Mental disorders, Leucorrhoea (*Vellai*), Sense of heat (*Utsoodu*)

"சொல்லி முடியாது துத்த நா கம்பொடியாய்

மெல்லத் துரத்தும் வியாதிகளை – நல்ல

உரனுடைரம யுண்டாக்கும் உண்டவரை யென்று

கிருமிமனை மண்மகிமை கேள்."

The zinc generally controls various diseases gradually and gives strength to the body.

Medicinal uses

- *Naaga Parpam* is taken with the oil of *Madhuca longifolia* (*Ilupai nei*) and hot water for intermittent fever, nausea, loss of appetite, abdominal pain, diarrhoea, tumor below the knee joint, septic ulcers and severe blood discharge with itching.
- The zinc powder should be used for two months to obtain good result. It is also used for fissures of the tongue.
- It has a specific control over epilepsy, cholera and other spasmodic diseases as whooping cough, asthma, hysteria, dipsomania etc.
- It is a good remedy to check profuse sweating.
- For its astringent property it is given in bronchorrhea and in colliquative sweats of phthisis.
- *Naaga Parpam* prepared by castor leaf juice (*Ricinus communis*) is useful in the treatment of gastric ulcer and hemorrhoids. It improves spermatogenesis.
- *Naaga Parpam* is also used for the treatment of *kapha*, gonorrhoea (*Vettai*), leucorrhoea (*Vellai*) abdominal tumors and hepatomegaly.

- *Naaga Chendooram* when given in cow's butter twice a day for 21 days, cures hemorrhoids, ano rectal diseases, venereal diseases and bilious disorders.
- In modern medicine also zinc is considered as an essential trace element and an essential component of a large number of enzymes. It has many therapeutic applications in the treatment of various external conditions of the body as a precipitating germicide.

ZINC [29]

Zinc is a chemical element with symbol Zn and atomic number 30. It is the first element in group 12 of the periodic table. It is an essential element needed by our body and is commonly found in nutritional supplements. However, taking too much zinc into the body can affect our health. The levels of zinc that produce adverse health effects are much higher than the Recommended Dietary Allowances (RDAs) for zinc of 11 mg/day for men and 8 mg/day for women.

Vernacular name

English	:	Spelter or impure commercial zinc; zinc –ore.
Tamil	:	*Tutanagam*
Hindi	:	Jasta
Malayalam	:	*Nagam*

Occurrence

Zinc found in small amounts in soil, water, living organisms and is present in all foods. Zinc is also found in ores of the Earth's crust.

The element is normally found in association with other base metals such as copper and lead in ores. In its pure elemental (or metallic) form, zinc is a bluish-white, shiny metal.

Physical properties

- ✓ Zinc is a bluish-white, lustrous, diamagnetic metal, though most common commercial grades of the metal have a dull finish.
- ✓ The metal is hard and brittle at most temperatures but becomes malleable between 100 and 150 °C.
- ✓ Above 210 °C, the metal becomes brittle again and can be pulverized by beating.
- ✓ Zinc is a fair conductor of electricity.
- ✓ For a metal, zinc has relatively low melting (419.5 °C) and boiling points (907 °C).
- ✓ Metallic Zinc on cooling becomes brittle ad may then be reduced to powder.
- ✓ Pure Zinc becomes tarnished by exposure to air.
- ✓ When melted with copper it forms an alloy known as Brass.

Chemical properties

Atomic number	30
Atomic mass	65.37 g.mol $^{-1}$
Electronegativity according to Pauling	1.6
Density	7.11 g.cm^{-3} at 20°C
Melting point	420 °C

- ✓ Zinc has an electron configuration of [Ar]3d^{10}4s^{2} and is a member of the group 12 of the periodic table.
- ✓ It is a moderately reactive metal and strong reducing agent.
- ✓ Zinc burns in air with a bright bluish-green flame, giving off fumes of zinc oxide.
- ✓ Zinc reacts readily with acids, alkalis and other non-metals.

Recommended Dose level (35)

S. No	Age	Dose
1	1 – 8	2 mg/day
2	7 months to 3 years	3 mg/day
3	4 to 8 years	5 mg/day
4	9 to 13 years	8 mg/day
5	14 to 18 years (girls)	9 mg/day
6	14 and older (boys)	11 mg/day
7	Women 19 and older	8 mg/day
8	Pregnant women 14 to 18	13 mg/day
9	Lactating women 14 to 18	14 mg/day
10	Pregnant women 19 and older	11 mg/day
11	Lactating women 19 and older	12 mg/day

If large doses of zinc (10–15 times higher than the RDA) are taken by mouth even for a short time, stomach cramps, nausea and vomiting may occur. Ingesting high levels of zinc for several months may cause anemia, damage the pancreas and decrease levels of high-density lipoprotein (HDL) cholesterol. Without enough zinc in the diet, people may experience loss of appetite, decreased sense of taste and smell, decreased immune function, slow wound healing, and skin sores. Too little zinc in the diet may also cause poorly developed sex organs and retarded growth in young men. If a pregnant woman does not get enough zinc, her babies may have birth defects [(44)].

Zinc rich foods

Zinc is present in a wide variety of foods, particularly in association with protein foods. A vegetarian diet often contains less zinc than a meat based diet and so it is important for vegetarians to eat plenty of foods that are rich in this vital mineral.

- ✓ Meat, fish, shellfish, fowl, eggs, dairy, wheat and various seeds such as sesame, poppy, alfalfa, celery, mustard contain zinc.
- ✓ Zinc is also found in beans, nuts, almonds, whole grains, sunflower seeds and blackcurrant.
- ✓ Pumpkin seeds provide one of the most concentrated vegetarian food sources of zinc.

RESEARCH REVIEW

Toxicological studies

[A] In vitro cytotoxicity studies of *Naga Parpam*

The L929 fibroblast cells were seeded in 24 well plates at a density of 5 x [10.sup.5] cells/well, cultured for 24 h in incubator at 37[degrees]C under 5% C[O.sub.2]. The medium was replaced with *Naga Parpam* particle suspension in the medium at a concentration of 5 mg/ml/ well and incubated for 20h. Medium alone was used as control. The particles were removed and 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was done. The results revealed that *Naga Parpam* is slightly toxic. As compared to control (medium) *Naga Parpam* exhibited 75 [+ or -] 5% cell viability [(37)].

[B] Histopathological studies of the effect of *Naga Parpam*, a zinc based drug of *siddha* medicine, in rats

Male albino rats of wistar strain (180-200gm) were selected. The animals were divided into five groups each containing 6 rats. Group I served as control and to the other four groups, the drug *Naga Parpam* was orally administered in doses of 5, 10, 20 and 40 mg/kg body wt, as a suspension in 1% carboxymethyl cellulose by intubation for 15 consecutive days. To find the effect of chronic administration on various tissues, the drug was also administered to two other sets of animals in the same manner respectively for 30 and 60 days.

The results revealed that in 15 days treatment, liver and kidney had normal histology with no marked changes in the tissue architecture. Liver and kidney were selected for evaluation as they are sensitive to metal toxicity. In 30 days study, the kidney tissues remained normal while the liver tissues exhibited a few apoptotic cells with mild focal and lobular inflammation. On 60^{th} day treatment, liver showed mild lobular inflammation and apoptotic cells were found in all doses. However brain, kidney and testis remained normal[(38)].

[C] Safety and pharmacological profile of *Naga Chenduram*

(i) Acute toxicity study

Female wistar rats weighing 150-200gm were selected and divided into 4 groups containing three animals in each group. The single dose of *Naga Chenduram* starting from 5mg/kg, 50mg/kg, 300mg/kg and 2000mg/kg was administered orally. The drug treated animals were carefully observed individually for the toxicity signs and mortality. The result revealed no abnormal signs and behavioral changes in rats at the dose of 5, 50, 300, 2000mg/kg body wt administered orally and in Gross necropsy no abnormalities seen in external observation and examination on the dose level of 5, 50, 300, 2000mg/kg body weight. All vital organs were normal [(39)].

(ii) Sub chronic toxicity study of *Naga Chenduram*

24 Wistar Albino Rats (12M + 12F) were selected and divided into 4 groups. Each group consist of 6 animals (3 Male and Female 3). First group treated as a control and other three groups were treated with test drug of dose 90mg/kg, 450mg/kg, 900mg/kg for 90 days. The results revealed that there was no significant change in the body weight and all animals

from control and all the treated dose groups survived throughout the dosing period of 90 days. The results of hematological and biochemical investigations conducted on day 91th revealed no significant changes values when compared with those of respective controls. The vital organs were removed from the test groups at the end of the study and carefully observed macroscopically to find any observable gross lesions compared with the control group and did not reveal any abnormal macroscopic changes [39].

Pharmacological activities

[A] Clinical, immunological, anti-inflammatory and antioxidant roles of zinc

Oxidative stress and chronic inflammation are important contributing factors for several chronic diseases attributed to aging, such as atherosclerosis and related cardiac disorders, cancer, neurodegeneration, immunologic disorders and the aging process itself. Zinc is very effective in decreasing reactive oxygen species (ROS).

In order to understand the mechanism of zinc effect on cell-mediated immunity, we utilized RT-PCR analysis to determine PHA (phytohemagglutinins) induced expression of IL-2 mRNA in isolated MNCs in elderly subjects before and after zinc supplementation.

Zinc deficiency not only affects adversely the thymulin (a thymic hormone) activity but also decreases the generation of IL-2 and IFN-γ from Th1 cells. Zinc deficiency also decreases IL-12 generation from macrophages. IL-12 and IFN-γ are required for optimal phagocytic activity of macrophages against parasites, viruses and bacteria.

Since zinc supplementation to younger subjects decreased the generation of inflammatory cytokines and decreased oxidative stress markers, we hypothesized that zinc supplementation would not only increase the generation of IL-2 in MNCs but also decrease the generation of inflammatory cytokines and decrease oxidative stress in the elderly. This study in the elderly showed that zinc supplementation decreased the incidence of infections significantly.

Thus in this experimental human zinc deficiency model, it showed that zinc supplementation improved Th1 cells cytokines production, decreased generation of inflammatory cytokines, and decreased oxidative stress [40].

[B] Beneficial effect of nanoparticles over standard form of zinc oxide in enhancing the anti-inflammatory activity of ketoprofen in rats

Zinc is known as an anti-inflammatory agent. Recent studies demonstrated the usage of zinc ions for enhancing the anti-inflammatory effect of Non-Steroidal Anti-inflammatory Drugs (NSAIDs). This study compares the influence of chronic administration of zinc oxide nanoparticles (ZnO-NPs) and zinc oxide standard form (ZnO-S) on the anti-inflammatory and gastric activity of ketoprofen in rats. Both ZnO-S and ZnO-NPs were administered at doses of 7 or 14mg/kg (intraperitoneally (ip) and per os (po)) for 2 weeks followed by single po ketoprofen administration (in three doses: 5, 10, and 20mg/kg).

The results revealed that the ZnO-NPs (but not ZnO-S) at a dose of 14mg/kg ip reduced the carrageenan-induced paw edema at the second hour after carrageenan administration and enhanced the ketoprofen anti-inflammatory activity at second and third hour after carrageenan administration. A dose of 7mg/kg of both forms administered ip was ineffective in these measures. ZnO-NPs and ZnO-S administered po did not affect the carrageenan-induced paw edema or ketoprofen anti-inflammatory activity. ZnO-S and ZnO-NPs administered po and ip at both doses (7 and 14mg/kg) protected the gastric mucosa from ketoprofen-induced gastric ulcer.

Thus Zinc oxide nanoparticles demonstrated beneficial effects over standard form in enhancing the anti-inflammatory activity of ketoprofen [(41)].

[C] Analgesic, anti-inflammatory and ulcerogenic activity of a zinc – naproxen complex in mice and rats

Naproxen, a non-steroidal anti-inflammatory drug (NSAID), was complexed with zinc (II) metal. Tests were performed to determine the analgesic, anti-inflammatory and ulcerogenic effects of zinc-naproxen compared with naproxen.

Compared with naproxen, on a molar equivalent basis, the zinc-naproxen complex was found to have greater analgesic activity (acetic acid-induced abdominal constriction and tail-flick tests in mice) and comparable anti-inflammatory activity (rat paw oedema). The zinc-naproxen complex was also less ulcerogenic than naproxen in a chronic gastric injury model. Thus the complexation of naproxen with zinc markedly reduces its ulcerogenic effect with better analgesic and comparable anti-inflammatory effects [(42)].

Other Pharmacological activities

- Antioxidant [43]
- Antidepressant [44]
- Anti-inflammatory [40]
- Anti hyperlipidemic [45]
- Anti-bacterial [46]

Test for zinc

Cobalt cyanide paper (Rinmann's test for Zn) can be used as a chemical indicator for zinc. 4 g of $K_3Co(CN)_6$ and 1 g of $KClO_3$ is dissolved on 100 ml of water.

Paper is dipped in the solution and dried at 100 °C. One drop of the sample is dropped on the dry paper and heated. A green disc indicates the presence of zinc.

Medicinal uses

- Zinc speed up the healing process after an injury.
- Zinc is an essential mineral, including to prenatal and postnatal development
- It is used for boosting the immune system, treating the common cold and recurrent ear infections and preventing lower respiratory infections.
- It is used for malaria and other diseases caused by parasites.
- Some people use zinc for an eye disease called macular degeneration, for night blindness and for cataracts. It is used to treat skin conditions such as psoriasis, eczema, and acne.
- Some people use zinc for benign prostatic hyperplasia (BPH), male infertility, erectile dysfunction(ED), weak bones (osteoporosis), rheumatoid arthritis and muscle cramps associated with liver disease.
- Apart from other medicinal uses, zinc compounds are used by the drug industry as ingredients in some common products, such as vitamin supplements, sun blocks, diaper rash ointments, deodorants, athlete's foot preparations, acne and poison ivy preparations and antidandruff shampoos.

Parpam [47, 48]

Parpam is equivalent to calyx, which is prepared by a process of calcination. The term *parpam* is apparanently a Tamilazed form of the Sanskrit word *Bhasma*. *Parpam* has held the ground in *Siddha* medicine.

Classification of *Parpam*

- Metal-based *Parpam*
- Mineral-based *Parpam*
- Herbal *Parpam*

Steps used to prepare *Parpam*

The following processes are involved

- Elimination of harmful matter from the drug
- Modification of undesirable physical properties of the drug
- Conversion of some of the characteristics of the drug to different stages
- Enhancement of the therapeutic action.

Parpam - nano particle

Animal derivatives such as horns, shells, feathers, metallic and nonmetallic minerals are normally administered as *Parpam*. *Parpam* means an ash obtained through incineration. The starter material undergoes an elaborate process of purification followed by the reaction phase, which involves incorporation of some other mineral and herbal extracts. Then the material in pellet form is incinerated in a furnace.

For the complete transformation of the material into the *Parpam* state, the process of grinding, drying and calcification may have to be repeated several times or at least as many times as directed in the recipe. However, the calcination is repeated until a satisfactory product is obtained. But in those instances where the number of calcinations is definitely indicated, the

process should be repeated accordingly, even if a satisfactory *parpam* is obtained within a few calcinations.

While preparing the *parpams* of Lead, Tin and Zinc, the number of dung cakes used as fuel, should always be comparatively lesser than the number used for other metals, because, excessive heating will result in the reversion of the *parpam* to the metallic state.

