STANDARDIZATION AND PHARMACOLOGICAL PROFILE OF POONEERU CHUNNAM

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"Standardization and Pharmacological profile of *Pooneeru Chunnam*"** is a bonafide and genuine research work carried out by me under the guidance of Dr.S.Sivakkumar M.D(s), Ph.D, Associate professor, Department of Gunapadam, National Institute of Siddha, and Chennai– 47 and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

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Place: Chennai 47 Dr.U.Madhunitha

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **"Standardization and Pharmacological profile of *Pooneeru Chunnam***" is submitted to the Tamilnadu Dr.M.G.R.Medical University, Chennai-32 in partial fulfillment of the requirements for the award of degree of M.D (Siddha) is the bonafide and genuine research work done by Dr.U.Madhunitha under my supervision and guidance and the dissertation has not formed the basis for the award of any Degree, Diploma, Associate ship, Fellowship or other similar title.

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BONAFIDE CERTIFICATE

This is to certify that the dissertation entitled "**Standardization and Pharmacological profile of *Pooneeru Chunnam*"** is a bonafide work done by, Dr.U.Madhunitha a candidate of the National Institute of Siddha, Chennai-47 in partial fulfillment of the University rules and regulations for award of M.D (Siddha) - Gunapadam during the academic year of 2019.

Signature of the Head of the Department

Signature of the Director

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1. INTRODUCTION

Siddha system of medicine is the most primitive medical system, it is usually considered as the oldest medical system known to mankind. Siddha system is an integrated part of Indian system, which is very potent and unique. Siddha medicine works by revitalizing and rejuvenating the organs. The Siddha system helps to correct the dysfunctions responsible for causing the diseases[(1)]. It restores the normal functioning of the organs and maintains the ratio of the *mukkutram- vadham, pittam, kapham*, there providing a healthy state of equilibrium of the body.

According to Siddhars healthy soul can only be developed through a healthy body. So they developed methods and medication that are believed to strengthen their physical body and thereby their souls. The drugs used in Siddha medicine were classified on the basis of five properties.

- *Suvai* (Taste)
- *Gunam* (Character)
- *Veeryam* (Potency)
- *Pirivu* (Class)
- *Mahimai* (Action).

Since this system was bestowed to as a time when science was not developed Siddhars were designed the treatment according to the above mentioned parameters. Siddhars have classified the disease into 4448 types.

Siddha system is focused on "*Ashtamahasiddhi"* the eight supernatural powers. Those who attained or achieved these powers are known as Siddhars. There were 18 Siddhars in olden days and they developed this system of medicine. The Siddhars wrote their knowledge in palm manuscripts, fragments of which were found in parts of south India. Agastyar is considered the first Siddhar and Guru of all Siddhars. The Siddha system is believed to have been handed over to him by Siva[(2)].

The Siddha system strongly advocates proper food habits and hygiene. This system not only aims at curing a disease but also claims the prevention of the disease, maintenance and promotion of health as well said "prevention is better than cure".

Food habit and daily activities of an individual play a major role in causing disease. The physical functions of the body is mediated and maintained by three vital forces. They are *Vali, Azhal* and *Iyam.* In normal state they are called three forces or *Mutthathu* that sustain and nourish the body. In disease state when the forces are vitiated they are called *Mukkutram.* When the three forces are in balance one is healthy. When vitiated singly or combination bring about disease. Emotion and stress also stimulates the *Uyir thathukal* (7 physical constitutions) ending up in a disease.[3]

The treatment aspect involves the neutralization of affected humours.

‘‘விரேசனத்தால் வாதம் தாழும்

வமனத்தால் பித்தம் தாழும்

நசிய அஞ்சனத்தால் கபம் தாழும”.

நோய் நாடல் நோய் முதல் நாடல்- பாகம் 1

Siddha system also considers the human body as a conglomeration of 3 humours, seven basic tissues and the waste products of the body such as faeces, urine and sweat. The food is considered to be basic building material of human body which gets processed into humours, body tissues and water products. The equilibrium of humours is considered as health and its disturbance or importance leads to disease or sickness.

Vatham:

Vatham is associated with elements of space and air, which governs sensory and motor activities and movements in the body and mind.

Pittham:

pittham is associated with the element of fire and is responsible for maintaining the body heat.

Kapam:

Kapam is associated with the elements of earth and water which is responsible for strength and endurance.

The diagnoses of diseases involve identifying its causes. Identification of causative factors is through the examination of Pulse (*Naadi*), Skin (*Sparisam*), Tongue (*Naa*), colour of body (*Niram*), Nature of voice (*Mozhi*), Eyes (*Vizhi*), Bowel habit (*Malam*) and Urine (*Moothiram*).[4]

The drug used by the Siddhars could be classified into 3 groups- *Thavaram* (herbal product), *Thathu* (inorganic substances), *Sangamam* (animal products)[5]. Apart from the vast herbal sources some idea about the depth of knowledge the system possesses in the field of mineral, material medica has been formed from the detailed drug classification, briefly described below:

- There are 25 varieties of water soluble inorganic compounds called *Uppu.*
- There are 64 varieties of mineral drugs. 32 of these are natural and remainings are synthetic.
- There are 32 types of internal medicine like tablet, decoction, *parpam, chenduram*, etc.. and 32 types of external therapy such as non-invasive surgery, setting of bones, cauterization, blood letting, leach therapy, etc..

Saint Thirumular, the one among eighteen Siddhars define medicine as follows

“மறுப்பதுடல் நோய் மருந்தெனலாகும்

மறுப்பதுள நோய் மருந்தெனச்சாலும்

மறுப்பதினி நோய் வாராதிருக்க

மறுப்பது சாவையு மருந்தெனலாமே.”[6]

Dysmenorrhoea is one of the most common gynaecologic disorders and a frequently observed cause of anxiety and discomfort among female adolescents. Dysmenorrhoea is painful menstrual cramps of uterine origin; it is commonly divided into primary dysmenorrhoea (pain without organic pathology) and secondary dysmenorrhoea (pelvic pain associated with an identifiable pathological condition such endometriosis, ovarian cyst, PID, adenomyosis, fibroids, uterine polyps)[7]. primary dysmenorrhoea affects more than 50% post pubescent women in the age group of 18-25 years with ovulatory cycles[8].

Spasmodic dysmenorrhoea is the most prevalent and manifests as cramping pains, generally most pronounced on the first and second day of menstruation. Congestive

dysmenorrhoea manifests as increasing pelvic discomfort and pelvic pain a few days before menses begin. It is commonly seen in PID, IUCD wearers, pelvic endometriosis and fibroids. Membranous dysmenorrhoea is a special group in which the endometrium is shed as a cast at the time of menstruation.

Prostaglandins are released during menstruation, due to the destruction of the endometrial cells, and the resultant release of their contents [(9)]. Release of prostaglandins and other inflammatory mediators in the uterus cause the uterus to contract. These substances are thought to be a major factor in primary dysmenorrhoea. This results in uterine cramping, nausea, vomiting, backache, diarrhoea, giddiness, syncope and fainting. It is responsible for the highest incidence of absenteeism, resulting in loss of work hours and economic loss.

The prevalence of dysmenorrhoea worldwide ranges 15.8-89.5% with higher prevalence rates reported in the adolescent population.[(10)]

In our Siddha system of medicine besides herbals, metals, minerals and animal products have been used to prepare the medicine. Medicine cures disease through bring back the deranged *kutrams* into balanced state.

Nowadays the usage of herbal medicines in tremendously increased because of its therapeutic potency without or less side effects. In traditional siddha literatures so many preparations are available which are more valuable and clinically very effective. Among the drug *Pooneeru Chunnam* is one of the herbo mineral Siddha formulation mentioned in Siddha literature *Anuboga Vaithiya Navanetham*, indicated for Soothagavali (Dysmenorrhoea), Gunmam (Gastric ulcer), Seriyamai (Indigestion). *Soothagavali* is due to alteration of *Vaatha* humour. In our siddha literature to prescribe salt preparations for dysmenorrhoea.