Physical characters

Color

A specific color is mentioned for each *Parpam*. They are generally white and pale. The color of the preparation primarily depends on the parent material.

Lusterless

Parpam must be lusterless before therapeutic application. For this test, *Parpam* is observed under bright sunlight whether luster is present are not, if luster is still present, it indicates further incineration.

Lightness and Fineness

Parpam floats on stagnant water surface. This test is based on law of surface tension. Properly incinerated *Parpam* need to float on water surface.

Tactile sensation

Tactile sensation can be absorbed and assimilated in the body without producing any irritation to mucous membrane of gastrointestinal tract.

Particle size

Prepared *Parpam* should be in powder form. Particle of *Parpam* should be like pollen grains of *Pondanus odoratissimus* flower.

Physiologically, the particle fineness is of great importance. Most compounds of metals and minerals are not absorbed by the body from the digestive tract, because under ordinary circumstances, these substances could not be reacted upon by the secretions of the digestive

system, so as to render them absorbable by the organism. This difficulty is overcome when the individual particles of these compounds are very minute. This concurrently has a say in the matter of dosage in that the dose could be reduced to a great degree as a major part of the finely particulate drug is absorbed into the system.

Quality control of *Parpam*

Traditionally, the end points of incineration of a metal and its conversion to a *Parpam state* are evaluated based on the following criteria.

- When a *Parpam* is spread between the index finger and thumb and rubbed, it should be so fine as to get easily into the lines and crevices of the fingers and should not be washed out from the lines of the fingers
- When a small quantity is spread on cold and still water, it should float on the surface
- The *Parpam* should not revert to the original state
- *Parpam* should be tasteless
- The *Parpam* should not produce nausea when administration.
- The *Parpam* if satisfactory completed, is irreversible to its metallic waste when heated with a mixture of cane jaggery, hemp powder, ghee and honey.

Importance of *Parpam*

- Maintain optimum alkalinity for optimum health
- Provide easily absorbed and usable calcium
- Cleanse the kidneys, intestines and liver
- Maintain stronger bones and healthier teeth
- Alleviate insomnia, depression
- Keeps rhythmic heart beating
- Keeps arrhythmias and minerals balance
- Help metabolize iron in body
- Aid nervous system
- Breakdown heavy metals and drug residues in body
- Neutralize harmful acids that lead to illness
- Achieve a healthy alkaline level by neutralizing acid
- Protect body from free radical damage.

Storage of *Parpam*

- ✓ *Parpams* are usually stored in glass bottles.
- ✓ For smaller packing, vials of glass may be employed.
- ✓ It is highly desirable that these preparations be stored and retained in relevantly labelled containers.
- ✓ They are said to retain their potency for 100 years, if properly stored.

Analytical study of the drug *Naaga Sangu Parpam* brings the validation to be used as a medicine by subjecting the drug to many analysis and determining its quality and effectiveness. Analytical study includes many studies such as its organoleptic properties, physicochemical properties, phytochemical properties and also to assess the active principles and elements present in the drug. Thus analytical study brings the efficacy and potency of the drug.

As per AYUSH protocol, the following analytical parameters were evaluated for *Naaga Sangu Parpam*.

1. **Organoleptic characters**
2. **Phytochemical analysis**
3. **Physicochemical analysis**
4. **Chemical analysis**
5. **Heavy metal analysis**
6. **Scanning Electron Microscopy (SEM) with Energy Dispersive X-ray analysis (EDAX)**
7. **Fourier transform infrared (FTIR)**
8. **X- ray Diffraction**
9. **Thermogravimetric analysis (TGA)**

The organoleptic characters of the *Naaga Sangu Parpam* were evaluated. 1gm of the test drug was taken and the following characters were seen.

- Colour
- Odour
- Texture
- Taste

Other morphology characters were viewed by naked eye under sunlight. Then the results were noted.

Colour

The medicine was taken into watch glasses and placed against white back ground in white tube light. It was observed for its colour by naked eye.

Odour

The medicine was smelled individually. The time interval among two smelling was kept 2 minutes to nullify the effect of previous smelling.

Taste

Small amount of *Naaga Sangu Parpam* was kept over the tip of the tongue.

Results

The results of Organoleptic characters were showed in Table - 1

The preliminary phytochemical screening test was carried out for each extracts of *Naaga Sangu Parpam* as per the standard procedure. The Experimental Procedure was done at The T.N. Dr. M.G.R Medical university, Guindy, Chennai-32.

[1] Detection of Alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

i) Mayer's Test

- Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide).
- Formation of a yellow colored precipitate indicates the presence of alkaloids.

ii) Wagner's Test

- Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide).
- Formation of brown / reddish precipitate indicates the presence of alkaloids.

iii) Dragendroff's Test

- Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide).
- Formation of red precipitate indicates the presence of alkaloids.

iv) Hager's Test

- Filtrates were treated with Hager's reagent (saturated picric acid solution).
- Presence of alkaloids confirmed by the formation of yellow colored precipitate.

[2] Detection of Carbohydrates

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

i) Molisch's Test

- To 2 ml of sample extract, two drops of alcoholic solution of α- naphthol are added.
- The mixture is shaken well and few drops of concentrated sulphuric acid is added slowly along the sides of test tube.
- A violet ring indicates the presence of carbohydrates.

ii) Benedict's Test

- Filtrates were treated with Benedict's reagent and heated gently.
- Orange red precipitate indicates the presence of reducing sugars.

[3] Detection of Glycosides

Extracts were hydrolyzed with dil. HCl, and then subjected to test for glycosides.

i) Modified Borntrager's Test

- Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes.
- The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution.
- Formation of rose-pink color in the ammonical layer indicates the presence of anthranol glycosides.

ii) Cardiac glycoside (Keller-Killiani test)

- Extract was shaken with distilled water (5 mL). To this, glacial acetic acid (2 mL) containing a few drops of ferric chloride was added, followed byH2SO4 (1 mL) along the side of the test tube.
- The formation of brown ring at the interface gives positive indication for cardiac glycoside and a violet ring may appear below the brown ring

[4] Detection of Saponins

i) Froth Test

- Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes.
- Formation of 1 cm layer of foam indicates the presence of saponins.

ii) Foam Test

- 0.5 gm of extract was shaken with 2 ml of water.
- If foam produced persists for ten minutes it indicates the presence of saponins.

[5] Detection of Phytosterols

Salkowski's Test

- Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand.
- Appearance of golden yellow color indicates the presence of triterpenes.

[6] Detection of Phenols

Ferric Chloride Test

- Extracts were treated with 3-4 drops of ferric chloride solution.
- Formation of bluish black color indicates the presence of phenols.

[7] Detection of Tannins

Gelatin test

- The extract was dissolved in 5 ml of distilled water and 2 ml of 1% solution of Gelatin containing 10% NaCl was added to it.
- White precipitate indicates the presence of phenolic compounds.

[8] Detection of Flavinoids

i) Alkaline Reagent Test

- Extracts were treated with few drops of sodium hydroxide solution.
- Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

ii) Lead acetate Test

- Extracts were treated with few drops of lead acetate solution.
- Formation of yellow color precipitate indicates the presence of flavonoids.

[9] Detection of Proteins and Aminoacids

i) Xanthoproteic Test

- The extracts were treated with few drops of conc. Nitric acid.
- Formation of yellow color indicates the presence of proteins.

ii) Ninhydrin Test

- To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes.
- Formation of blue color indicates the presence of amino acid.

[10] Detection of Diterpens

Copper Acetate Test

- Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution.
- Formation of emerald green color indicates the presence of diterpenes

[11] Gum and Mucilage

- To 1ml of extract add 2.5ml of absolute alcohol and stirring constantly.
- Then the precipitate was dried in air and examine for its swelling properties.
- Swelling was observed that will indicate presence of gum and mucilage.

[12] Test for Fixed oils and Fats

Spot test

- A small quantity of extract was pressed between two filter papers.
- Oil stain on the paper indicates the presence of fixed oils.

[13] Test for Quinones

Extracts were treated with sodium hydroxide; blue or red precipitate indicates the presence of Quinones.

The results of phytochemical analysis were presented in Table No. 2

Physicochemical studies of the trial drug have been done according to the WHO guidelines. The physical properties of *Naaga Sangu Parpam* was analyzed at The T.N. Dr. M.G.R Medical university, Guindy, Chennai-32.

Physicochemical studies of the drugs are necessary for standardization, as it helps in understanding the significance of physical and chemical properties of the substance being analyzed in terms of their observed activities and especially for the determination of their purity and quality. The analysis includes the determination of ash value, Loss on drying at 105ºC, pH value, Extractive value. These were carried out as per guidelines [(49)].

1. Determination of pH:

Five grams of *Naaga Sangu Parpam* was weighed accurately and placed in clear 100 ml beaker. Then 50 ml of distilled water was added to it and dissolved well. After 30 minutes it was then applied in to pH meter at standard buffer solution of 4.0, 7.0, and 9.2. The test was repeated for four times and average was recorded.

2. Loss on drying of the sample at 105ºC

4g of test drug was weighed in a previously weighed 100ml beaker and heated in an oven at 105ºC for 5hours. Cooled in a desiccator and weighed. The procedure was repeated till constant weight was obtained. The percentage of loss in weight of the test drug was calculated by the following formula.

Calculation:

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

3. Ash content

Total ash content

4g of test drug was weighed accurately in a previously ignited and tared silica dish. The material was evenly spread and ignited in a muffle furnace at 600^{0}C until it became white indicating the absence of carbon. The dish was cooled in a desiccator and weighed. As carbon

free ash cannot be obtained in this manner, the dish was cooled and the residue moistened with 2 sufficient quantity of water. Dried on a water bath and then ignited in the electric furnace to get the constant weight. The dish was cooled in a desiccator and then weighed. The percentage of total ash of air-dried materials was calculated as per the formula given below.

Calculation:

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

4. Acid-insoluble ash

The total ash of the test drug was found out as described above. To the dish containing the total ash was added 45 ml of 1: 5 hydrochloric acid in three portions of 13 ml each time. Boiled gently for 5 minutes and filtered. The insoluble matter was collected on an ashless filter paper (Whatman No.41) and washed with distilled water until the residue was free from acid. Transfer the filter paper containing the insoluble mater to the original dish. Dried and ignited to the constant weight. The dish was cooled in a desiccator and then weighed. Calculation was made by given formula.

Calculation:

$$\text{Percentage of acid-insoluble ash} = \frac{\text{Weight of the acid-insoluble residue}}{\text{Weight of test drug taken}} \times 100$$

5. Water soluble ash

Total ash 1g was boiled for 5min with 25ml water and insoluble matter collected on an ash less filter paper was washed with hot water and ignited for 15 min at a temperature not exceeding 450^0C in a muffle furnace. The amount of soluble ash was determined by drying the filtrate.

6.Water-soluble extractive of the test drug

4 g of the test drug was weighed accurately in a glass stoppered flask. 100 ml of distilled water and shacked occasionally for 6 hours and then allowed to stand for 18 hours. Filtered rapidly taking care not to lose any solvent and pipetted out 25 ml of the filtrate in a pre-weighed 100 ml beaker and evaporated to dryness on a water bath. Kept in an air oven at 105ºC for 6

hours. Desiccator was cooled and weighed. The experiment was repeated twice and the average value was noted. The percentage of water soluble extractive was calculated by the formula given below.

Calculation:

$$\text{Percentage of water soluble extract} = \frac{\text{Weight of the extract}}{\text{Weight of sample taken}} \times \frac{100}{25} \times 100$$

7. Alcohol-soluble extractive of the sample

4 g of the sample was weighed accurately in a glass stoppered flask. 100 ml of distilled alcohol (approximately 95%) was added and shaken occasionally for 6 hours and then allowed to stand for 18 hours. Filtered rapidly taking care not to lose any solvent and pipetted out 25 ml of the filtrate in a pre-weighed 100 ml beaker and evaporated to dryness on a water bath. Kept in an air oven at 105ºC for 6 hours and cooled in a desiccator and weighed. The experiment was repeated twice and the average value was noted. The percentage of alcohol soluble extractive was calculated by the formula given below.

Calculation:

$$\text{Percentage of alcohol soluble extract} = \frac{\text{Weight of the extract}}{\text{Weight of sample taken}} \times \frac{100}{25} \times 100$$

Results

The results of physicochemical properties were represented in Table No. 3

The chemical analysis of *Naaga Sangu Parpam* was carried out in Biochemistry Lab, National Institute of Siddha.

S. No	Experiment	Obsevation	Inference
1.	Physical Appearance of extract	Grey in colour	
2.	**Test for Silicate** a. A small amount of the sample was shaken well with distilled water. b. A small amount of the sample was shaken well with Conc. HCL/ Con. H_2SO_4	Sparingly soluble	**Presence of silicate**
3.	**Action of Heat** A small amount of the sample was taken in a dry test tube and heated gently at first and then strong.	No White fumes evolved. No brown fumes evolved.	Absence of Carbonate Absence of Nitrate.
4.	**Flame Test** A small amount of the sample was made into a paste with con. HCl in a watch glass and introduced into non-luminous part of the Bunsen flame.	No bluish green flame appeared	Absence of copper
5.	**Ash Test** A filter paper was soaked in a mixture of extract and dil. cobalt nitrate solution and introduced into the Bunsen flame and ignited.	No yellow colour flame appeared	Absence of sodium

Preparation of Extract:

5 gm of *Naaga Sangu Parpam* was taken in a 250 ml 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 100 ml volumetric flask and made up to 100 ml with distilled water. This preparation was used for the qualitative analysis of acidic/basic radicals and chemical constituents in it.

S. NO	Experiment	Observation	Inference
	I. Test For Acid Radicals		
1.	**Test for Sulphate** a.2 ml of the above prepared extract was taken in a test tube to this added 2 ml of 4% ammonium oxalate solution b. 2 ml of the above prepared extract was added with 2 ml of diluted HCL, until the effervescence ceases off. Then 2 ml of Barium Chloride solution was added.	Cloudy appearance present White precipitate insoluble in con. HCL was obtained.	**Presence of Sulphate** **Presence of Sulphate was confirmed**
2.	**Test for Chloride** 2 ml of the above prepared extracts was added with 2 ml of dil- HNO_3 till the effervescence ceases. Then 2ml of silver nitrate was added.	No Cloudy appearance present.	Absence of Chloride
3.	**Test for Phosphate** 2 ml of the extract was treated with 2 ml of ammonium molybdate solution and 2 ml of con.HNo3	No Cloudy yellow appearance formed	Absence of Phosphate

4.	**Test for Carbonate** 2 ml of the extract was treated with 2 ml diluted magnesium sulphate solution	Cloudy yellow appearance present	**Presence of carbonate**
5.	**Test for Nitrate** 1 gm of the substance was heated with copper turning and concentrated H_2So_4 and viewed the test tube vertically down.	No Brown gas was evolved	Absence of nitrate
6.	**Test for Sulphide** 1 gm of the substance was treated with 2 ml of con. HCL	Rotten egg smelling gas evolved	**Presence of sulphide**
7.	**Test for Fluoride & Oxalate** 2 ml of extract was added with 2 ml of dil. Acetic acid and 2 ml calcium chloride solution and heated**.**	No cloudy appearance.	Absence of fluoride and oxalate
8.	**Test for Nitrite** 3 drops of the extract was placed on a filter paper, on that-2 drops of acetic acid and 2 drops of Benzidine solution were placed.	No characteristic changes	Absence of nitrite
9.	**Test for Borate** 2 Pinches (50mg) of the extract was made into paste by using sulphuric acid and alcohol (95%) and introduced into the blue flame.	Bluish green colour flame not appeared	Absence of borate

S.No	Experiment	Observation	Inference
II. Test For Basic Radicals			
1.	**Test for Lead** 2ml of the extract was added with 2ml of potassium iodine solution.	No Yellow precipitate appeared	Absence of lead
2.	**Test for Copper** a. One pinch (25 mg) of extract was made into paste with con. HCl in a watch glass and introduced into the non-luminous part of the flame. b. 2ml of extract was added with excess of ammonia solution.	No blue colour flame appeared No blue colour precipitate appeared	Absence of copper Absence of copper
3.	**Test for Aluminium** To the 2 ml of extract sodium hydroxide was added in drops to excess.	No characteristic changes	Absence of Aluminium.
4.	**Test for Iron** a. To the 2 ml of extract add 2 ml of ammonium thiocyanate solution. b. To the 2ml of extract 2ml ammonium thiocyanate solution and 2ml of con HNO3 were added	Mild Red colour appeared. Blood Red colour appeared	Presence of Iron
5.	**Test for Zinc** To 2 ml of the extract sodium hydroxide solution was added in 5 drops to excess and ammonium chloride was added.	No White precipitate was formed	Absence of Zinc

6.	**Test for Calcium** 2 ml of the extract was added with 2 ml of 4% ammonium oxalate solution	Cloudy appearance and white precipitate was obtained	Presence of calcium
7.	**Test for Magnesium** To 2 ml of extract sodium hydroxide solution was added in drops to excess.	No White precipitate was obtained	Absence of magnesium
8.	**Test for Ammonium** To 2 ml of extract 1 ml of Nessler's reagent and excess of sodium hydroxide solution were added.	No Brown colour appeared	Absence of ammonium
9.	**Test for Potassium** A pinch (25 mg) of extract was treated off with 2 ml of sodium nitrite solution and then treated with 2 ml of cobalt nitrate in 30% glacial acetic acid.	Yellow precipitate was obtained	Presence of potassium
10.	**Test for Sodium** 2 pinches (50 mg) of the extract was made into paste by using HCl and introduced into the blue flame of Bunsen burner.	No yellow colour flame evolved	Absence of sodium
11.	**Test for Mercury** 2 ml of the extract was treated with 2 ml of sodium hydroxide solution.	No Yellow precipitate obtained	Absence of Mercury
12.	**Test for Arsenic:** 2 ml of the extract was treated with 2 ml of sodium hydroxide solution.	No Brownish red precipitate obtained	Absence of arsenic

S.No	Experiment	Observation	Inference
III. Miscellaneous			
1.	**Test for Starch:** 2 ml of extract was treated with weak Iodine solution	No Blue colour developed	Absence of starch
2.	**Test for Reducing Sugar:** 5 ml 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 and again boil it for 2 minutes. The colour changes were noted.	No Brick red colour developed	Absence of reducing sugar
3.	**Test for The Alkaloids**: a) 2 ml of the extract was treated with 2ml of potassium lodide solution. b) 2 ml of the extract was treated with 2ml of picric acid. c) 2 ml of the extract was treated with 2ml of phosphotungstic acid.	No Yellow colour developed	Absence of Alkaloid
4.	**Test for Tannic Acid:** 2 ml of extract was treated with 2 ml of ferric chloride solution	No Black precipitate obtained	Absence of Tannic acid
5.	**Test for Unsaturated Compound:** To the 2 ml of extract 2 ml of Potassium permanganate solution was added.	Potassium permanganate was not decolourised	Absence of unsaturated compound
6	**Test for Amino Acid:** 2 drops of the extract were placed on a filter paper and dried well. 20ml of Burette reagent was added.	No violet colour	Absence of amino acid

7.	**Test for Type of Compound:** 2ml of the extract was treated with 2 ml of dil.ferric chloride solution.	No green and red colour No red colour NoViolet colour developed No Blue colour developed.	Absence of quinoa epinephrine and pyrocatechol Antipyrine, Aliphatic amino acid and meconic acid are absent. Apomorphine salicylate and Resorcinol are absent Morphine, Phenol cresol and hydrouinone are present.

Results

The results of acid and basic radicals were showed in Table No. 4 and Table No. 5

The analysis of heavy metals and trace elements were estimated by using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). The Experimental Procedure was done at SAIF, IIT Madras, Chennai-36.

Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) Analysis

ICP, abbreviation for Inductively Coupled Plasma, is one method of optical emission spectrometry. When plasma energy is given to an analysis sample from outside, the component elements (atoms) are excited. When the excited atoms return to low energy position, emission rays (spectrum rays) are released and the emission rays that correspond to the photon wavelength are measured. The element type is determined based on the position of the photon rays and the content of each element is determined based on the ray's intensity.

To generate plasma, first argon gas is supplied to torch coil and high frequency electric current is applied to the work coil at the tip of the torch tube. Using the electromagnetic field created in the torch tube by the high frequency current, argon gas is ionized and plasma is generated. This plasma has high electron density and temperature (10000k) and this energy is used in the excitation-emission of the sample. Solution samples are introduced into the plasma in an atomized state through the narrow tube in the center of the torch tube.

Sample preparation – Microwave Digestion

Inductively Coupled Plasma Spectroscopy techniques are the so-called "wet" sampling methods whereby samples are introduced in liquid form for analysis. Solids cannot be analyzed directly. Such samples should be made into clear aqueous medium quantitatively.

- 0.37 g of test sample *Naaga Sangu Parpam* was weighed and transferred into a liner provided with instrument.
- 9ml of Nitric acid was slowly added, such that no piece of sample sticks on the slide.
- It was mixed thoroughly and allowed to react for few minutes.
- The liner was placed in the vessel jacket.
- The screw cap was closed hand- tight in clockwise direction.
- The vessel was sealed and placed in the rotor fixed in microwave.

- The temperature was set to 180°C for 5 minutes and holded at 180°C for least 10 minutes.
- The vessel was allowed to cool down to a vessel interior temperature below 60°C and to a vessel surface temperature (IR) below 50°C before removing the rotor.
- The digested sample was made upto 100ml with Millipore water.
- If visible insoluble particles exist, solution could be filtered through whatmann filter paper.
- The digested solution was transferred into plastic containers and labelled properly.

In ICP intensity of light emitted when the sample "sprayed or aspirated into an argon plasma" is measured at different wavelengths. The intensity of light at a given wavelength will be proportional to a particular elemental ion concentration. The intensity is calibrated with known standard concentration. For accurate quantitative results it is necessary to stimulate the sample matrix condition with that of the standard. Each element generally will have many emission lines and the sensitivity is different for each of this wave length. When more than one element is present it is quite common that some emission lines interfere due to overlapping.

Result

The analytical result of heavy metals and trace elements in *Naaga Sangu Parpam* using ICP-OES were showed in Table No. 6.

Scanning Electron Microscopy (SEM), Energy Dispersive X-ray Spectrometry (EDAX)

The particle size of the *Naaga Sangu Parpam* was determined by using High resolution scanning electron microscopy (HR SEM). The Experimental Procedure was done at Department of Material Science, Madurai Kamaraj University, Madurai-21.

A Scanning Electron Microscope (SEM) is a type of electron microscope that produces images of a sample by scanning the surface with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals that contain information about the sample's surface topography and composition. The electron beam is scanned in a raster scan pattern, and the beam's position is combined with the detected signal to produce an image. It is a powerful and mature technique in the examination of materials, widely in metallurgy, geology, biology and medicine.

The Quanta 200 FEG Scanning Electron Microscope (SEM) is a versatile high resolution scanning electron microscope with three modes of operation namely,

1. High vacuum (HV) mode for metallic (electrically conducting) sample
2. Low vacuum (LV) mode for insulating, ceramic, polymeric (electrically insulating)
3. Environment scanning electron microscope (ESEM) for biological samples

Apart from giving the high resolution surface morphological images, the Quanta 200 FEG also has the analytical capabilities such as detecting the presence of elements down to boron on any solid conducting materials through the Energy Dispersive X-ray spectrometry (EDX) providing crystalline information from the few nanometer depth of the material surface via electron back scattered detection (BSD) system attached with microscope and advanced technological PBS (WDS) for elemental analysis. EDX analysis is useful in identifying materials and contaminants, as well as estimating their relative concentrations on the surface of the specimen. The EDX analysis system works as an integrated feature of a scanning electron microscope (SEM) and cannot operate on its own without the latter.

Principle

The primary electron beam interacts with the sample in a number of key ways: -

- Primary electrons generate low energy secondary electrons, which tend to emphasize the topographic nature of the specimen.

- Primary electrons can be back scattered which produces images with a high degree of atomic number (Z) contrast.
- Ionized atoms can relax by electron shell-to-shell transitions, which lead to either X-ray emission or Auger electron ejection.
- The X-ray emitted are characteristic of the elements in the top few µm of the sample and are measured by the EDX detector.

Resolution **:** 1.2 nm gold particle separation on a carbon substrate

Magnification **:** From a min of 12 X to greater than 1,00,000 X.

Application **:** To evaluate grain size, partical size distributions, material homogeneity and inter metallic distributions.

Sample required

- Any dimension (Height or Diameter) less than 10mm.
- The ideal shape of a sample was that of a button on a shirt.
- If the sample was not electrically conducting, it will require silver or gold coating.
- If the sample was a powder, make a normal button size pellet of the sample.
- If the sample was insulator (or) polymeric (or) electrically non-conducting it needs to be coated with carbon.

Procedure

A representative portion of *Naaga Sangu Parpam* was sprinkled on to a double side carbon tape and mounted on aluminium stubs, in order to get a higher quality secondary electron image for SEM examination.

Calculation of the particle size

The horizontal line in the right corner of the micrograph corresponds to micro in length would be given. A comparison could be made between the length of the particles visible in the micrograph with this line and the length of the particles was calculated.

Results

The results were represented in Figure No. 1, Figure No. 2 and Table No. 7

The Fourier Transform Infrared Spectroscopy test was carried out for *Naaga Sangu Parpam* as per the standard procedure. The Experimental Procedure was done SAIF, IIT Madras, Chennai-36.

Fourier Transform Infrared Spectroscopy is a powerful tool for identifying types of chemical bonds in a molecule by producing an infrared absorption spectrum that is like a molecular "fingerprint". This property is used for characterization of organic, inorganic and biological compounds. The band intensities are proportional to the concentration of the compound and hence qualitative estimations are possible. The IR spectroscopy is also carried out by using Fourier transform technique.

Description:

The Perkin Elmer Spectrum FTIR instrument consists of globar and mercury vapour lamp as sources, an interferometer chamber comprising of KBr and mad Mylar beam splitters followed by a sample chamber and detector. Entire region of 400-4500 cm-1 is covered by this instrument. The spectrometer works under purged conditions. Solid samples are dispersed in KBr or polyethylene pellets depending on the region of interest. This instrument has a typical resolution of 1.0 cm -1 cm. Signal averaging, signal enhancement, base line correction and other spectral manipulations are possible.

The interference pattern obtained from a two beam interferometer as the path difference between the two beams is altered, when Fourier transformed, gives rise to the spectrum. The transformation of the interferogram into spectrum is carried out mathematically with a dedicated on- line computer.

Model	:	Spectrum 1 FTIR spectrometer
Scan range	:	MIR 450-4500 cm-1
Resolution	:	1.0 cm-1
Sample required	:	50 mg solid or liquid.

Method

Solid	:	KBr or Nujol mull method
Liquid	:	Cal / TIBr cells
Gas	:	Gas cells

Procedure

The Perkine Elmer Spectrum One Fourier Transform Infrared (FTIR) Spectrometer was used to derive the FTIR Spectra of *Naaga Sangu Parpam* in Potassium Bromide (KBr) matrix with scan rate of 5 scan per minute at the resolution 4cm-1 in the wave number region 450-4000cm-1. *Naaga Sangu Parpam* was grounded to fine powder using agate motor and pestle and then mixed with KBr. They were then Pelletized by applying pressure to prepare the specimen (the size of specimen about 13 mm diameter and 0.3 mm in thickness) to recorded the FT- IR Spectra under Standard conditions. FTIR Spectra were used to determine the presence of the functional groups and bands in the *Naaga Sangu Parpam*.

Applications

Infrared spectrum is useful in identifying the functional groups like – OH, -CN, -NH2, etc. Also quantitative estimation is possible in certain cases for chemical, pharmaceuticals, petroleum products etc. Resins from industries, water and rubber samples can be analysed. Blood and food materials can also be analysed [51].

Result

The result of FTIR was represented in Figure No. 3 and Table No. 8

The X-ray powder diffraction test was carried out for *Naaga Sangu Parpam* as per the standard procedure. The Experimental Procedure was done SAIF, IIT Madras, Chennai-36.

X-ray powder diffraction (XRD) is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. It is a compact advanced instrument. When X-rays falls over a crystal, it diffracts in a pattern characteristic to its structure. A diffraction pattern plots Intensity against the angle of detector. Diffraction occurs when light is scattered by a periodic array with the range of order, producing constructive interference at specific angles. The pattern contains information about the atomic arrangement in crystal. Amorphous materials like glass do not have periodic array with long range order, so they do not produce any significant diffraction pattern.

Sample required

25 gm to be submitted

Sample preparation

1. 200 mg of test sample *Naaga Sangu Parpam* was taken.
2. Powder less than ~10 μm (or 200-mesh) in size was preferred.
3. So the sample was grinded manually with a mortar and pestle and was reduced to silt size.
4. Then the sample was gently pressed with the sample holder on a glass slide.
5. The excess powder was removed from the edges of the sample holder and carefully placed in the appropriate XRD slot and subjected for reading.

Benefits

It serves a major role in all stage of drug development, testing and production. It is an essential part of analytical research and development, quality control of the active ingredients, excipients and final products. It helps in elucidation of the relevant polymorphic and pseudo-polymorphic forms in pharmaceutical development.

Advantage

The PXRD analysis of crystalline compounds gives a diffraction pattern consisting of a well-defined, narrow, sharp and significant peak while amorphous materials do not give clear peaks rather the pattern has noise signals, smeared peak or it can have some short order bumps. Powder XRD is used to determine the crystallinity by comparing the integrated intensity of the background pattern to that of the sharp peaks.

Strengths

- Powerful and rapid (< 20 min) technique for identification of an unknown mineral.
- In most cases, it provides an unambiguous mineral determination.
- XRD units are widely available and minimal sample preparation is required.
- Data interpretation is relatively straight forward.

Limitations

- Homogeneous and single phase material is best for identification of an unknown.
- Must have access to a standard reference file of inorganic compounds (d-spacings, *hkl*s).
- Requires tenths of a gram of material which must be ground into a powder.
- For mixed materials, detection limit is ~ 2% of sample.
- For unit cell determinations, indexing of patterns for non-isometric crystal systems is complicated.
- Peak overlay may occur and worsens for high angle 'reflections'.

Result

The result of X-ray diffraction was represented in Figure No. 4

The TGA test was carried out for *Naaga Sangu Parpam* as per the standard procedure. The Experimental Procedure was done SAIF, IIT Madras, Chennai-36.