The ingredient of *Pooneeru Chunnam* is *Pooneeru, Veliparuthi* (Percularia deamia) and this medicine is not evaluated so far in the aspect of pharmacological activity. Ingredients of *Pooneeru Chunnam* are easily available and the preparation of the medicine is also cost effective to explicit the safety and pharmacological effect of trial medicine to the scientific world.

Hence, I have chosen *Pooneeru Chunnam* to evaluate the Antispasmodic activity, Anti inflammatory activity and Analgesic activity in animal model.

2. AIM AND OBJECTIVES

Aim:

To evaluate the Anti Spasmodic, Anti Inflammatory and Analgesic activity of "*Pooneeru Chunnam*" in animal model.

Objectives:

- To prepare the *Pooneeru Chunnam* as per standard Opereating Procedure.
- To study the analytical standardization parameters as per AYUSH- PLIM Guidelines.
- To evaluate the Anti Spasmodic, Anti Inflammatory and Analgesic activities as per standard methods.

3. REVIEW OF LITERATURE

3.1 GUNAPADAM REVIEW

பூநீறு

பூநீறு என்பது பூமியில் இருந்து பூத்து வருகின்ற ஒரு வகையான சுண்ணாம்பு படிந்த மண். இதனை பொதுவாக உவர்மண், வண்ணார்மண் என்றும் அழைப்பர். சித்தமருத்துவத்தில் இம்மண்ணிலிருந்து எடுக்கபடும் உப்பு பெரிய மருந்துகளை செய்ய பயன்படுகிறது.

பூநீற்றின் வேறுபெயர்கள்:

வழலை, பூமிநாதம், மாதுநாதம், சக்திநாதம், கரு, சுத்தகங்கை, பிரம்மகற்பம், சாகாமூலி, சவுக்காரம், நாதம், நிலவுப்பு, மண்ணுப்பு, ஆயினாதம், அன்னையுமை நாதம், சுயம்புநீறு, பிறப்பகற்று மூலி, கோவானூர் மண்.

பூநீறு எடுக்கும் விதம்:

"பூவது போல் பூநீறு பூமிவேலி கட்டப் பூக்கும்" பூநீறு பூமியில் இருந்து பூத்து வரும் ஒரு வகை சுண்ணம் படிந்த உப்பு. இது உவர் மண் பூமியில் பங்குனி, சித்திரை, வைகாசி மாதத்தில் பொங்கி வரும். சிவகங்கை, காளாஸ்திரி, மோசூர், புதுக்கோட்டை (வாராப்பூர்), பூம்புகார், திருநெல்வேலி (தருவை), சேரன்மாதேவி (உப்பளூர்). அம்பாசமுத்திரம், திருச்சி போன்ற இடங்களில் கிடைக்கிறது.

தமிழ்நாட்டில் சிவகங்கை மாவட்டம் கோவாணூரில் விளையும் பூநீற்றையே மருத்துவத்தில் மிகவும் சிறப்பாக கொள்கின்றனர். இதனை

"கோவாணூர் கோவிலிலே
குண்டுமணி அம்மன் சந்நிதியிலே
சாகாமூலி ஒன்றிருக்கு அதை
சார்ந்து கொள்ளடி ஞானப்பெண்ணே"

என்ற அடிகளினால் அறியலாம்.

"பார்த்திட்ட பூநீற்றின் பருவங்கேளு
பங்குனியுஞ் சித்திரைவை காசிக்குள்ளே
பூர்த்திட்ட ரவிசுருக்கிற் பொங்கிநீறும்
பூப்போன்மே னிற்குமதை வாரிக்கொள்ளு"

-போகர் 7000

தை, மாசி, பங்குனி, சித்திரை ஆகிய மாதங்களில் தசமியில் இருந்து பவுர்ணமி வரை ஆகிய 6 நாட்களில் பூநீற்றை எடுக்கலாம். இரவில் மூன்று மணி முதல் சூரியோதயம்

வரையில் எடுக்கலாம். உடல் தூய்மை, மனத்தூய்மை ஆகியவற்றை கடைபிடித்து பின் பூநீறு எடுப்பது உத்தமம். பூநீறு எடுக்கப்படும் பூமியானது செம்மண் பூமியானால் ஒரு மூங்கில் குழாயிலாவது அல்லது கண்ணாடி பாத்திரத்திலாவது எடுத்து கொள்ளலாம்.குழியினுள் ஆவி தோன்றி அந்த மூங்கிலுக்குள்ளே சென்று நீராக தங்கிவிடும்.

தை, மாசி,பங்குனி என்று கூறியிருப்பினும் சித்ரா பவுர்ணமி அன்று பூநீற்றை சேகரிப்பது மிகவும் நல்லது. மணி மந்திர அவிழ்தம் செய்வதற்கு சித்ரா பவுர்ணமி அன்று எடுக்கும் பூநீறேசிறந்தது[11].

"பனி பெய்யுங்காலம்

பாருங்களர் மன்னில்

பூநீறாய் புனிதமாய்ப் பிறக்கும்."

விடியற்காலம் சூரியன் தோன்றுமுன் எடுக்க வேண்டும் என்பதை

"சரிவிடியற் காலம்

அந்தமுள்ள பானு

அதுமுகம் படுமுன்

சொந்தமொன்று பூவைத்

தொட்டெடுப்பாய் நீயே."

-போகர் 7000

பூநீறு உப்பு:

சித்திரை வைகாசி மாதங்களில் களர் பூமியில் அதாவது உழமண் விளையும் இடத்தில் பௌர்ணமி தினத்தன்று காலையில் சூரிய உதயத்திற்குமுன் கதிர்போல் ஒரு அங்குலம் முதல் 2 அங்குலம் வரையில் பூத்து கிளம்பி இருக்கும். இந்த பூநீரை மண்படாமல் எடுத்து ஒரு பீங்கானில் வைத்து அல்லது ஒரு மண் பாத்திரத்தில் வைத்து எடுத்துக்கொண்ட பிறகு அதில் சேர்க்க வேண்டிய மருந்தை ஒரு சிட்டிகை கலந்து இரவில் சந்திர ஒளியில் வைக்க வேண்டும்.

-நம் நாட்டு வைத்தியம[12].

பூநீறு தீட்சை (சுத்தி):

பூநீற்றை காடியில் கரைத்து தெளிவு எடுத்து அதனை வெயிலில் உப்பு உறையும் வரை வைக்க வேண்டும். மீண்டும் அதனை காடியில் கரைத்து வெயிலில் வைக்க வேண்டும். இது போல் பத்து முறை செய்ய வேண்டும்.

ஒரு படி (1.3 லிட்) பூநீற்றுக்கு நான்கு படி (5.2 லிட்) பனி நீர் சேர்த்து பாண்டத்திலிட்டு தெளியவிட்டு காலையிலிறுத்துக் கடைந்து ஆடை போக்கி பீங்கான் தட்டுகளிலிட்டு வெயிலில் வைக்க உறைந்து உப்பாகும். இதற்கு தீட்சை செய்தல் என்று பெயர். இதனை,

"பூருவத்தில் சுத்தி பூவெடுக்கும் சித்தி
மாறுந்தேய் பிறையில் மன்னுசிவன் கூறு
பாருமோர் படிக்குப் பனிச்சலந்தா னாலு
மாருயது பாண்டம் அவைதெளியு மட்டும".