It is a method of thermal analysis in which the mass of a sample is measured over time as the temperature changes. This measurement provides information about physical phenomena, such as phase transitions, absorption and desorption as well as chemical phenomena including chemisorption, thermal decomposition and solid- gas reactions.

Principle

The principle of thermogravimetry is based on the simple fact that the sample is weighed continuously as it is being heated to elevated temperatures and changes in the mass of a sample are studied.

Changes in temperature affect the sample. Not all thermal changes/events bring a change in mass of sample *i.e.* melting, crystallization but some thermal events *i.e.* desorption, absorption, sublimation, vaporization, oxidation, reduction and decomposition bring a drastic change in mass of sample. It is used in analysis of volatile products, gaseous products lost during the reaction in thermoplastics, thermosets, elastomers, composites, films, fibres, coatings, paints, etc.

Types of Thermogravimetry

1.Isothermal/ Static Thermogravimetry

In this technique the sample weight is recorded as a function of time at constant temperature.

2. Quasistatic Thermogravimetry

In this technique the sample is heated to constant weight at each of the series of increasing temperature.

3. Dynamic Thermogravimetry

In this technique a sample is heated in an environment whose temperature is changing in predetermine manner generally at linear rate. Most of the studies are generally carried out with dynamic thermogravimetry. Therefore, it is generally referred to as thermogravimetry.

Procedure

12.4 mg of sample drug *Naaga Sangu Parpam* was taken and it was evenly distributed in the bottom of the sample crucible (holder). While filling the crucible, no sample material should be left remaining on the edge of the crucible. The sample crucible was placed on the front- hand sample support and subjected for reading. Good thermal contact between the sample and heat- flux sensor is an indispensable requirement for optimum results.

Factors affecting TGA curve

1. Instrumental factors

- Heating rate
- Effect of furnace atmosphere
- Sample holder

2. Sample characteristics

- Weight of the sample
- Sample particle size
- Heat of reaction
- Compactness of the sample
- Previous history of the sample

Applications of TGA

1. From TGA, we can determine the purity and thermal stability of both primary and secondary standards.
2. Determination of the composition of complex mixture and decomposition of complex.
3. For studying the sublimation behavior of various substances.
4. TGA is used to study the kinetics of the reaction rate constant.
5. Used in the study of catalyst. The change in the chemical states of the catalyst may be studied by TGA techniques. Zinc- Zinc chromate is used as the catalyst in the synthesis of methanol.

Result

The result of Thermogravimetric analysis was represented in Figure No. 5

Styptic activity of *Naaga Sangu Parpam* in Wistar albino rats

Aim

To evaluate the styptic activity of *Naaga Sangu Parpam* in Wistar albino rats by Tail cutting method.

Materials and methods

Test Substance	**:**	*Naaga Sangu Parpam*
Animal Source	**:**	The Tamilnadu Veterinary and Animal Sciences University, Madhavaram.
Animals	**:**	Wistar albino rats (Male -12, female -12)
Age	**:**	8-10 weeks.
Body Weight	**:**	200-250gm.
Acclimatization	**:**	7 days prior to dosing.
Veterinary examination	**:**	Prior and at the end of the acclimatization period.
Identification of animals	**:**	By cage number, animal number and individual marking by using Picric acid.
Diet	**:**	Pellet feed
Water	**:**	Aqua guard portable water in polypropylene bottles.
Housing & Environment	**:**	The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	**:**	24-28°C
Relative humidity	**:**	Between 30% and 70%,
Air changes	**:**	10 to 15 per hour
Dark and light cycle	**:**	12**:**12 hours.

Selection of animals

Healthy Wistar albino rats (200-250gm) of both sex were used for this study with the approval of the Institutional Animal Ethics Committee and obtained from the animal laboratory. **IAEC approved no: NIS/IAEC-III/04/29092016**

The animals were kept in plastic cages and maintained at 24-28°C. All the rats were housed individually with free access to food, water and libitum. They were fed with standard diet and kept in well ventilated animal house and they were also maintained with alternative dark-light cycle of 12 hrs throughout the study. Rats were allowed an acclimatization period of 7 days before actual experiment. The rats were closely observed for any infection and if they show any signs of infection they were excluded from the study. The animal experiment was performed with accordance legislation on welfare.

Experimental design

The animals were divided into 4 groups of six animals in each group.

Group I : Vehicle Control (Ghee)

Group II : Standard drug - Adrenochrome (10 µg/animal/i.p)

Group III : *Naaga Sangu Parpam* Dose I (12mg/kg)

Group IV : *Naaga Sangu Parpam* Dose II (24mg/kg)

Experimental procedure

The animals were kept overnight fasting before the experiment**.** First group received 1ml of Ghee, second group received 10 mg/kg of Adrenochrome, third group and fourth group received 12mg/kg and 24mg/kg of *Naaga Sangu Parpam* respectively. After 1 hour, the following parameters such as clotting time, bleeding time, prothrombin time, activated partial prothrombin time and fibrinogen time were screened.

Clotting time

The tail of the animal was warmed for 1 min in water at 40°C, dried and cut at the tip with a razor blade. A 25 µl sample of blood was collected into a micro hematocrit glass capillary tube. The time of appearance of the first drop of the blood on the cut tail was noted. The blood was left to flow by gravity between the two marks of the tube, 45 mm apart, by

tilting the capillary tube alternately to +60° and -60° angles with respect to the horizontal plane until blood ceased to flow (reaction end point). The glass tube was then kept between the palms of both the hands for 30 seconds to maintain it at body temperature. After 30 seconds the tube was taken out and small portion of the capillary tube was broken at regular intervals of 10 seconds, until a thread of clotted blood appears between the two pieces of capillary glass tube. The time interval between the appearance of the drop of the blood and the thread of the blood clot was noted as the clotting time of rat expressed in minutes.

Bleeding time method (BT)

The tail of the rat was warmed for 1min in water at 40°C and then dried. A small cut was made in the middle of the tail with a scalpel. Bleeding time was calculated from the time of first blood appeared till bleeding was stopped. Spots were made with the bleeding tail on a blotting paper every 15 seconds, till the bleeding stopped and the bleeding time was calculated accordingly.

Animal blood collection

For the remaining parameters like prothrombin, activated partial prothrombin time and fibrinogen time, blood was collected from the lateral tail vein. The blood sample was immediately emptied into a plastic tube containing 0.11M sodium citrate at a ratio of 1:10 anticoagulant blood, gently mixed and centrifuged at 2500g at 4c for 10 min. The plasma was separated and maintained in ice bath throughout this process.

Prothrombin time (PT)

0.1 ml of plasma was mixed with 0.2 ml of PT reagent (calcium thromboplastin) and maintained at 37°C and observed until the formation of fibrin clot and the time was noted.

Activated Partial Thromboplastin time (APTT)

0.1ml of plasma was mixed with 0.1ml of APTT reagent (cephalin-karolin suspension) and was incubated at 37°C for 5 minutes and then 0.1ml of 0.025ml cacl2 solution was added and observed until the formation of fibrin clot visually detected and the time was noted.

Fibrinogen time

0.25ml of animal blood plasma was mixed with 0.05 ml of saline and was incubated at 37°C. After 30's, 0.1ml of streptokinase solution was added and waited for 30's, then 0.1ml of bovine thrombin was added. The Stopwatch was started. 30 or later, the time when the fibrinogen clot formed was noted.

Statistical analysis

N= 6, Values are expressed as mean ± SD, analysis was done by using One-Way ANOVA followed by Dunnett's Test. Test for significance is *P < 0.05, **P < 0.01, ***P < 0.001.

Result

The results of Styptic activity were showed in Table No. 9, Chart No. 1, Chart No. 2 and Chart No. 3

Anti – inflammatory activity of *Naaga Sangu Parpam* in Wistar albino rats

Aim

To evaluate the anti - inflammatory activity of *Naaga Sangu Parpam* in Wistar albino rats by Carrageenan induced paw oedema method**.**

Materials and methods

Test Substance	**:**	*Naaga Sangu Parpam*
Animal Source	**:**	The Tamilnadu Veterinary and Animal Sciences University, Madhavaram.
Animals	**:**	Wistar albino rats (Male -12, female -12)
Age	**:**	6-8 weeks
Body Weight	**:**	150-200gm.
Acclimatization	**:**	7 days prior to dosing.
Veterinary examination	**:**	Prior and at the end of the acclimatization period.
Identification of animals	**:**	By cage number, animal number and individual marking by using Picric acid.
Diet	**:**	Pellet feed
Water	**:**	Aqua guard portable water in polypropylene bottles.
Housing & Environment	**:**	The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	**:**	24-28°C
Relative humidity	**:**	Between 30% and 70%,
Air changes	**:**	10 to 15 per hour
Dark and light cycle	**:**	12**:**12 hours.

Selection of animals

Healthy Wistar albino rats (150-200gm) of both sex were used for this study with the approval of the Institutional Animal Ethics Committee and obtained from the animal laboratory. **IAEC approved no: NIS/IAEC-III/04/29092016**

The animals were kept in plastic cages and maintained at 24-28°C. All the rats were housed individually with free access to food, water and libitum. They were fed with standard diet and kept in well ventilated animal house and they were also maintained with alternative dark-light cycle of 12hrs throughout the study. Rats were allowed an acclimatization period of 7 days before actual experiment. The rats were closely observed for any infection and if they show any signs of infection they were excluded from the study. The animal experiment was performed with accordance legislation on welfare.

Experimental Design

The animals were divided into 4 groups of six animals in each group.

Group 1 : Vehicle control (Ghee)

Group II : Standard drug- Indomethacin (10mg/kg/p.o)

Group III : Received test drug *Naaga Sangu Parpam* (12mg/kg)

Group IV : Received test drug *Naaga Sangu Parpam* (24mg/kg)

Experimental procedure

First group (vehicle control) received 1ml of Ghee, second group (Standard drug) received 10 mg/kg of indomethacin p.o., third group received 12mg/kg of *Naaga Sangu Parpam* and fourth group received 24mg/kg of *Naaga Sangu Parpam* respectively. After 1hr, the rats were administered with subcutaneous injection of 1ml of 1% w/v solution of carrageenan into the plantar side of the right hind paw. The paw was marked with ink at the level of lateral malleolus and immersed in plethysmograph apparatus. The paw volume was measured initially after the administration of carrageenan at 0^{th}, 1^{st}, 2^{nd} and 3^{rd} hr by using plethysmographic method. The difference between the initial and subsequent reading gave the actual oedema volume.

Statistical analysis

N= 6, Values are expressed as mean ± SD, analysis was done by using One-Way ANOVA followed by Dunnett's Test. Test for significance is *P < 0.05, **P < 0.01, ***P < 0.001.

Result

The results of anti-inflammatory activity were showed in Table No. 10 and Chart No. 4

Analgesic activity of *Naaga Sangu Parpam* in Swiss albino mice

Aim

To evaluate the Analgesic activity of *Naaga Sangu Parpam* in Swiss albino mice by Eddy's Hot plate method**.**

Materials and methods

Test Substance	**:**	*Naaga Sangu Parpam*
Animal Source	**:**	The Tamilnadu Veterinary and Animal Sciences University, Madhavaram
Animals	**:**	Swiss albino mice (Male -12, female -12)
Age	**:**	6-8 weeks
Body Weight	**:**	20-25gm.
Acclimatization	**:**	7 days prior to dosing.
Veterinary examination	**:**	Prior and at the end of the acclimatization period.
Identification of animals	**:**	By cage number, animal number and individual marking by using Picric acid.
Diet	**:**	Pellet feed
Water	**:**	Aqua guard portable water in polypropylene bottles.
Housing & Environment	**:**	The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	**:**	24-28°C
Relative humidity	**:**	Between 30% and 70%,
Air changes	**:**	10 to 15 per hour
Dark and light cycle	**:**	12**:**12 hours.

Selection of Experimental animals

The experimental protocol was submitted and approved by institutional Ethical Committee, **(IAEC approved no: NIS/IAEC-III/04/29092016)**. Swiss albino mice (20-25 gm) of approximate same age were employed in this investigation.

The animals were kept in plastic cages and maintained at 24-28°C. All the mice were housed individually with free access to food, water and libitum. They were fed with standard diet and kept in well ventilated animal house and they were also maintained with alternative dark-light cycle of 12hrs throughout the study. Mice were allowed an acclimatization period of 7 days before actual experiment. The mice were closely observed for any infection and if they showed any signs of infection they were excluded from the study. The animal experiment was performed with accordance legislation on welfare.

Evaluation of Analgesic activity

Pain is the part of a defensive reaction against dysfunction of an organ or imbalance in its functions against potentially dangerous stimulus. The ascending pathway of pain includes the contralateral spinothalamic tract, lateral pons, mid brain to thalamus and ultimately through the somatosensory cortex of the brain that determines the locations, intensity and depth of pain

Eddy's Hot plate method

Principle

Painful reactions can be produced in experimental animals by applying noxious stimuli such as thermal – using radiant heat as a source of pain, chemical – using irritants such as acetic acid and bradykinin and physical pressure – using tail compression.

The hot plate test was a test of the pain response in animals. It was used in basic pain research and in testing the effectiveness of analgesics by observing the reaction to pain caused by heat.

They used a behavioral model of nociception where behaviors such as jumping and hind paw-licking are elicited following a noxious thermal stimulus. Licking was a rapid response to painful thermal stimuli that was a direct indicator of nociceptive threshold. Jumping represents a more elaborated response, with a latency and encompasses an emotional component of escaping.

Experimental design

The animals were divided into 4 groups of six animals in each group.

Group I : Vehicle control (Ghee)

Group II : Standard drug - Pentazocine (10mg/kg/i.p)

Group III : *Naaga Sangu Parpam* (13mg/kg)

Group IV : *Naaga Sangu Parpam* (26mg/kg)

Equipment

Eddy's Hot plate

Experimental procedure

Animals were weighed and placed on the hot plate. Temperature of the hot plate was maintained at 55 ± 1° C. Jumping response was seen. The time period (latency period), from when the animals were placed and until the responses occurred, were recorded using a stopwatch. To avoid tissue damage of the animals, 10 seconds was kept as a cut off time. The time obtained was considered the basal / normal reaction time in all the untreated groups of animals. Increase in the basal reaction time was the index of analgesia.

All the animals were screened initially at least three times in this way and the animals showing a large range of variation in the basal reaction time were excluded from the study. A final reading of the basal reaction time was recorded for the included animals. After selecting the animals, the drugs were administered to all the groups at the stipulated doses. The reaction time of the animals were then noted at 0, 30, 60, 120 and 180 mins interval after drug administration.

Statistical analysis

N= 6, Values are expressed as mean ± SD, analysis was done by using One-Way ANOVA followed by Dunnett's Test. Test for significance is *P < 0.05, **P < 0.01, ***P < 0.001.

Result

The results of analgesic activity were showed in Table No. 11 and Chart No. 5

Many studies have been carried out to bring the efficacy and potency of the drug *Naaga Sangu Parpam*. Traditional remedies are advantageous; it does suffer some limitations.

The main limitation is the lack of standardisation of raw materials, processing methods, the final products, dosage formulation and the non- existence of criteria for quality control.

Standardization of the drug is more essential to derive the efficacy, potency of the drug by analysing it through various studies.

Following tables and charts are the results of physicochemical and phytochemical analysis. Physical characterization and estimation of basic and acidic radicals have been done and tabulated. Pharmacological activities of the drug were derived. Its result has been tabulated below.

5.1 ORGANOLEPTIC CHARACTERS

Table No. 1: Organoleptic characters of *Naaga Sangu Parpam*

Organoleptic characters		
1	**Colour**	**Grey colour**
2	**Odour**	**Odourless**
3	**Texture**	**Fine powder**
4	**Taste**	**Tasteless**

5.2 PHYTOCHEMICAL ANALYSIS

Table No. 2: Phytochemical analysis of *Naaga Sangu Parpam*

S. No	Phytochemicals	Test Name	H_2O Extract
1.	Alkaloids	Mayer's Test	Negative
		Wagner's Test	Negative
		Dragendroff's Test	Negative
		Hager's Test	Negative
2.	Carbohydrates	Molisch's Test:	Negative
		Benedict's Test	Negative
3.	Glycoside	Modified Borntrager's Test	Negative
		Keller Killiani	Negative
4.	Saponin	Froth Test	Negative
		Foam Test	Negative
5.	Phytosterol	Salkowski's Test	Negative
6.	Phenols	Ferric Chloride Test	Negative
7.	Tannins	Gelatin Test	Negative
8.	Flavonoids	Alkaline Reagent Test	Negative
		Lead acetate Test	Negative
9.	Proteins and amino acids	Xanthoproteic Test	Negative
10.	Diterpenes	Copper Acetate Test	Negative
11.	Gum & Mucilage	Extract + Alcohol	Negative
12.	Fat & Fixed Oil	Spot Test	Negative
13.	Quinones	NAOH + Extract	Negative

Interpretation: Bioactive compounds are not present in *Naaga Sangu Parpam*

*****Phytochemical analysis was done at The T. N. Dr. M. G. R Medical university, Chennai-32

5.3 PHYSICOCHEMICAL ANALYSIS

Table No. 3: Physicochemical properties of *Naaga Sangu Parpam*

S. No	Parameters	Results
1.	Ph	9.5
2	Loss on drying	Less than 1%
3.	Total Ash value	98.21%
4.	Acid insoluble ash	76.49 %
5.	Water soluble ash	Less than 1%
6.	Water soluble extractive	Less than 1%
7	Alcohol soluble extractive	1.02 %

Interpretation

In the physicochemical analysis, the pH of the drug is 9.5. It denotes that, it is alkaline in nature. So that, in the oral administration, the drug will get ionized in stomach and absorbed in intestine and sent directly to the portal system.

The total of volatile content and moisture present in the drug was established in loss on drying. Moisture content of the drug reveals the stability and its shelf-life. High moisture content can adversely affect the active ingredients of the drug. Thus low moisture content could get maximum stability and better shelf life.

The loss on drying value of *Naaga Sangu Parpam* is found to be less than 1%, hence the drug will not lose much of its volume on exposure to the atmospheric air at room temperature.

Ash constitutes the inorganic residues obtained after complete combustion of a drug. Thus Ash value is a validity parameter describe and to assess the degree of purity of a given drug.

Total ash value indicates the amount of minerals and earthy materials present in the plant material. The total inorganic content (Potassium, Calcium, Chloride, Iron, etc.,) present in the drug is measured through the Total ash and it is of 98.21% for *Naaga Sangu Parpam*.

The acid insoluble ash value of the drug denotes the amount of siliceous matter present in the plant. It is 76.49% for *Naaga Sangu Parpam*.

***** The experimental procedure was analyzed at The T. N. Dr. M. G. R Medical university, Guindy, Chennai-32

5.4 CHEMICAL ANALYSIS *

Table No. 4: Results of acid radical studies of *Naaga Sangu Parpam*

S. No	Parameter	Observation	Result
1	Test for Sulphate	Cloudy appearance present. White precipitate insoluble in Con.HCL was obtained	**Positive**
2	Test for Chloride	-	Negative
3	Test For Phosphate	-	Negative
4	Test For Carbonate	Cloudy yellow appearance present	**Positive**
5	Test For Nitrate	-	Negative
6	Test for Sulphide	Rotten egg smelling gas evolved	**Positive**
7	Test For Fluride & oxalate	-	Negative
8	Test For Nitrite	-	Negative
9	Test For Borax	-	Negative

Interpretation

The acidic radicals test shows the presence of **Sulphate, Carbonate and Sulphide**

***** The chemical analysis was carried out in Biochemistry Lab, NIS, Chennai – 47

Table 5: Results of basic radicals studies of *Naaga Sangu Parpam

S. No	Parameter	Observation	Result
1	Test for Lead	-	Negative
2	Test for Copper	-	Negative
3	Test For Aluminium	-	Negative
4	Test For Iron	Mild red colour appeared Blood red colour appeared	**Positive**
5	Test For Zinc	-	Negative
6	Test for Calcium	Cloudy appearance and white precipitate was obtained	**Positive**
7	Test For Magnesium	-	Negative
8	Test For Ammonium	-	Negative
9	Test For Potassium	Yellow precipitate was obtained	**Positive**
10	Test For Sodium	-	Negative
11	Test For Mercury	-	Negative
12	Test For Arsenic	-	Negative

Interpretation

The basic radical test shows the presence of **Iron, Calcium, Potassium** and absence of heavy metals such as lead, arsenic and mercury.

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* The chemical analysis was carried out in Biochemistry Lab, National Institute of Siddha, Chennai- 47

5.5 ELEMENTAL ANALYSIS

Table 6: ICP-OES study results of *Naaga Sangu Parpam*

S. No	Elements	Wavelength in nm	mg/L
1	Aluminium	Al 396.152	BDL
2	Arsenic	As 188.979	BDL
3	Calcium	Ca 315.807	502.180 mg/L
4	Cadmium	Cd 228.802	BDL
5	Copper	Cu 327.393	BDL
6	Iron	Fe 238.204	01.376 mg/L
7	Mercury	Hg 253.652	BDL
8	Potassium	K 766.491	03.821 mg/L
9	Magnesium	Mg 285.213	01.104 mg/L
10	Sodium	Na 589.592	06.320 mg/L
11	Nickel	Ni 231.604	BDL
12	Lead	Pb 220.353	BDL
13	Phosphorus	P 213.617	86.341 mg/L
14	Sulphur	S 180.731	41.254 mg/L
15	Zinc	Zn 206.200	421.018 mg/L

BDL -Below Detection Limit, PPM - Parts per million

Interpretation

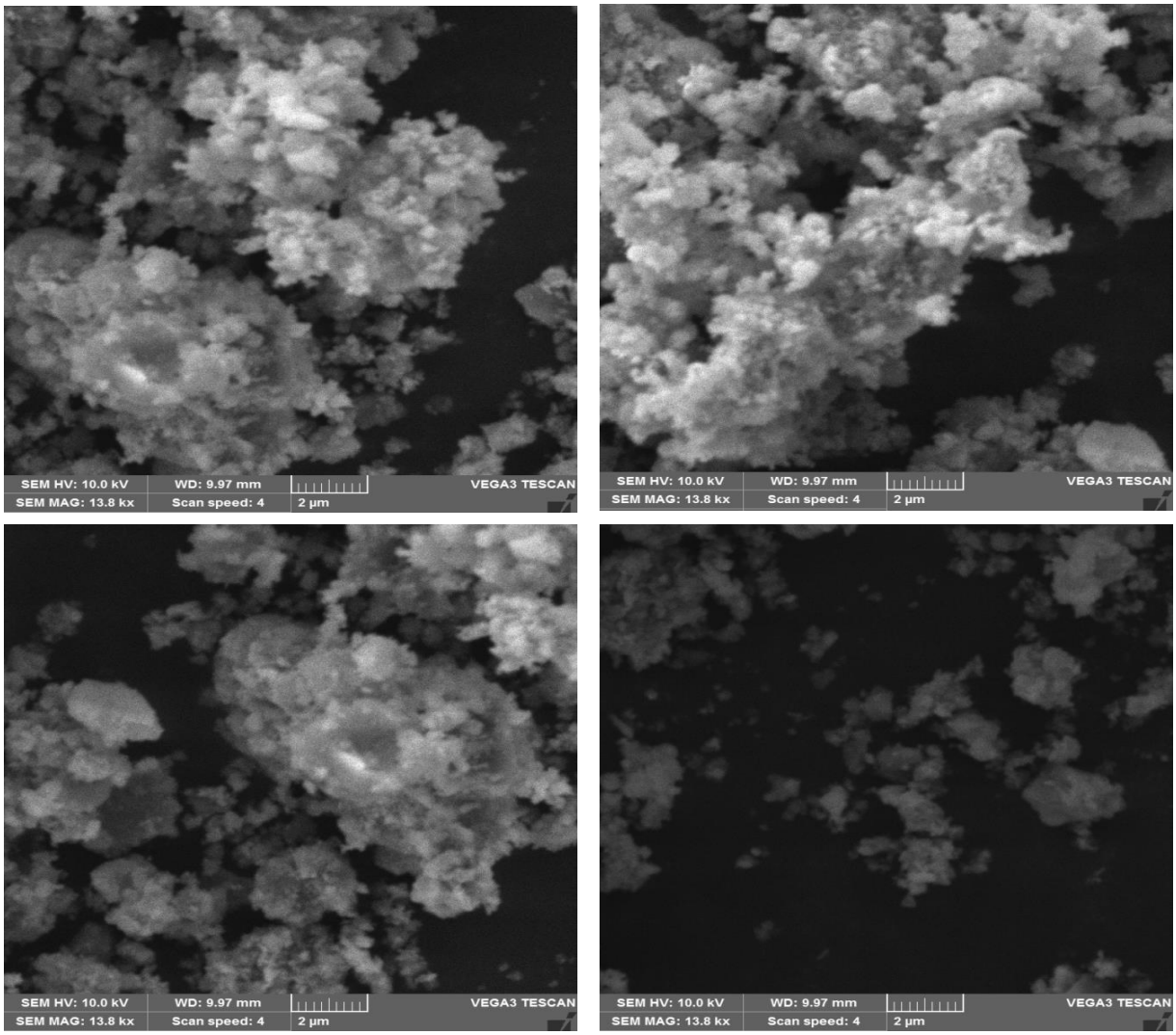
The heavy metals like Arsenic, Mecury, Lead and Cadmium were found detected limits. The presence of other elements shows the therapeutic value of *Naaga Sangu Parpam.*

***** The experimental procedure was analyzed at SAIF, IIT Madras, India.

5.6 ANALYSIS OF PARTICLE SIZE AND EDAX *

a) Scanning Electron Microscopy (SEM)

Figure No. 1: Determination of Particle size of *Naaga Sangu Parpam*



Interpretation

The SEM imaging of the sample shows that the particles are small and are in and around 100 nm as shown in Fig No. 1. The image shows the different magnification of the sample. Morphology of the sample revealed that the particles are in spherical shape with slight agglomeration with denser. The particles are aggregate and individual particles are seen on the top of the clusters. The particle size is low because of the grounding for more than 12 hours

b) Energy Dispersive X ray spectrometry (EDAX)

Figure No. 2: EDAX analysis of *Naaga Sangu Parpam*

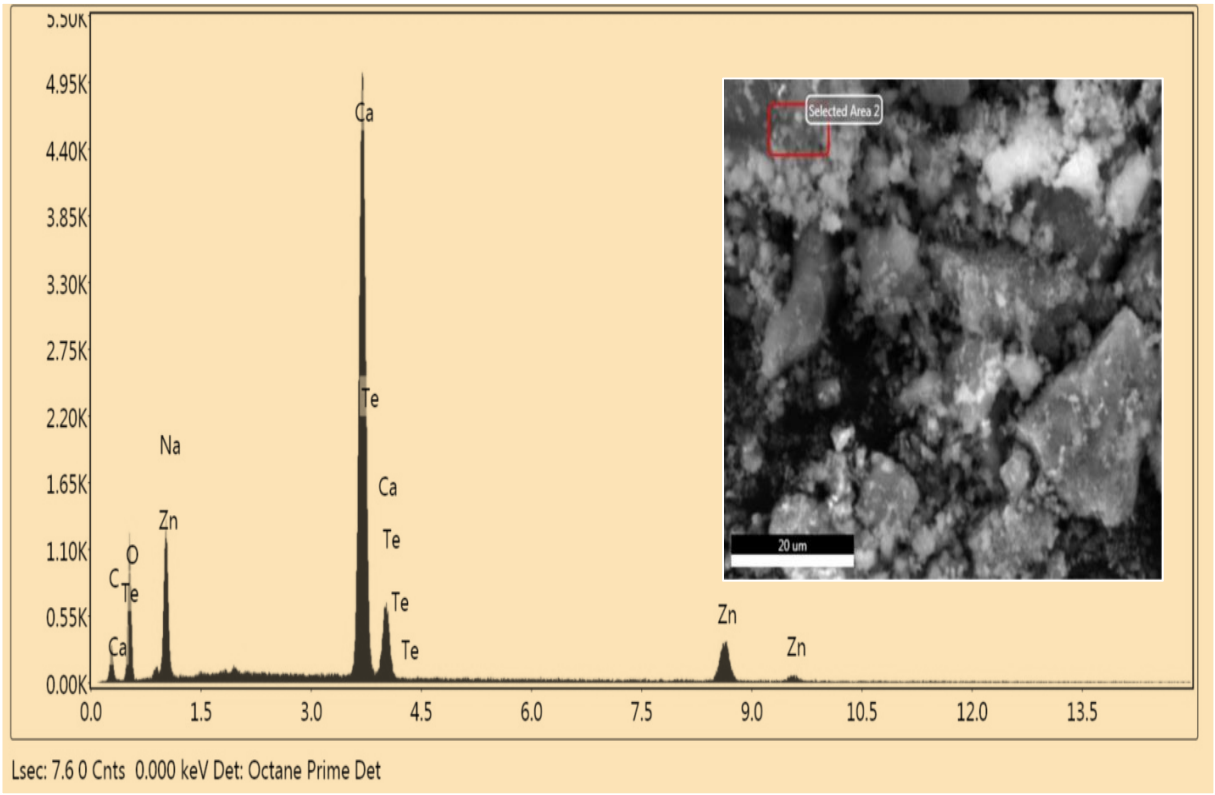


Table No. 7: Weight and atomic percentage of *Naaga Sangu Parpam*

S.no	Element	Weight %	Atomic %	Net Int.	Error %
1	C K	4.65	9.74	99.57	13.30
2	O K	34.15	53.62	888.16	10.77
3	NaK	11.31	12.36	639.48	11.05
4	CaK	29.46	18.46	6205.73	1.97
5	TeL	10.86	2.14	935.68	5.59
6	ZnK	9.57	3.68	679.08	4.35

Interpretation

EDAX analysis shows the elements present in the *Naaga Sangu Parpam* as shown in Fig No. 2 and the Table No.7 represents the weight and atomic percentage of *Naaga Sangu Parpam.*

The presence of calcium and sodium is 30% is due to the usage of purified Conch shells. The presence Zinc is about 10 wt % is contributed by the presence of purified *Naagam* in the sample.

Since the *Naagam* is subjected to overheat and it became zinc oxide, the presence of tellurium is minimum and may be from conch cell and the *Uthamani* leaf juice. The carbon and oxygen is present due to the calcination process.