"காலையி லிறுத்துக் கடைந்தாடை போக்கி
வாலையிலே விட்டு வற்றுமட்டும் வெய்யில்
சூலதுபோல் காய்ந்து சுண்டியநீ ரெல்லாம்
கோலமிது வாகுங் கொடியமுதல் தீட்சை" (13)

இம்முறையின்படி பத்து முறை தீட்சை செய்து, பளிங்குக் குப்பியில் அடைத்து கொள்ள வேண்டும் என்று கூறப்பட்டிருக்கிறது.

போகர், கீழ்க்காணுமாறு சுத்தி செய்து உப்பாக்கும் வகையை கூறுகின்றார்.

பூநீற்றுக்கு எலுமிச்சம் பழச்சாறு விட்டு கரைத்து தெளிவை வாங்கி அடுப்பேற்றி காய்ச்சி உப்பாக்கிக் கொள்ள வேண்டும். இதனை,

பூர்த்திட்ட ரவிசுருக்கிற் பொங்கி நீறும்
பூப்போன்மெ னிற்குமதை வாரிக்கொள்ளு
ஏர்த்திட்ட எலுமிச்சைச் சாறு விட்டு
இடுத்துமே கரைத்து நன்றாய் தெளிவை வாங்கி
ஆர்த்திட்ட அடுப்புக்குள் வைத்துக் காய்ச்சில்
அடங்கியே யுப்பாக்கும் பருவம் வாங்கே

-போகர் 7000

குணம்:

பூநீற்றால் கடுவன், சீதளம், வாயு, வலி குன்மம் நீங்கும்.

இதனால் பேதியாகும் என்க.

செய்கை:

ஆம்ல நாசினி, மூத்திரவர்த்தனகாரி.

பயன்:

பூநீறும் கற்சுண்ணாம்பும் சமனெடை சேர்த்து தெளிநீர் வாங்கி, அதில் ஆமை ஓடு, முட்டை ஓடு, முத்துச்சிப்பி, கல்நார், நண்டுக்கல், சங்கு, முதலிய பொருட்களை இட்டு எரித்து கழுவி எடுக்க சுத்தியாகும்.இது பாடாணங்களை சுத்தி செய்யவும் பயன்படும்.

பூநீற்றை வெந்நீரில் கலந்து அந்நீரில் வாதம் கண்ட குதி காலை சில நிமிடம் அமிழ்த்தி வைத்தெடுக்க நீங்கும்.

ஐந்து உப்புகளுள் இவ்வுப்பு, ஆகாச கூற்றுப்பாய் சேர்க்கபட்டிருக்கின்றது.

இவ்வுப்பு குன்மத்திற்கு வழங்கப்படும் குன்மக் குடோரி மெழுகிலும், அசீரண பேதிக்காக வழங்கப்படும் தயிர்ச்சுண்டி சூரணத்திலும் சேர்க்கபட்டிருக்கின்றது.

இதனை கொண்டு ஊசர பற்பம் செய்யபடுகின்றது.

"கரப்பானைச் சீதத்தைக் கண்டிக்கும் பேதி
யுரப்பாக்கும் வாயுதனை யோட்டும்- சுரப்பாக்கும்
உந்திவலி குன்மம் ஒழிக்கும்பூ நீறெனவே
செந்தா மரைமுகத்தாய் செப்பு." (13)

- **- பதார்த்த குண விளக்கம் .**

இதிலிருக்கும் எண்ணெய் என்று சொல்லப்படும் வழுவழுப்பு பண்பை போக்கி சுண்ணமாக்கி, மருந்துகளுக்குக் காரமுண்டாக்கும் பொருட்டு எல்லா மருந்துகளிலும் சேர்க்கின்றனர்.

இதனை கொண்டு செய்யப்படும் சுண்ணம், செயநீர், முப்பு மார்க்கம், இவைகளை

- ஞான வெட்டியான்
- நவரத்தின வைத்திய சிந்தாமணி,
- பஞ்சரத்தினம் 500,
- போகர் ஏழாயிரம் இரண்டாம் காண்டம்,
- சட்ட முனிவாத காவியம்,
- ஏணி ஏற்றம், போன்ற நூல்களில் காணலாம்.[11]

வேலிப்பருத்தி - *Pergularia daemia*[14]

Synonyms:

- *Uthamani*
- *Uthamamaakani*
- *Thamakannigal*

Botanical name: *Pergurlaria daemia* (forsk) chiov.

Eng : Dog's bane, whitelow plant

Tel : Juttu-paku, dushtupu- chettu

Mal : Velip- parithi

Kan : Hala koratige

Sans : Phala antaka

Hindi : Utran

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 *Veliparuthi.*

Parts used:

Leaf, Root.

Organoleptic characters:

Taste : Bitter

Nature : Hot

Division : Hot

Action:

- Expectorant
- Anthelmintic
- Emetic.

உத்தாமணி

“வேலிப்பருத்தி முழு தாலாற் பற்றாதுவேலிப்பாரு”

Veliparuthi contains lead content.

“சீதை முத்திருக்கஞ் செவிவெள்ளச் சார்வேளை
பாதுகைவே லிப்பருத்தி முஸ்தையும்- கோதில்சுரை
சீந்தில் விழுதி சிறுபீளை வெள்ளறுகும்
ஏந்திழயீ ரீய மூலி.”

-குணப்பாடம் தாது ஜீவ வகுப்பு

General characters

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

-அகத்தியர் குணவாகடம்

உத்தா மணியிலையால் உள்வயிற்றுக் குன்மமொடு
குத்தாம் வலியுங் குளிரும்போம் - பற்றி
இசிக்கும் வலியிரைப்பும் எத்தடிப்பும் ஏகும்
பசிக்கும்மதி 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.

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

Medicinal Uses:

- Decoction of the leaves is given to children as an Anthelmintic.
- In one to two ounce doses it is a good expectorant.
- Decoction or juice of leaves is useful also in asthma and snake bite.
- Powdered leaves in doses of 5 to 10 grains are also good expectorant.
- Externally the juice combined with lime is applied to rheumatic swellings.
- A mixture of the juices of these leaves on the palms of hands is a stimulating emetic.
- Honey is also added to the decoction of the leaves to help the expectorant effects.
- Combined with ginger, the juice of the leaves is given for rheumatism.
- Fresh leaves made into a pulp are used as a stimulating poultice in carbuncle.
- Juice of the leaves is employed in the preparation of medicinal oil used in rheumatism, amenorrhoea and dysmenorrhoea and the root bark is used as a purgative in rheumatic cases in doses of 1 to 2 drachmas mixed with cow's milk.
- The plant is extensively used in Bombay presidency for its emetic and expectorant properties.
- The decoction is used to treat liver problems, diarrhoea, dysentery, colic, painful joints and limbs, cramps in the legs, malaria, appendicitis, amenorrhoea and venereal diseases.
- The crushed leaves, or sometimes the crushed young fruits, are applied externally to boils, abscesses, subcutaneous worm infections and eczema.
- The latex is applied to sore eyes and teeth ache.
- An infusion of the roots is taken against stomach ache, cough, and as an abortifacient.
- The roots may also simply by chew to treat cough.

Other uses of root:

The root of the Pergularia is one of the ingredients of *kaayathirumeni thylam, kaayaraajangam* and *kaayasarvangam oil.*and also in decoction of *rajasanjeevi* and *kabha sanjeevi.*

Preparations:

Decoction of the leaves- dose	:	1 ounce
Juice of the leaves – dose	:	1 drachm
Powder of the root or root bark	:	5 to 10 grains.

காடிநீர்[15]

காடி, புளித்த நீர். இனிய பழவகைகள், அரிசி வகைகள், சர்க்கரை, தேன் முதலிய வஸ்துகளை புளிக்க வைத்து அவைகளினின்று இறக்கும் நீர். இதற்குப் பொதுவாக காடி என்று பெயர். இது குளிர்ச்சியுண்டாக்கி, வயிற்றுக் கிருமிகளை போக்கும்.