***** The experimental procedure was analyzed at Dept. of Material science, Madurai Kamaraj University, Madurai.

5.7 FOURIER TRANSFORM INFRARED

Figure No. 3: FTIR curve of *Naaga Sangu Parpam*

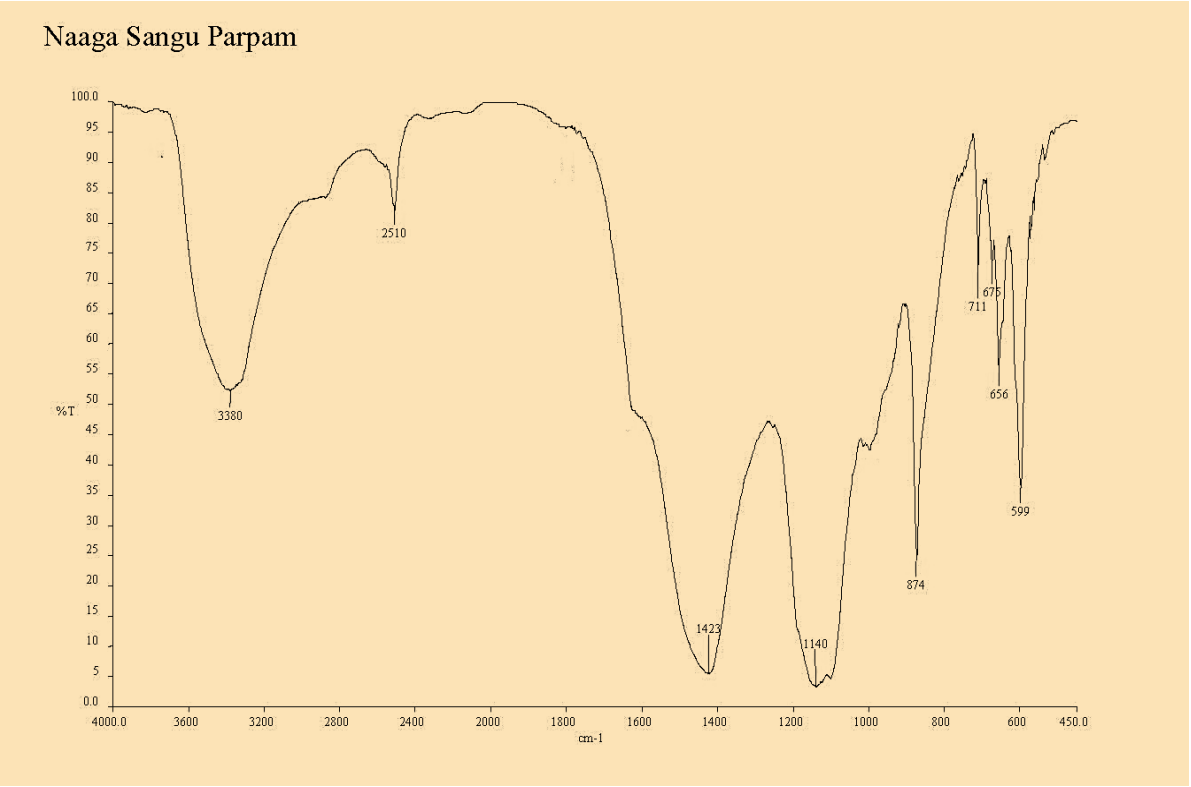


Table No. 8: Vibrational modes and functional group of *Naaga Sangu Parpam* in FTIR

Wave number (cm-1)	Vibrational modes of *Naaga Sangu Parpam* in IR region	Functional group
3380	N-H Stretch	Amine
2510	O-H Stretch	Acid
1423	-C-H bending	Alkane
1140	C-O Stretch	Alcohol
874	=C-H bending	Alkene
711	C-Cl Stretch	Alkyl halide
675	=C-H bending	Alkene
656	C-Cl Stretch	Alkyl halide
599	C-Br Stretch	Alkyl halide

Interpretation

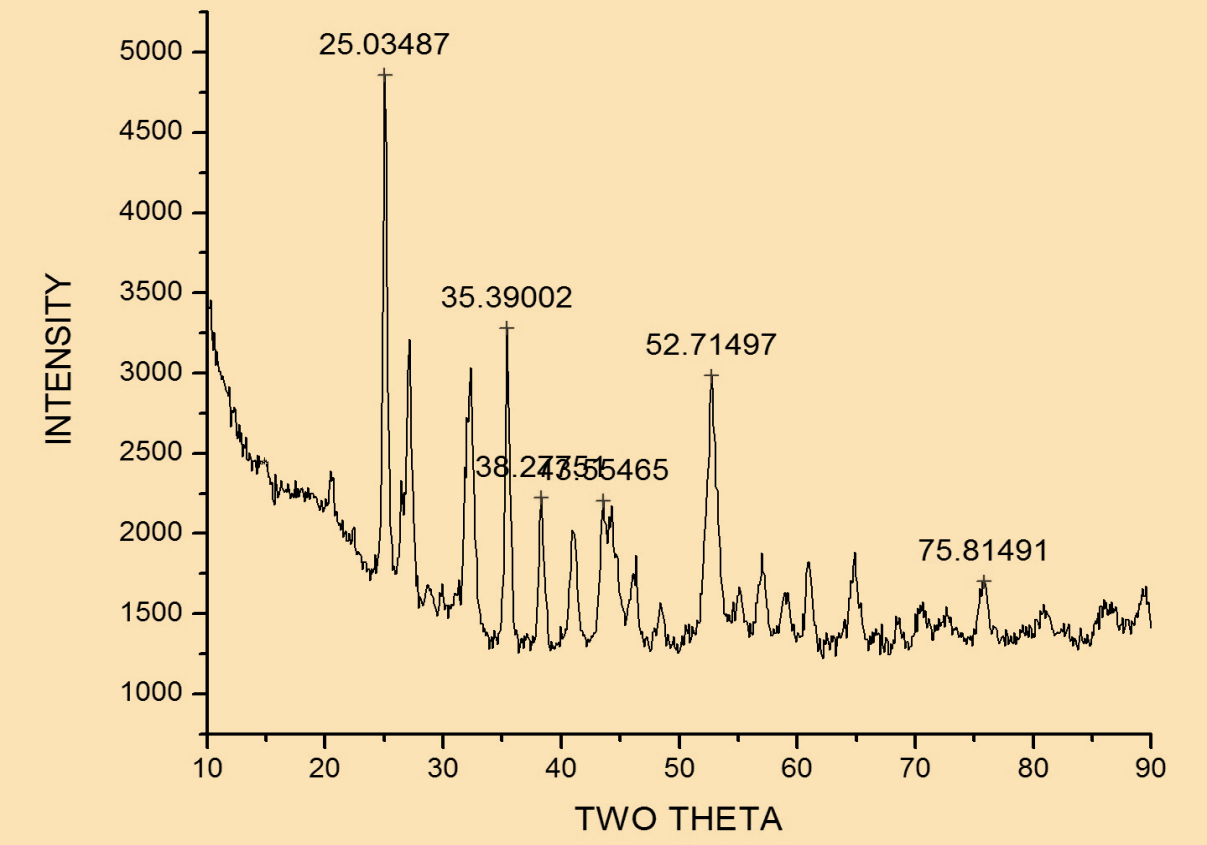
In the FTIR Spectra analysis, *Naaga Sangu Parpam* sample exhibits the peak value as shown in Table No. 8, at the wave number of 3380, 2510, 1423, 1140, 874, 711, 675, 656, 599 having N-H Stretch, O-H Stretch, -C-H bending, C-O Stretch, =C-H bending, C-Cl Stretch, =C-H bending, C-Cl Stretch, C-Br Stretch.

This indicates the presence of some organic functional groups such as amine, acids, alkane, alcohol, alkene, alkyl halide, alkene, alkyl halide and alkyl halide.

***** The experimental procedure was analyzed at SAIF, IIT Madras, India.

15.8 X-RAY DIFFRACTION*

Figure No.4: Xrd curve for *Naaga Sangu Parpam*



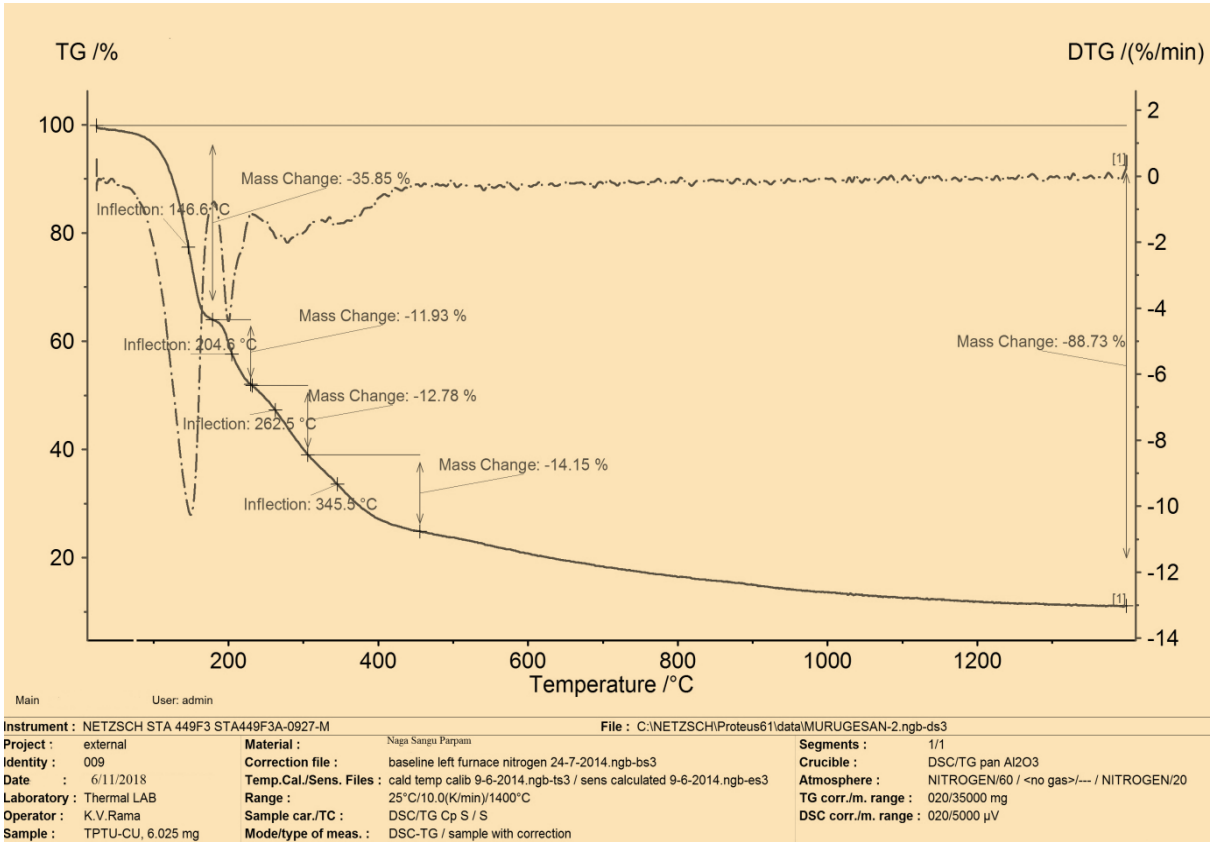
Interpretation

- The X-ray diffraction pattern of the prepared sample *Naaga Sangu Parpam* reveals the presence of major peak with 2- Theta value of 35.39 with the intensity of 3250 corresponds to Iron.
- Major peaks observed in test sample with 2-theta values of 52.71 and their corresponding intensities matching with the material Calcium.
- Major peaks observed in test sample with 2-theta values of 25.03 and their corresponding intensity of 4600 matching with the material Zinc.
- Further form this observation it was concluded that Zinc, Iron and calcium may be the key ingredients present in the sample *Naaga Sangu Parpam.*

***** The experimental procedure was analyzed at SAIF, IIT Madras, India.

5.9 THERMOGRAVIMETRIC ANALYSIS

Figure. No 5: TGA curve for *Naaga Sangu Parpam*



Interpretation

- Thermogravimetric analysis of *Naaga Sangu Parpam* carried out at the maximum of 1300 degree centigrade. The main objective of the study is to evaluate the decomposition and stability limit of the prepared formulation *Naaga Sangu Parpam*.
- Prepared formulation *Naaga Sangu Parpam* seems to be stable at the temperature varying from 50 ºC to 460 ºC.
- Point of decomposition begins when the temperature increases beyond 460 ºC.
- Weight of the final residual matter was observed with 88.73% of residual volume.
- From the result of the present investigation it was concluded that the formulation *Naaga Sangu Parpam* seems to be stable at varying temperature ranges from 50 to 460 ºC.

*The experimental procedure was analyzed at SAIF, IIT Madras, India.

6.1 STYPTIC ACTIVITY

Table No. 9: Styptic activity of *Naaga Sangu Parpam* by Tail cutting method

S.no	Parameters	Control	Group I	Group II	Group III
1	Bleeding time (s)	4.89±0.35	2.96±0.26***	3.82±0.28***	3.07±0.82***
2	Clotting time (s)	4.55±0.34	2.22±0.08***	2.84±0.31***	2.32±0.11***
3	Prothrombin time (s)	11.97±0.47	8.55±0.26***	10.2±0.32***	10.5±0.57***
4	Activated Thromboplastin time (s)	29.88±0.32	22.51±0.73***	27.6±0.48***	24.9±0.59***
5	Fibrinogen time (s)	175.6±7.36	271±11.17***	202.17±19.14**	223.8±16.86***

N= 6, Values are expressed as mean ± SD, analysis was done by using One-Way ANOVA followed by Dunnett's Test. Test for significance is *P < 0.05, **P < 0.01, ***P < 0.001.