காடி நீர்: கழுநீரை வெயிலில் வைத்து வடித்தெடுக்கப்படும் புளித்த தண்ணீர்.

(rice- water fermented by exposing it to the sun).

இதை சர்க்கரையோடு சேர்த்து சுரத்திற்கு உபயோகிக்க தாபத்தை குறைத்து குளிர்ச்சியை தரும். இதை தீச் சுட்ட புண், கொதிநீர் புண், வழுவுண்ட தோல், இவைகளில் மேற் பூச குணமுண்டு.

ஐரோப்பாவில் கோதுமையை புளிக்க வைத்தும், திராட்சை ரசத்தினின்றும் காடி எடுப்பதுண்டு. இக்காடி, ஊழித் தசை ரணம், மார்புக் கட்டி, சுளுக்கு முதலியவைகளுக்கு உபயோகப்படும்.

நமது நாட்டில் பனங்கள், தென்னங்கள் இவைகளிலும், கருப்பஞ்சாற்றினின்றும் காடி எடுப்பார்கள். உஷ்ண தேசத்தில் காடி வெகு சீக்கிரத்தில் பக்குவமடையும். சுளுக்கு, தலைநோய், விஷக்கடி முதலியவைகளுக்கு வலியுள்ள இடங்களில் கந்தையில் நனைத்துப்போட குணமாகும். சூதக சம்பந்தத்திலும், மூக்கில் ஏற்படும் இரத்த ஒழுக்கிற்கும் மேற்கண்ட மாதிரி செய்யலாம்.

சுடுகஞ்சியுடன் சேர்த்துக் குடிக்க, வியர்வையைத் தரும். கடலை புளிப்புக் காடி தான் மருந்திற்கு மிக்க விசேஷமானதென கருதப்படும்.

காடி எடுக்கப்படும் பொருட்களை கொண்டு பல வகையாக கூறப்படும்.

- பழக் காடி - Fruit vinegar
- அரிசிக் காடி - grain vinegar
- கள்ளுக் காடி - toddy vinegar
- சர்க்கரைக் காடி - sugar vinegar
- கடலைக் காடி (புளிப்பு) - Bengal gram vinegar
- ஈச்சங்காடி - date vinegar
- பனங்காடி - palm vinegar
- தென்னங்காடி - coconut vinegar
- திராட்சை காடி - grape vinegar

- கஞ்சிக் காடி - rice vinegar
- ஆறுமாதக் காடி - six months| vinegar
- சீமைக் காடி - malt vinegar.

காடிக் கூழ்: புளிக்க வைத்த கூழ்.

காடிச் சத்து: புளித்தக் காடியை வாலையிலிட்டு இறக்கப்படும் போதையுள்ள சாரம் அல்லது சத்து,

The intoxicating principle obtained from fermented liquors- alcohol.

காடிச் செயநீர்: இளநீரையும், இலவழுக்கையும் சேர்த்துக் கரைத்து தெளிவிறுத்து, அத்துடன் சுண்ணாம்பு நீர் விட்டு கலக்கி, சூரிய புடத்தில் வைத்துப் பிறகு தெளிவிறுத்து வடித்தக் காடி. இது செந்தூரம் செய்ய உதவும்.

A strong pungent fermented liquid filtered after exposing to the sun rays a solution of lime water and the coconut extract obtained from a mixture of tender coconut and its water. This is used for preparing red oxides.

காடிச் சோறு: புளித்த கஞ்சி, sour gruel; புளித்த கஞ்சியினின்று எடுக்கப்படும் சோறு. இச்சோற்றை அரைத்து கட்டிகளுக்குப் பூச பழுக்கும்.

Rice taken out of *sour conji*. This rice when made into a paste and applied to abscess is said to ripen it.

சொண்டிச் சோறு: '*sonti soru'* an intoxicating liquor extracted from the fermented of boiled rice.

காடித் தெளிநீர்: நோய்களுக்குக் கொடுக்கவும், புடம் போடுவதற்காக வேண்டி மருந்துகளை அரைப்பதற்க்கும் உபயோகப்படுத்த வேண்டி தெளிய வைத்த காடிநீர். இது கருங்குருவை அரிசியினின்று போகர் நூலின் முறைப் படி தயாரித்த பழச்சாறு என்னும் ஆறு மாதத்தியக் காடி.

Vinegar from black karuvai or dark-red paddy allowed to ferment in the summer after exposure to the sun and then filtered after six months according to the process laid down in Bogar works.

காடி செய்முறை:

ஒரு படி பச்சையரிசி அல்லது கருங்குருவை அரிசியை ஆற்று நீர் விட்டு 6 அல்லது 7 தடவை களைந்து தூயதாக்கி சமையல் பாத்திரத்திலிட்டு அதில் ஆற்றுநீர் அரிசின் மேல் நாலங்குலம் நிற்கும்படி ஊற்றி அடுப்பேற்றிப் பாகப்படி பொங்கிப் பக்குவமாய் சமைத்து சூட்டுடன் இருபதுபடி நீரிற்போட்டு மூன்று நாள் வெயிலில் மூடி வைத்து நாலாம் நாள் எடுத்து நன்றாய் பிசைந்து, அந்த நீரை வடிகட்டி கொள்ள வேண்டும். பின்னர், இந்த நீர் கொள்ளும்படியான நான்கு பெரும் பானைகளைத் தயாரித்து தூய்மை செய்து வைத்து கொள்ளவும். முன் தயாரித்த நீரை பாண்டங்களிலொன்றில் ஊற்றி, வாய்க்கு வேடு கட்டி வெயிலில் வைக்க வேண்டும். மறுநாள் மறுபாண்டத்தில் ஊற்ற வேண்டும். முன்னால் நீரை வார்த்து வைத்திருந்த பாண்டத்தை வெயிலில் ஈரம் வற்ற உலர்த்தி வர வேண்டும். அப்போதைக்கப்போது அந்தந்தப் பாண்டங்களையும் வெயிலில் வைத்து கொஞ்சமும் ஈரமில்லாமல் உலர்த்தி வர வேண்டும், இவ்விதம் 40 நாட்கள் செய்து கண்ணாடிக் குப்பியில் அடைத்து மூடியிட்டு ஆறுமாதம் வைத்திருந்து பின்பெடுத்து உபயோகிக்க வேண்டும். இதில் ஆரம்பத்தில் கொஞ்சம் கற்சுண்ணம் சேர்ப்பதனால், புழு முதலியவை உண்டாகாது.

வேறுமுறை

பச்சரிசி 2 படி, இதனைத் தவிடு முதலியவை இல்லாமல் தூய்மைபடுத்தி வைத்து கொள்ள வேண்டும். முள்ளங்கி இலை 1 வீசை (1400 கிராம்) இவ்விரண்டையும் சேர்த்து உரலில் நன்றாயிடித்து, 24 அல்லது 30 படி நீர் கொள்ளும்படியான ஒரு மட்பாண்டத்தில் இட்டு, அதில் 12 படி நீரிட்டு பாண்டத்தின் வாயை ஒரு மண் மூடியால் மூடி, பெரிய அடுப்பின்மேல் வைத்து கீழே தீ போட வேண்டும். விளக்கின் சுடர்போல சிறு தீயால் இரவு பகல் ஓயாமல் ஏழு நாட்கள் எரித்து எட்டாம் நாள் தீயை நிறுத்தி, மறுநாள் பிரித்துப் பாண்டத்திலுள்ள நீரை மாத்திரம் இறுத்து உரத்த துணியில் வடிகட்டிக் குப்பியில் நிறைத்து, மூடியிட்டு வைத்து நான்கு அல்லது ஆறு மாதங்களுக்குப்பின் பயன்படுத்தலாம். முன்னே கூறிய பாண்டத்தில் நீர் மாத்திரம் விட்டு வாய்புறத்தை துணியால் மூடி எரித்து நீரை இறுத்து கொள்ளலாம்.இதுவும் காடியாகவே இருக்கும். இவ்விதம் அந்தப் பாண்டத்தில் ஒருமுறை இட்ட பொருட்களிலிருந்தே ஓர் ஆண்டு வரையில் காடிநீர் எடுத்துக் கொண்டிருக்கலாம்.

வேறுமுறை

நம் நாட்டு வைத்தியத்தில் கூரிய காடி செய்முறை இந்த பூநீறு சுண்ணம் செய்முறையில் பயன்படுத்தப்பட்டுள்ளது. அதனை பின் வருமாறு காணலாம்.

ஒரு பெரிய மண்பாண்டத்தில் கால் படி பச்சரிசியை சமையல் செய்து கஞ்சியும் சாதமும் இரண்டையும் கொட்டி 6 படி சுத்த நீர் விட்டு துணியால் வாய்கட்டி சூரிய புடத்தில் வைத்து வர வேண்டும். வாரத்திற்கு ஒரு முறை வேறு பாத்திரத்தில் மாற்றிவிட வேண்டும். இப்படி ஒரு மாதம் கழித்த பின் பார்க்க முதலில் போட்ட அன்னமானது காணப்படாது.அந்த சமயம் மறுபடியும் கால்படி அன்னம் சமைத்து அதில் கலந்து விட வேண்டும்.கலந்த பின்

நாள் தவறாமல் சூரிய புடத்தில் வைக்கவும்.வாரத்திற்கு ஒரு முறை பானையை மாற்றுவதும் முக்கியமாய் கவனிக்க வேண்டும்.இதன் படி மாதம் ஒருமுறை அன்னம் சமைத்து கலந்து கொண்டுவர வேண்டும்.இப்படி செய்து கொண்டு வரும் காலத்தில் 3 மாதத்துக்குமேல் அந்த காடியை உபயோகித்து வரலாம்.எவ்வளவு காடி எடுத்துக் கொள்கின்றோமோ அவ்வளவு நீரை அதில் கலந்து விட வேண்டும்.இப்படி கலந்து வருவதால் எத்தனை ஆயிரம் பேருக்கும் நாம் கொடுத்தாலும் காடி குறைந்து விடாது.

ஆறு மாதத்திற்குப்பின் காடியில் அதிக புளிப்பு ஏறிவிடுமாகையால் 4 நாளைக்கு ஒரு முறை பானையை மாற்றுதல் வேண்டும்.

மழை காலத்தில் சூரியபுடத்தில் வைக்க இயலாத காரணத்தால் காடியில் பூசணம் பிடிக்காதபடி தடுக்க சமையல் செய்த பிறகு அடுப்பிலுள்ள நெருப்பை எடுத்து விட்டு அடுப்பில் காடியை வைத்து விட வேண்டும். இதன் படி செய்ய காடி கெடாமல் இருக்கும்.

காடிக்கு அதிகநாள் ஏற ஏற புளிப்பு அதிகரிக்கும், புளிப்பு அதிகரிக்க கொடுக்க வேண்டிய அளவை குறைத்தல் வேண்டும்.

ஆறு மாதத்திற்கு மேற்பட்ட காடியானது பௌர்ணமி தினத்தில் இரவில் ஒருவித சப்தத்தை உண்டு பண்ணும்.

வயிற்றுவலி, நீர்கடுப்பு, மார்புவலி, உஷ்ணபேதி, அஜீரணபேதி, விஷபேதி முதலிய வியாதிகள் நீங்கும்.சரீரத்தில் உள்ள வெக்கையை போக்கும[10].

காடியைக் கொண்டு செய்யப்படும் சுத்தி முறைகள்:

- முத்தை எலுமிச்சம் பழச்சாற்றிலும் காடியிலும் ஒரு நாள் ஊற வைத்து நீர்விட்டுக் கழுவி எடுக்க சுத்தியாகும்.
- இரும்பின் பொடியை எலுமிச்சம் பழச்சாறு, காடி, நாட்டு காட்டாமணக்குப்பால் இவை ஒவ்வொன்றிலும் மூன்று நாள் ஊற வைத்துக் கழுவியெடுக்கச் சுத்தியாகும்.
- காந்தத்தைப் பொடித்து முடிகட்டி காடியிலும் கொள்ளுக் குடிநீரிலும் முறையே அவித்து எடுத்துக் கழுவி உலர்த்திக் கொள்ளச் சுத்தியாகும்.

3.2 MODERN ASPECTS

FULLER'S EARTH[17), (18)]

There is a medley in naming the salt (and of the soil) as Fuller's Earth. The correct name would be dhobies soil / sand as this would correctly indicate the mineral cited by the author. This will be salty and alkaline.

Fuller's earth is usually highly plastic, sedimentary clays or clay-like earthy material used to decolorize, filter, and purify animal, mineral, and vegetable oils and greases.

In India this sand is used by washer men (dhobis) for cleaning and Bleaching the clothes. Usually heavy alkaline sand is collected by the dhobis' in appropriate season and used for cleaning purposes.

Chemical Composition of Fuller's Earth:

Fuller's earth usually has a high **Magnesium oxide** content. Fuller's earth mined, mainly comprise the minerals **Montmorillonite or Palygorskite** (Attapulgite) or a mixture of the two; some of the other minerals that may be present in fuller's earth deposits are calcite, dolomite, and quartz.

Chemical analysis of different samples of Fuller's earth show wide range of variation. This depends upon the geological location of the sample. The constitutions and biochemical vary to a great extent. Only thing to differentiate from the other clay is by its salty and alkaline nature. Like all other clays, fuller's earth is a hydrous, aluminium silicate containing small Proportion of other substances. Most fuller's earths contain a higher Percentage of water composition than other clays.

Properties of Fuller's earth:

- Nonplasticity
- Disintegrating in water
- Detergent action
- Large water content
- Property of adhering to the tongue

- Porosity
- Salty
- If the sample is touched with a neutral litmus paper the paper will turn red.
- If the clay is suspended in water and phenolphthalein is added-red Colour appears
- Specific gravity of fuller's earth is much the same as that of other clays.

Medicinal Uses:

- In pharmacy it makes an excellent substitute for talcum powder on account of its absorptive powers.
- Certain earths are even claimed to have special virtue as a poultice for Swellings, ulcers and sores.
- Arsenic compound may be purified with the solution.
- Fuller's earth is mixed with hot water. To curing the arthritis in the ankle joint and the foot is kept in the above solution for sometimes.

***Pergularia daemia* (*Veliparuthi*)**[19]

General information:

It is a slender, hispid, fetid- smelling perennial climber. Leaves opposite, membranous, 3-9 cm long and about as wide, broadly ovate, orbicular or deeply cordate, acute or short-acuminate at apex, pubescent beneath, petioles 2-9 cm long. Flowers greenish-yellow or dull white tinged with purple, borne in axillary, long-peduncled, drooping clusters. Fruits (follicles) lanceolate, long-pointed, about 5 cm long, covered with soft spines and seeds are pubescent, broadly ovate. Flowering may occur each year between August and January in central India, with fruits maturing from October to February. In central Indian deciduous forests, the stems typically die down in February and reappear with the onset of the rainy season.