Chart No. 1: The mean value of Bleeding time, Clotting time and Prothrombin time of control and treated groups of Wistar albino rats exposed to *Naaga Sangu Parpam*

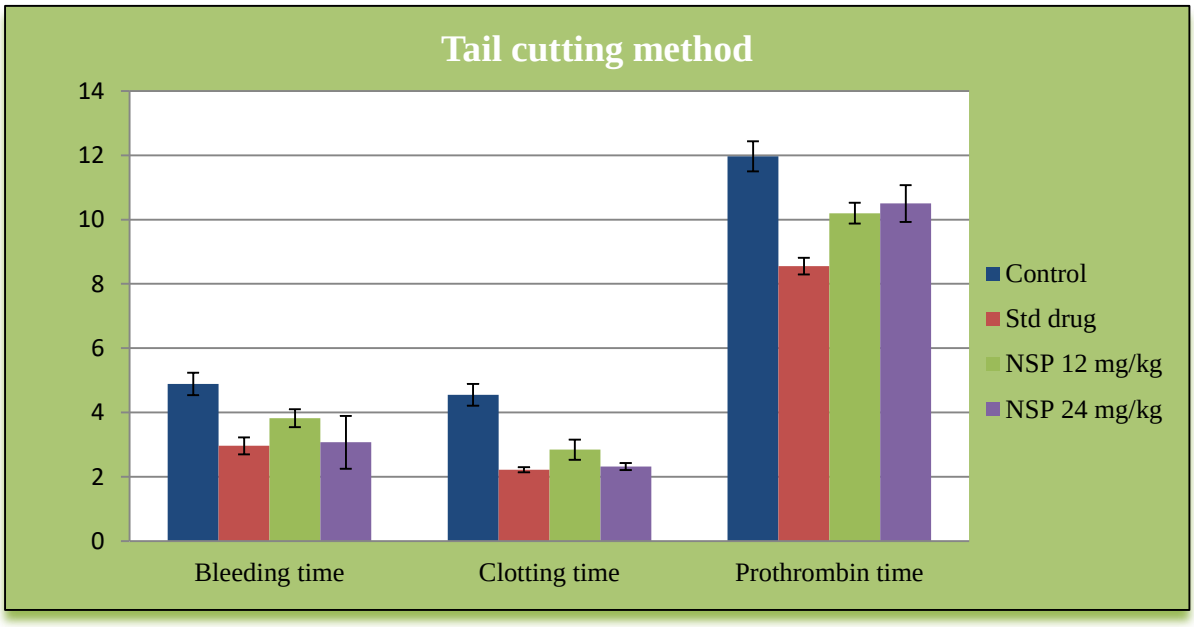


Chart No. 2: The mean value of Thromboplastin time of control and treated groups of Wistar albino rats exposed to *Naaga Sangu Parpam*

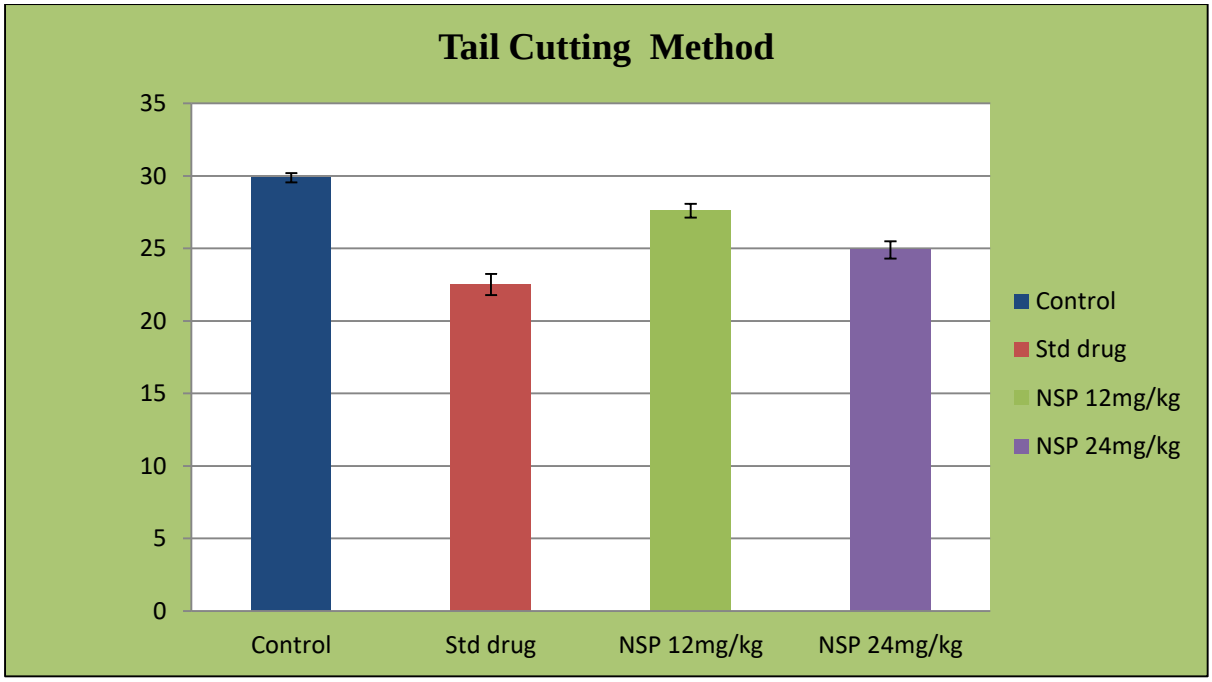
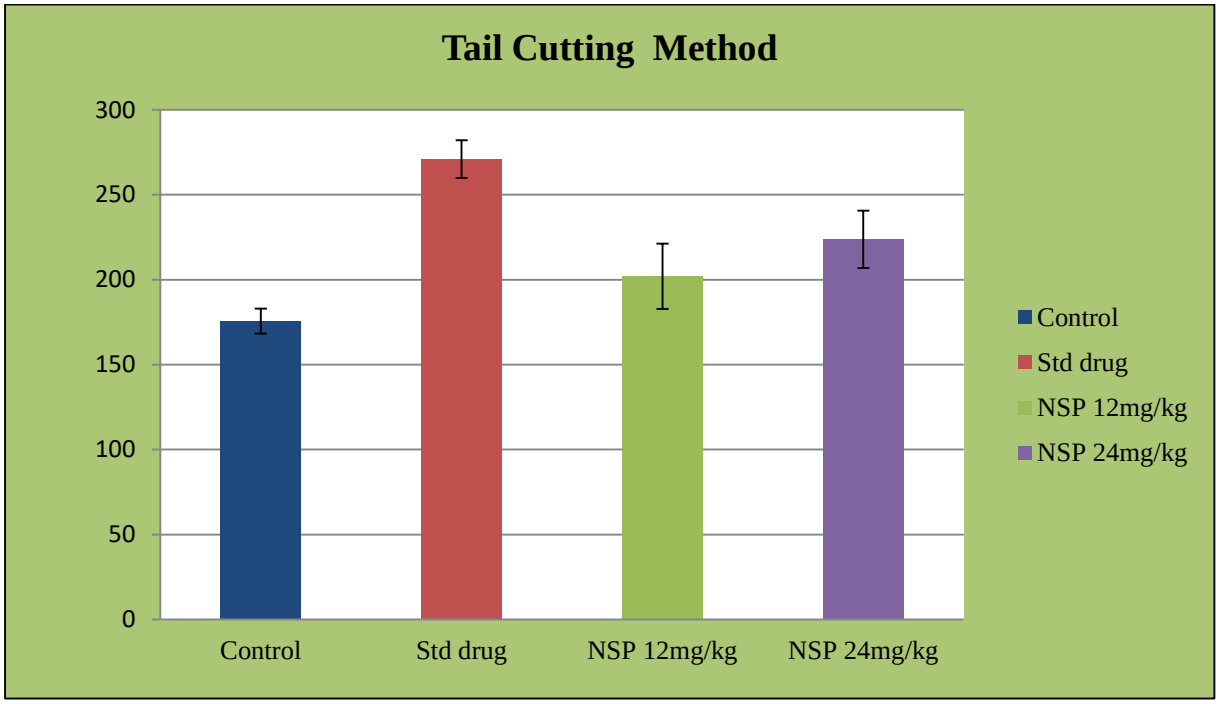


Chart No. 3: The mean value of Fibrinogen time of control and treated groups of Wistar albino rats exposed to *Naaga Sangu Parpam*



Results of Styptic activity

BleedingTime (BT)

In the vehicle treated control group the mean bleeding time is 4.89±0.35, while NSP at 12mg/kg it is 3.82±0.28*** and at 24mg/kg it is 3.07±0.82***. The result showed that *Naaga Sangu Parpam* at both 12mg/kg and 24mg/kg have more significant (***P < 0.001) effect, when compared to control group.

Clotting time (CT)

The mean clotting time in this vehicle treated control group is 4.55±0.34, while NSP at 12mg/kg it is 2.84±0.31*** and at 24mg/kg it is 2.32±0.11***. The result showed that *Naaga Sangu Parpam* at both 12mg/kg and 24mg/kg have more significant (***P < 0.001) effect, when compared to control group.

Prothrombin time (PT)

In the vehicle treated control group the mean prothrombin time is 11.97±0.47seconds while NSP at 12mg/kg it is 10.2±0.32***and at 24mg/kg it is 10.5±0.57***. The result showed that *Naaga Sangu Parpam* at both 12mg/kg and 24mg/kg have more significant (***P < 0.001) effect, when compared to control group.

Activated Thromboplastin

The mean Activated Thromboplastin time in this vehicle treated control group is 29.88±0.32, while NSP at 12mg/kg it is 27.6±0.48***and at 24mg/kg it is 24.9±0.59 The result showed that *Naaga Sangu Parpam* at both 12mg/kg and 24mg/kg have more significant (***P < 0.001) effect, when compared to control group.

Fibrinogen time

The mean Fibrinogen time in this vehicle treated control group is 175.6±7.36, while NSP at 12mg/kg it is 202.17±19.14*** and at 24mg/kg it is 223.8±16.86***. The result showed that *Naaga Sangu Parpam* at both 12mg/kg and 24mg/kg have more significant (***P < 0.001) effect, when compared to control group.

Conclusion

Thus it is concluded that administration *Naaga Sangu Parpam* at both 12mg/kg and 24mg/kg exhibits significant (***P < 0.001) Styptic activity in Wistar albino rats when compared with control group.

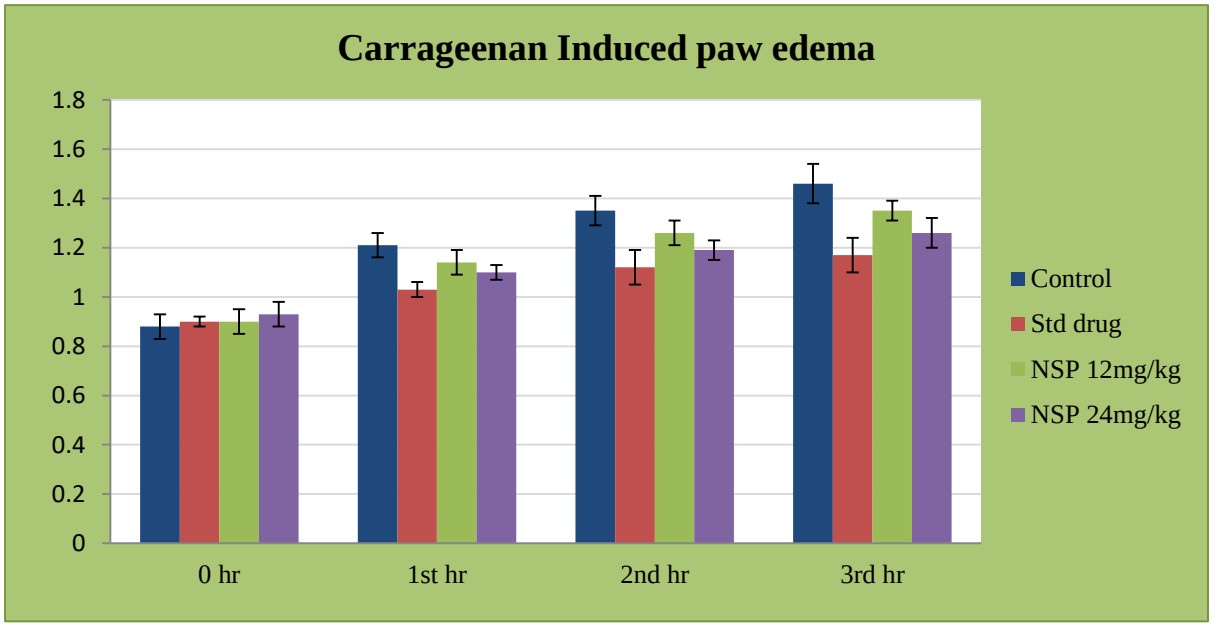
6.2 ANTI- INFLAMMATORY ACTIVITY

Table No. 10: Effect of *Naaga Sangu Parpam* on carrageenan induced paw edema method

Treatment	Percentage of inflammation after carrageenan injection at hr			
	0hr	1hr	2hr	3hr
Control	0.88±0.05	1.21±0.05	1.35±0.06	1.46±0.08
Indomethacin 10mg/kg	0.90±0.02	1.03±0.03***	1.12±0.07***	1.17±0.07***
NSP 12mg/kg	0.90±0.05	1.14±0.05*	1.26±0.05*	1.35±0.04*
NSP 24mg/kg	0.93±0.05	1.10±0.03**	1.19±0.04***	1.26±0.06***

N= 6, Values are expressed as mean ± SD, analysis was done by using One-Way ANOVA followed by Dunnett's method. Test for significance is *P < 0.05, **P < 0.01, ***P < 0.001.

Chart No. 4: Anti-inflammatory activity of *Naaga Sangu Parpam* by carrageenan induced paw edema method



Result of Anti-inflammatory activityK

Naaga Sangu Parpam at 12 mg/kg dose showed significant anti-inflammatory activity ($P < 0.05$) at 1^{st}, 2^{nd} and 3^{rd} hour when compared to control group. At 24 mg/kg the drug showed more significant ($P<0.01$) at 1^{st} hour and highly significant ($P < 0.001$) at 2^{nd} and 3^{rd} hour. Among the two doses of *Naaga Sangu Parpam*, 24mg/kg have shown better anti- inflammatory activity ($P < 0.001$) when compared with control group.

Conclusion

Thus it is concluded that administration of *Naaga Sangu Parpam* at the dose of 24 mg/kg exhibits significant ($p<0.001$) anti-inflammatory activity in Wistar albino rats when compared with control group.

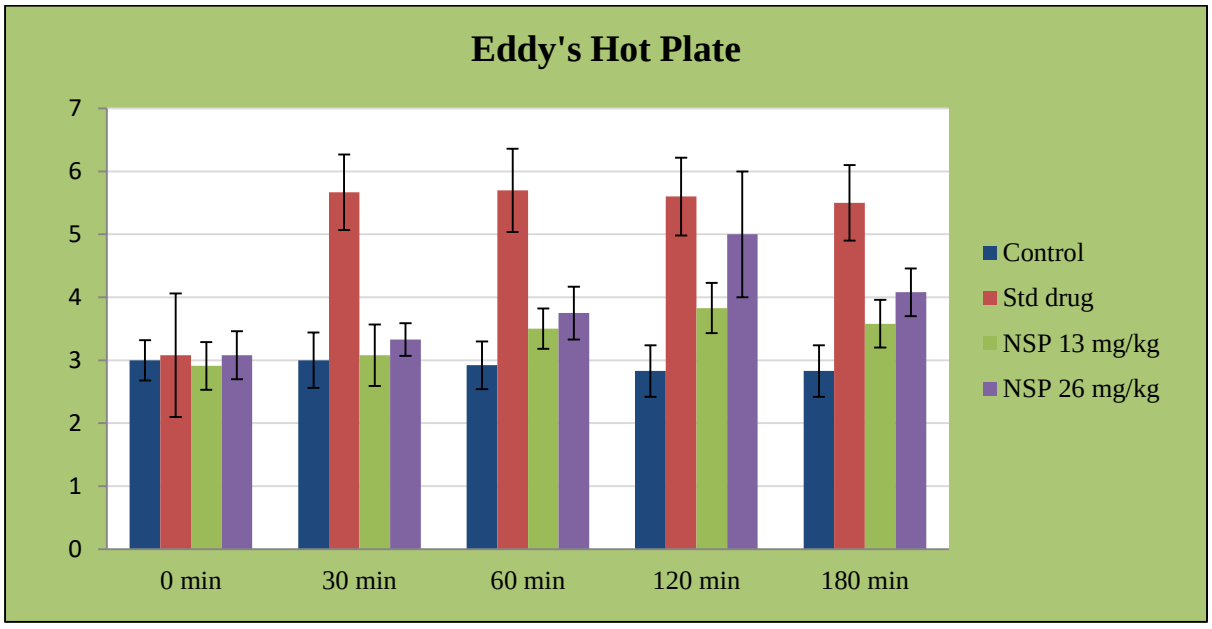
6.3 ANALGESIC ACTIVITY

Table No. 11: Analgesic activity of *Naaga Sangu Parpam* in Swiss albino mice

Groups	Treatment	Reaction time in sec				
		0min	30min	60min	120min	180min
I	Control	3±0.32	3±0.44	2.92±0.38	2.83±0.41	2.83±.41
II	Pentazocine (10mg/kg)	3.08±0.98	5.67±0.60***	5.7±0.66***	5.6±0.62***	5.5±0.6***
III	Low dose (13mg/kg)	2.91±0.38	3.08±0.49	3.5±0.32	3.83±0.40*	3.58±0.38*
IV	High dose 26mg/kg)	3.08±0.38	3.33±0.26	3.75±0.42**	5±1**	4.08±0.38***

N= 6, Values are expressed as mean ± SD, analysis was done by using One-WAY ANOVA followed by Dunnett's method. Test for significance is *P < 0.05, **P < 0.01, ***P < 0.001.

Chart No. 5: Analgesic activity of *Naaga Sangu Parpam* by Eddy's hot plate method



Result of Analgesic activity of *Naaga Sangu Parpam* in Swiss albino mice

Analgesic activity was carried out by Eddy's Hot plate method. *Naaga Sangu Parpam* at dose of 13 mg/kg showed analgesic activity with the statistical significance of ($P<0.05$) at 120 min and 180 min when compared to control group. At 26 mg/kg, the drug showed analgesic activity with significance ($P<0.01$) at 60 min and 120 min, highly significant ($P < 0.001$) at 180 min. Among the two doses of *Naaga Sangu Parpam*, 26 mg/kg have shown better analgesic activity ($P<0.01$) and ($P < 0.001$) when compared with control group.

Conclusion

Thus it is concluded that administration of *Naaga Sangu Parpam* at the dose of 26 mg/kg exhibits highly significant ($p<0.001$) analgesic activity in Swiss albino mice when compared with control group.

The drug *Naaga Sangu Parpam* was selected from the *Siddha* literature *Kannusamy Paramparai Vaithiyam* for Standardization and evaluation of pharmacological activities (Styptic, Anti-inflammatory and Analgesic) in animal models.

The ingredients of the test drug were identified and authenticated by *Siddha* experts. The drug was prepared as per the procedure and subjected to various studies such as qualitative, quantitative and pharmacological activities.

Qualitative analysis includes organoleptic characters, chemical analysis and Physicochemical properties of *Naaga Sangu Parpam*.

Quantitative analysis included ICP-OES, HR SEM with EDAX, FTIR, TGA, XRD analysis to reveal its potency and effectiveness against the disease. Also Styptic activity, Analgesic activity and Anti-inflammatory activity were carried out in Wistar albino models.

From the above analysis, the results indicate the presence of active ingredients responsible for its activity.

Literature collections include drug review, which consist of both Botanical aspect, *Gunapadam* aspect, Pharmacological review and Pharmaceutical aspect that supported the study.

In **Chemical analysis**, the drug *Naaga Sangu Parpam* revealed the presence of Iron, Sulphate, Carbonate, Sulphide, Calcium, Potassium. These chemical elements present in *Naaga Sangu Parpam* enhances the Styptic activity, Analgesic activity and Anti-inflammatory activity of the drug.

In **Physicochemical analysis**, the pH of the drug is 9.5. It denotes that, it is alkaline in nature. So that, in the oral administration, the drug will get ionized in stomach and absorbed in intestine and sent directly to the portal system.

The total of volatile content and moisture present in the drug was established in loss on drying. Moisture content of the drug reveals the stability and its shelf-life. High moisture content can adversely affect the active ingredients of the drug. Thus low moisture content could get maximum stability and better shelf life.

The loss on drying value of *Naaga Sangu Parpam* is found to be less than 1%, hence the drug will not lose much of its volume on exposure to the atmospheric air at room temperature.

Ash constitutes the inorganic residues obtained after complete combustion of a drug. Thus Ash value is a validity parameter describe and to assess the degree of purity of a given drug.

Total ash value indicates the amount of minerals and earthy materials present in the plant material. The total inorganic content (Potassium, Calcium, Chloride, Iron, etc.,) present in the drug is measured through the Total ash and it is of 98.21% for *Naaga Sangu Parpam*.

The acid insoluble ash value of the drug denotes the amount of siliceous matter present in the plant. It is 76.49% for *Naaga Sangu Parpam*.

The **ICP-OES** results showed the heavy metals like As, Pb, Cd and Hg were found below detection limit in *Naaga Sangu Parpam.* Hence, it may be safe for human consumption. The presence of calcium (502.180 mg/L) and zinc (421.018 mg/L) are observed in major quantities. Iron, sodium, sulphur, magnesium, potassium and phosphorus were observed at the level of 01.376 mg/L ,03.821 mg/L, 06.320 mg/L, 41.254 mg/L, 01.104 mg/L, 03.821 mg/L, 86.341 mg/L respectively are in a permissible amount in the *Naaga Sangu Parpam*. The drug *Naaga Sangu Parpam* which is rich in calcium, zinc and magneisum explains that this drug may help in the treatment of Hemorrhoids

Potassium

It is useful in maintaining the tissues in digestive tract which reduces inflammation of stomach and intestine.

Magnesium

It is focusing on repairing digestive system and preventing constipation.

Calcium

Taking calcium supplements will help in strengthening the veins and facilitating easy bowel movements. Calcium is necessary for the muscle contraction which helps to reduce muscle cramps during constipation.

Sodium

The sodium components of salt contribute to digestion.

Zinc

Zinc speed up the healing process after an injury.

In **HR SEM** analysis, the sample shows that the particles are small, spherical shape and around 100 nm. The particles are aggregate and individual particles are seen on the top of the clusters. The particle size is low because of the grounding for more than 12 hours. This ensures the absorption of the drug was more active and the drug have increased bio-availability.

In **EDAX** analysis shows the presence of calcium and sodium is 30% is due to the usage of Purified Conch shells. The presence Zinc is about 10 Wt % is contributed by the presence of purified *Naagam* in the sample. Since the *Naagam* is subjected to overheat and it became zinc oxide, the presence of tellurium is minimum and may be from conch cell and the *Uthamani* leaf juice. The carbon and oxygen is present due to the calcination process.

FTIR analysis indicates the presence of some organic functional groups such as amine, acids, alkane, alcohol, alkene, alkyl halide, alkene, alkyl halide and alkyl halide.

In **XRD** analysis it is clouded that Zinc, Iron and calcium may be the key ingredients present in the sample *Naaga Sangu Parpam*.

In **TGA** analysis it is concluded that the formulation *Naaga Sangu Parpam* seems to be stable at varying temperature ranges from 50 to 460 ºC.

Pharmacology activities

Styptic activity

Wistar albino rats of either sex were divided into 4 groups of 6 animals each. Group I animals received Vehicle Control (Ghee), Group II animals received Standard drug - Adrenochrome (10 µg/kg, i.p), Group III and Group IV received *Naaga Sangu Parpam* at doses (12mg/kg) and (24mg/kg) respectively. From the results, it is concluded that administration of *Naaga Sangu Parpam* at the dose of both 12 mg/kg and 24mg/kg exhibits highly significant ($p<0.001$) Styptic activity in Wistar albino rats when compared with control group.

Anti- inflammatory activity

Wistar albino rats of either sex were divided into 4 groups of 6 animals each. Group I received Vehicle control (Ghee), Group II received Standard drug- Indomethacin (10mg/kg),

Group III and Group IV received *Naaga Sangu Parpam* at doses (12mg/kg) and (24mg/kg) respectively. From the results it is concluded that administration of *Naaga Sangu Parpam* at the dose of 24mg/kg exhibits highly significant ($p<0.001$) anti- inflammatory activity in Wistar albino rats when compared with control group.

Analgesic activity

Wistar albino mice of either sex were divided into 4 groups of 6 animals each. Group I received Vehicle control (Ghee), Group II received Standard drug- Pentazocine (10mg/kg, i.p.), Group III and Group IV received *Naaga Sangu Parpam* at doses (13mg/kg) and (26mg/kg) respectively. From the results it is concluded that administration of *Naaga Sangu Parpam* at the dose of 26mg/kg exhibits highly significant ($p<0.001$) analgesic activity in Swiss albino mice when compared with control group.

Thus by scrutinizing all the above mentioned factors it is concluded that the test drug *Naaga Sangu Parpam* has been scientifically validated and it is a safe and a potent Styptic drug. It also possesses rich Anti- inflammatory and analgesic activity which supports the effective treatment for managing *Moolam* (Hemorrhoids), *Powthiram* (Fistula in ano), *Vellai* (Leucorrhoea), *Vettai* (Venereal disease).

- The test drug *Naaga Sangu Parpam* was selected from the *Siddha* literature *Kannusamy paramparai vaithiyam* to Standardize and evaluate the Styptic, Anti-inflammatory and Analgesic activities.

- The dissertation started with an introduction explaining about the *Siddha* concept, prevalence of Hemorrhoids and role of the test drug in treating hemorrhoids.

- All the ingredients were identified and authenticated by the experts and were purified and the medicine was prepared as mentioned in the *Siddha* literature.

- Review of literature in various categories were carried out. *Siddha* aspect, botanical aspect, mineralogical aspect and zoological aspect, disclosed about the ingredients of the drug and strongly supported that it possesses Styptic, anti-inflammatory and analgesic activities

- The drug was subjected to qualitative analysis such as physicochemical, phytochemical, chemical analysis. Quantitatively with FTIR, ICP-OES, HR-SEM with EDAX, XRD and TGA analysis which provided the key ingredients present in the drug. Thus it accounts the efficacy of the drug.

- Pharmacological studies revealed that the drug *Naaga Sangu Parpam* exhibited significant styptic, anti-inflammatory and analgesic activities in animal models.

- Results and discussion gives the necessary justifications to prove the potency of the drug.

- Conclusion gives a compiled form of the study and explains the synergistic effect of all the key ingredients and activities that supports the study.

From the above analytical studies (i.e. qualitative and quantitative analysis) and pharmacological activities (i.e. Styptic, Anti-inflammatory and Analgesic activities), this study is concluded that the study drug *Naaga Sangu Parpam* possesses a good Styptic, Anti-inflammatory and Analgesic activities. Finally concluded that the study drug *Naaga Sangu Parpam* can be used for *Moolam* (Hemorrhoids), *Powthiram* (Fistula in ano), *Vellai* (Leucorrhoea) and *Vettai* (Venereal disease).

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This Certificate is awarded to Dr/Mr/Mrs.....KINGSLEY.J.....

For participating as ~~Resource Person~~ / Delegate in the Twenty First Workshop on

"RESEARCH METHODOLOGY & BIOSTATISTICS"

*For **AYUSH Post Graduates & Researchers***

Organized by the Department of Siddha

The Tamil Nadu Dr. M.G.R. Medical University From 25th to 29th April 2016.

Dr.N.KABILAN, MD(S).
PROF & HEAD
DEPT.OF SIDDHA

Prof.**Dr.P.ARUMUGAM,** M.D.,
REGISTRAR i/c

Prof. **Dr.S.GEETHALAKSHMI,** M.D., Ph.D.,
VICE CHANCELLOR

CERTIFICATE

This is certify that the project title **Standardization and Pharmacological screening of *"Naaga Sangu Parpam (NSP)"*** has been approved by the IAEC. 24 Rats (12 M + 12 F) 24 Mice (12 M + 12 F). Approval No: NIS/IAEC-III/04/29092016

Prof. Dr. V. Banumathi
Name of Chairman/~~Member Secretary~~ IAEC:

Prof. Dr. K. Nachimuthu
Name of CPCSEA nominee:

Signature with date

V. Banumathi 17.11.16
Chairman/~~Member Secretary~~ of IAEC:

CPCSEA nominee:

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by Office)

Name of the PI: Dr. J. Kingsley

Name of the Dept: Gunapadam - Branch II

CERTIFICATE OF AUTHENTICATION OF METAL SAMPLES

Certified that the metal submitted for identification by Dr.J.Kingsley, II year PG Scholar, Department of Gunapadam, National Institute of Siddha,Tambaram sanatorium,Chennai-47 is identified as Zinc metal with below microscopic and macroscopic characteristics based on Danas metal descriptions.

Dr.M.Suresh Gandhi
Assistant Professor
Department of Geology
Guindy Campus, Chennai-600025

Dr. M. SURESH GANDHI, M.Sc.,M.Phil.,Ph.D.,
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Department of Geology
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Guindy Campus, Chennai - 600 025

Date: 31.01.2017

SAMPLE I-ZINC

Symbol	: Zn
Atomic number	: 30
Appearance	: Silver-gray
Streak	: light gray
Crystal structure	: Hexagonal close-packed
Melting point	: 692.68 K (419.53 °C, 787.15 °F)
Boiling point	: 1180 K (907 °C, 1665 °F)
Density	: 7.14
Atomic weight	: 65.39

M. Suh Gandh

Dr. M. SURESH GANDHI, M.Sc.,M.Phil.,Ph.D.,
Assistant Professor
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NATIONAL INSTITUTE OF SIDDHA, CHENNAI – 600047

BOTANICAL CERTIFICATE

Certified that the following plant drug used in the Siddha formulation **Naaga Sangu Parpam** (Internal) taken up for Post Graduation Dissertation studies by **Dr.J.Kingsley** M.D.(S), II year, Department of Gunapadam, 2017, is identified through Visual inspection, Experience, Education & Training, Organoleptic characters, Morphology, Micromorphology and Taxonomical methods as

Pergularia daemia (Forssk) Chiov (Asclepiadaceae), Leaf

MEDICINAL BOTANY ★ National Institute of Siddha ★ CHENNAI 600 047

Certificate No: NISMB2842017

Date: 08-03-2017

Authorized Signatory

Dr. D. ARAVIND, M.D.(s),M.Sc.,
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GOVERNMENT OF INDIA
Ministry of Environment, Forest and Climate Change
ZOOLOGICAL SURVEY OF INDIA

F.No 4-49/2015/Tech./ 24 May 2017

Dr S.M Murugan
Dy. Director
National Institute of Siddha
Ministry of Ayush
Tambaram Sanatorium,
Chennai 600 047

Identification Report

With reference to the molluscan specimen brought to this Centre by Dr. J. Kingsley, Department of Gunapadam, National Institute of Siddha, please find the identity of which is confirmed as *Turbinella pyrum* (Linnaeus, 1767), under Phylum: Mollusca; Class: Gastropoda; Order: Neogastropoda; Family: Turbinellidae.

The voucher specimen submitted may be received from the center. The service rendered by ZSI may be acknowledged appropriately in your reports, thesis and scientific publications.

(Dr. Rajkumar Rajan)
Scientist-D
Officer-in-Charge
Officer in Charge
Marine Biology Regional Centre
Zoological Survey of India
Chennai - 600 028.

Copy to 1. HOD I/C Gunapadam
2. Dr. J. Kingsley, Ilyear, Department of Gunapadam

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