Scientific classification[20]

Kingdom : Plantae
Order : Gentianales
Family : Asclepiadaceae
Subfamily : Asclepiadaceae
Genus : *Pergularia*
Species : *Pergularia daemia*

Vernacular Names[21]

Bengali : Chagulbanti, Changulbati
Hindi : Utranajutuka, Utran, Dudhi, Dudhibel, Jutuk, Sagovani
Kannada : HaaluKoratige, Hala koratige, Juttute balli, Kurudig
Malayalam : Veliparatti, Veliparuti
Marathi : Utaranavel, Uturhi
Oriya : Juktiruhi, Uttruri, Uturdi
Sanskrit : Uttaravaruni, Kurutakah, Yugaphala, Yugmaphala
Tamil : Beliparti, Nandamani, Uthamani, Veliparuthi
Telegu : Dushtupatige, Gurtichettu, Guruti, Jittupaku

ACTIONS:

- Emmenogogue
- Emetic
- Antiseptic
- Expectorant
- Anti rheumatic
- Abortifacient
- Antipyretic

Phytochemical properties

Terpenoids, Flavonoids, Sterols and Cardenolides are among the chemicals that have been isolated from either the leaves, stems, shoots, roots, seeds or fruit.

Traditionally it has been used as an anthelmintic, laxative, antipyretic and expectorant, besides treatment of infantile diarrhoea, malarial fever, intermittent fever, toothache and cold.

Chemical constituents[22]

Plant - lupeol, sterol, hentriacontane, α- amyrin, β-amyrin, β- sito Sterol.

Root - β- sitosterol and its glocoside, α- amyrin and its acetates, Calcitin.

Seeds - calcitin, calotropin and caloteropagenin.

Stems - uzarigenin, Coroglucigenin.

VINEGAR (ACETIC ACID) [23], [24]

Vinegar is a liquid substance consisting mainly of acetic acid (CH3CO2H) and water. The acetic acid is produced through the fermentation of ethanol by acetic acid bacteria. It is today mainly used in the kitchen as a general cooking ingredient, but historically, as the most easily available mild acid, it had a great variety of industrial, medical and domestic uses, some of which (such as a general household cleaner) are still promoted today.

Commercial vinegar is produced either by fast or slow fermentation processes. In general, slow methods are used with traditional vinegars, and fermentation proceeds slowly over the course of weeks or months. The longer fermentation period allows for the accumulation of a nontoxic slime composed of acetic acid bacteria. Fast methods add mother of vinegar (i.e., bacterial culture) to the source liquid before adding air using a venture pump system or a turbine to promote oxygenation to obtain the fastest fermentation. In fast production processes, vinegar may be produced in a period ranging from 20 hours to three days.

TYPES OF ACETIC ACID OR VINEGAR:

- Apple cider vinegar
- Balsamic vinegar
- Beer vinegar
- Cane vinegar
- Coconut vinegar
- Distilled vinegar
- East Asian black vinegar
- Flavoured vinegar
- Fruit vinegar
- Honey vinegar
- Job’s tears vinegar
- Kiwifruit vinegar
- Kombucha vinegar
- Malt vinegar
- Palm vinegar
- Raisin vinegar

- Rice vinegar
- Seamark vinegar
- Spirit vinegar
- Sherry vinegar
- White vinegar
- Wine vinegar

3.3 SCIENTIFIC REVIEW

TOXICITY STUDY

Pooneeru Chunnam

Acute toxicity study:(OECD Guideline- 423) [25]

In acute oral toxicity study, at different dose of Levels in all groups did not show any adverse effects or mortality. There was no change the behavioural pattern also.

Even the administration of 3200mg of the drug did not produce any mortality. Higher dose or the lethal has not been tried out.

Chronic toxicity study: [26]

Usually siddha drugs are administered for a long time in order to cure the chronic diseases. So chronic toxicity study in animals is essential. The dose levels of 200mg and 400mg were given to two groups. Each consisting of five albino rats was considered for this study. Duration of administration was 90 days. The two dose levels, 200mg and 400mg produce only little malformations and pathological changes.

There is no significant or notable variations produced in the haematogical evaluation also.

Bio statistical measures to the acute and chronic toxicity of the drug are safe up to 3200mg/100gm body weight. Lethal dose of the drug cannot be calculated as there is no mortality.

PHARMACOLOGICAL STUDIES:

Pergularia daemia;

Acute oral toxicity study of *Pergularia daemia*[27]

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* (forsk.) was determined by using wistar albino rats of either sex weighting between 120±02gm 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* and observed for its mortality upto 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.

Analgesic activity and anti-inflammatory activity[28]

Vk Bhaskar et el., studied the analgesic activity of *Pergularia daemia* in animal model. The ethanol and aqueous extracts from roots of *Pergularia daemia* exhibited significant analgesic and anti-inflammatory activities at the doses of 100 and 200mg/kg body weight.in analgesic activity, the highest reaction time was observed(9.8sec) from ethanol extracts of *Pergularia daemia* at a dose of 200mg/kg body weight. The ethanol and aqueous extracts of *P.daemia* was found to reduce significantly the formation of edema induced by carrageenan after 2 hrs.

Anti inflammatory and anti arthritic activity: (*Pergularia daemia*)[29]

The invitro anti- inflammatory and anti arthritic activity of leaves and roots of *Pergularia daemia* by membrane stabilization assay, protein denaturation assay was performed. Three different concentrations of ethanolic extract of leaves and roots were used in this study. In membrane stabilization assay, the maximum stabilization was observed in ethanolic extract of leaves than roots. Similarly, in the protein denaturation assay, the ethanolic extract of roots showed maximum inhibition than leaves. Thus we concluded that the ethanolic extract of the leaves of *Pergularia daemia* possess strong anti inflammatory and anti arthritic activity than the roots.

Analgesic activity: (*Pergularia daemia*)[30]

Lokesh T Nikajoo et el., studied the analgesic activity root extract of *Pergularia daemia* in animal model. The present study was undertaken to evaluate the analgesic activity of the aqueous and alcohol root extracts of *Pergularia daemia* (forsk) chiov. Using Eddy's hot plate and Heat conduction method. In Eddy's hot plate method the aqueous extract showed significant analgesic activity at the doses of 500mg/kg ($p<0.01$) and 1000mg/kg ($p<0.001$) and alcohol extract showed significant analgesic activity at the doses of 500 and 1000mg/kg ($p<0.001$). In Heat conduction method both extracts showed significant analgesic activity at the doses of 500 and 1000mg/kg ($p<0.001$) as compared to control group, when analysed statistically by Tukey Kramer multiple comparison test.

Phytochemical study – *Pergularia daemia*[31]

The present study aims at comparative analysis of qualitative and quantitative phytocomponents present in the leaves of *Pergularia daemia* in different solvents. The methanolic extract of leaves showed the presence of high number of phytocomponents when compared with ethanol, petroleum ether, chloroform and aqueous. Presence of alkaloids, steroids, terpenoids, saponins, phenols, tannins, glycosides, amino acids, proteins, carbohydrates and reducing the sugars.it has high amount of tannins and they play a major role in the treatment of intestinal disorders like diarrhoea and dysentery. The leaves also have flavonoids which play a major role as antioxidant. Thus , the study reveals the presence of various medicinally valued bioactive components of *Pergularia daemia* which has many curing abilities.

Hepatoprotective activity[32]

S.V. Sureshkumar et el., studied the hepatoprotective activity in animal model. To study the hepatoprotective effect of crude ethanolic and aqueous extracts from the aerial parts of *Pergularia daemia*. The ethanolic extract at an oral dose was evaluated for hepatoprotective activity in rats by inducing liver damage by carbon tetrachloride. The ethanolic extract at an oral dose of 200mg/kg exhibited a significant protective effect by lowering serum levels of glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, alkaline phosphatase, total bilirubin and total cholesterol and increasing the levels of total protein and albumin levels as compared to silymarin used as a positive control. Furthermore, the acute toxicity of the extracts showed no signs of toxicity up to dose level of 200mg/kg.

Diuretic activity[33]

Pergularia daemia was extracted with 50% alcohol and a fresh batch of the plant material was successively extracted with petroleum ether, ethyl acetate and n-butanol to determine its diuretic activity. The diuretic activity of the different extracts at a dose of 400mg/kg was assessed orally in rats with furosemide as a standard drug using lipschitzs test. All extracts except the petroleum ether extract showed significant increase ($p<0.001$) in urine output. The alcoholic, ethyl acetate and n-butanol extract caused an increase in the urinary excretion of sodium and potassium ions. These findings suggest that among the mentioned extracts, ethanolic extract has the maxium diuretic activity followed by n-butonal extract.

Assessment of antioxidant potential and acute toxicity studies of whole plant extract of *Pergularia daemia* (forsk.)[34]

Matured *P. daemia* plant was collected from river banks of Pudukottai District, Tamil Nadu. 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 medical 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 and in-vitro study revealed that the methanolic extract showed higher antioxidant activity at 400 µg/ml than ethyl acetate extract of *Pergularia daemia.*

Antioxidant and Antimicrobial capacities of ethanolic extract of *Pergularia daemia* leaves: a possible substitute in diabetic management[35]

The leaves of *Pergularia daemia* provide alternative plant- based treatments for the management of diabetes mellitus. This study therefore sought to investigate the anti-hyperglycaemic activity of the 70% ethanolic extract of *P.daemia* using streptozotocin (STZ)- induced diabetic male spargue- Dawley rats. The total phenolic content, total flavonoids content, radical scavenging activity and reducing power assays were estimated using Folin- Ciocalteu method.

The result showed that *P. daemia* extract caused anti- hyperglycaemic activity in STZ-induced rats at doses of 30, 60 and 90mg/kg body weight with significant reduction in blood glucose levels. The phytosterols, saponins, phenols, alkaloids, tannins and triterpenes found in the extract may be responsible for the observed anti- hyperglycaemia and antioxidant activities. The extract also showed antimicrobial activity against Escherichia coli, pseudomonas aeruginosa, staphylococcus aureus and bacillus sabtilis.

Anti bacterial activity of a novel quinine from the leaves of *pergularia Daemia*, a traditional medicine.[36]

The anti bacterial activity of crude extract was determined using the disc diffusion method and the minimum inhibitory concentrations (MIC) of the isolated compound was tested by the broth microdilution method. The crude hexane, chloroform and ethyl acetate extracts of *P. daemia* leaves were tested for antibacterial activity. The ethyl acetate extract showed growth inhibitory activity against Bacillus subtilis (15mm), staphylococcus aureus (17 mm) and proteus vulgaris (20 mm) at 400 µg/ disc. The minimum inhibitory concentrations for the ethyl acetate crude extract were determined against staphylococcus aureus (300µg\ml), bacillus subtilis (200µg\ml) and proteus vulgaris (500 µg\ml). The results revealed the importance of the compound, 6-(4, 7-dihydroxy-heptyl) quinine, as a novel agent with significant antibacterial activitity. Several benzoquinones, naphthoquinones and athraquinones have also shown antibacterial activity.

Pooneeru dravagam[37]

Acute and subacute toxicity study

Acute toxicity study was carried out in healthy wistar albino female rat. The study was carried out in three female rats under fasting condition; signs of toxicity were observed for every one hour for 24 hours and every day for fortnight from the beginning of the study. Sub acute toxicity study was carried in swiss albino rats at two dosage levels 0.2 ml and 0.4 ml of 28 days continuous drug administration (oral route).

The result of sub-acute toxicity on 29^{th} day did not show any evidence of toxic changes. Physiological, haematological as well as histopathological parameters remained unaltered when compared with control animals throughout the dosing period.

Karunguruvai khadi[38]

The composition of *karunguruvai* rice was compared with samba rice and it is indentified the absence of heavy metals in *samba* as well as *karunguruvai* rice. The bacterial and fungal activities were assessed during the different stages of *khadi* preparation- no activities were found in any form of the *khadi*. This suggests that *khadi* may have a preventive effect against fungal and bacterial infection. The result of the chemical analysis of *khadi* extracts showed that *khadi* prepared from the *karunguruvai* paddy grains was the best base solvent for iron exchange in the preparation of *muppu* than *samba khadi*. *Karunguruvai khadi* show that it is a good solvent for the eliminaton of heavy metals. These characteristics enhance the therapeutic potential and safety of the drugs for healing chronic diseases.

4. MATERIALS AND METHODS

Standard operative procedure for preparation of *"Pooneeru Chunnam"* [39]

The test drug *Pooneeru Chunnam* is a herbo mineral Siddha preparation mentioned in Siddha literature *Anuboga Vaidya Navaneetham* which is indicated for S*oothagavali* (Dysmenorrhea), *Gunmam* (Gastric ulcer) and *Seriyamai* (Indigestion).

Ingredients

- Purified *Pooneeru*
- The fresh juice of *Veliparuth*i (Pergularia daemia)

Collection of Raw drugs:

The *Pooneeru* was collected from Suththamalli Village, Uthramerur, Tamil Nadu. The herb *Veliparuthi* (*Pergularia daemia*) was collected from in and around Pudukkottai District, Tamil Nadu. All the ingredients were purified and the medicine was prepared in the Gunapadam Laboratory of National Institute of Siddha.

Identification and authentication of the drug:

The *Poonneru* was identified and authenticated by competent authority of Gunapadam Department, National Institute of Siddha, Tambaram sanatorium, Chennai.

The herb *Veliparuthi (Pergularia daemia)* was identified and authenticated by Botanist, National Institute of Siddha, Tambaram Sanatorium, Chennai.

Purification:

The earth is considered to be a source of valuable drugs. *Pooneeru* not only enriched with pharmaceutically useful compounds but also contain toxic materials. One of the most important tasks is removal of these toxic substances, which is called purification (*suddhi)* of raw materials by Siddhars. Otherwise it may be results in toxicity. *Suddhi* contributes the following changes in the raw drug;

- Elimination of unwanted / toxic elements
- Reduction in particle size.
- Conjugation of trace elements
- Formation of desirable compounds
- Enhance the therapeutic potency

***Pooneeru*:**[40]

The *Pooneeru* collected from the field is to be purified in order to get it's almost efficacy. Sufficient quantity of *Pooneer*u was taken in an earthen pot and dissolved in required quantity of *Kaadineer* (vinegar). This was agitated well with a bamboo stick. This process was done thrice a day for three consecutive days. Then it was filtered in a cotton cloth and then it was poured in a porcelain plate and kept in Sun and Moon to obtain the salt.

Then the salt was dissolved with sufficient quantity of *Kaadi neer*. The process was repeated for ten times and this supremely purified *Pooneeru* was obtained.

***Veliparuthi* (*Pergularia daemia*):**

Veliparuthi was washed in running tap water and the soil and impurities were removed.

Preparation of *Pooneeru Chunnam*:

Procedure:

The purified *Pooneeru* was placed in stone mortar and powdered well. The fresh juice of *Veliparuthi (Pergularia daemia)* was instilled over the *Pooneeru* powder and ground well for six hours (2 *Samam*). Then the pellets (*villai*) were made and dried in sun shadow. These pellets were subjected to *pudam* with 300 number of cow dung cakes. This process was repeated for two more times and the *Pooneeru Chunnam* was obtained finally the *Chunnam* was powdered well and stored in an air tight container.

Labelling:

Name of the preparation	:	*Pooneeru chunnam*
Dose	:	4 to 8 *kundri edai* (520 to 1040 mg)
Adjuvant/ vehicle	:	Ghee, Honey, Chukku kudinner, Cucumber Seed juice.
Route of administration	:	Oral.
Indications	:	*Soothagavali* (Dysmenorrhea), *Gunnam* (Gastric ulcer), *Seriyamai* (Indigestion).
Date of expiry	:	100 years from the date of manufacture.

INGREDIENTS OF *POONEERU CHUNNAM*

Fig No: 1 *Kaadi* – 1st day

Fig No: 2 After 6 month

Fig No: 3 *Pooneeru* collection

Fig No: 4 Purification of *Pooneeru*

Fig No: 5 After purification of *Pooneeru*

Fig No: 6 *Uththamani*

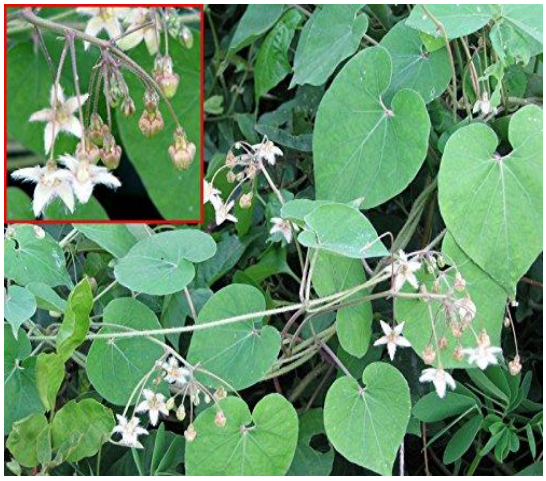


Fig No: 7 *Seelai*

Fig No:8 *Pooneeru Chunnam*

5. STANDARDIZATION OF THE DRUG[41]

5.1 STANDARDIZATION OF THE DRUG *POONEERU CHUNNAM* AS PER SIDDHA CLASSICAL LITERATURE[42]

- **Analysis as per classical Siddha literature:**

  - Floating on Water
  - Fine enough to enter the crevices of finger
  - Irreversible reaction
  - Tasteless
  - Lusterless

1. Floating on Water:

A pinch of *Parpam* gently placed on the still surface of water in a vessel, did not sink immediately. It was found that the *Pooneeru Chunnam* particles floated over the surface of water indicated lightness of the trial.

2. Fine enough to enter the crevices of finger:

Parpam in well prepared form should be fine. When taken between thumb and index finger, the fine powder will fill up the lines of the finger print. A pinch of *Pooneeru Chunnam* was taken in between the thumb and index finger and rubbed. It was found that the *Pooneeru Chunnam* entered into the lines of the finger, and was not easily washed out from the lines, confirmed its fineness.

3. Irreversible reaction:

The well prepared *Parpam* does not reversible to its metallic state when heated with a mixture of cane jaggery, hemp powder, ghee and honey. A pinch of *Pooneeru Chunnam* was taken and mixed with cane jaggery, ghee and honey. It was observed that the *Pooneeru Chunnam* did not reversible to its metallic state.

4. Tasteless:

The well prepared *Parpam* should be completely tasteless. Presence of any taste like sweet or bitter indicate incomplete preparation which needed another calcination process. When a small amount of *Pooneeru Chunnam* was kept on the tip of the tongue, no specific taste was found.

5. Lusterless:

If any shining particles present in *Parpam*, it indicates that the *Parpam* is not manufactured properly and contains unchanged substances like minerals, metals and other toxic substances. There should be no shining particles present in the well manufactured *Parpam*. The *Pooneeru Chunnam* was taken in a Petri bowl and observed for any luster in day light through magnifying glass. No luster was observed in the *Pooneeru Chunnam.*

5.2 STANDARDIZATION OF THE DRUG *POONEERU CHUNNAM* BY USING MODERN TECHNIQUES:

Standardization of drugs helps to prove its identity and determination of its quality and potency. Standardization of the Siddha formulation is based on the qualitative and quantitative analysis through physico- chemical investigations and instrumental analysis.

As per AYUSH protocol for standardization, the following parameters were evaluated.

- **Organoleptic characters**
    - Colour
    - Odour
    - Taste

- **Physicochemical analysis**
    - Moisture content (Loss of Drying)
    - Determination of Total Ash Value
    - Determination of Acid insoluble Ash
    - Determination of Water soluble Extractive
    - Determination of Alcohol soluble Extractive

- **Instrumental Analysis**
    - Fourier Transform Infra-Red Spectroscopy (FTIR)
    - Atomic absorption spectrophotometric analysis of heavy metals(AAS)
    - X-Ray Fluorescence (XRF)
    - Thin- layer chromatography Analysis (TLC)

The physico-chemical analysis of the prepared Siddha formulation was done at VS clinical research and hospital (P)Ltd.

Identification of organic, polymeric and inorganic functional groups was engaged by using modern analytical technique Fourier Transform Infra-Red Spectroscopy (FTIR). To

determining trace and ultra trace levels of elements or metal using accepted technique Atomic absorption spectrophotometric analysis of heavy metals (AAS).

Identify and determine the concentrations of elements present in solid, powered and liquid samples by using non- destructive analytical technique X-Ray Fluorescence (XRF). TLC can be used to monitor the progress of a reaction, determine the purity of a substance and to allow more accurate quantitative analysis.

The thermal analysis of the sample measured over time as the temperature changes by Thermo Gravimetric analysis or Thermal Gravimetric analysis (TGA). The above mentioned elemental analysis have been done at VS clinical research and hospital (P) Ltd,Taramani, chennai- 600 113.

5.2.1 ORGANOLEPTIC CHARACTERS

Colour

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

Taste

Small amount of *Pooneeru Chunnam* was kept over the tip of the tongue.

5.2.2 PHYSICO CHEMICAL ANALYSIS[43]

Physicochemical properties of *Pooneeru Chunnam* was analyzed at VS clinical research and hospital (P)ltd, CSIR Road, Taramani, Chennai-600113.

Physico-chemical studies of the plant 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 of the sample at 105ºC, pH value and Extractive value. These were carried out as per guidelines.

1. Loss on drying of the sample at 105ºC

5g of the drug without preliminary drying was weighed accurately in a tared evaporating dish, dried at 105ºC for 5 hours, cooled in desiccators and weighed. Later the drying and weighing process was continued at one hour interval until difference between two successive weighing of sample corresponds to not more than 0.25 precent. When the constant weight was obtained the percentage of moisture content was calculated with reference to the air dried drug.

Calculation:

$$\text{Percentage of Loss on Drying at 105°C} = \frac{\text{Loss in weight of the sample}}{\text{Weight of the test drug taken}} \times 100$$

2. Ash content

2. a. Total ash content

2 to 3 g of *Pooneeru chunnam* was weighed in the pre weighed and tared Gooch crucible was kept in the muffle furnace at a temperature not exceeding 450ºC until free from carbon then cooled and weighed and the percentage of the total ash content were calculated with reference to the air dried drug.

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

2. b. Acid-insoluble ash

The ash obtained from total ash boiled with 25ml of dilute hydrochloric acid for 5 minutes and insoluble matter were collected in an ash less filter paper, washed with hot water and ignited to constant weight. Later the percentage of the acid insoluble ash content was calculated with reference to the air dried drug.

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

3. Extractive value of the test drug

4g of *Pooneeru Chunnam* was weighed accurately in a glass stoppered flask. Added 100 ml of distilled water 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. Cooled in a dessicator and weighed. Repeated the experiment twice, and taken the average value. The percentage of water soluble extractive was calculated by the formula given below.

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

3. a. Water-soluble extractive of the test drug

3g of *Pooneeru Chunnam* in a glass stoppered flask and add 100 mL of distilled water, shake occasionally for 6 h and then allow standing for 18 h, then filter rapidly taking care not to lose any solvent and pipette out 25 mL of the filtrate in a pre weighed 100 mL beaker and evaporate to dryness on a water bath. Keep it in an air oven at 105°C for 6 h, cool in a desiccator and weighed. Repeat the experiment twice and take the average value.

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

3. b. Alcohol-soluble extractive of the sample

4g of *Pooneru Chunnam* was weighed accurately in a glass stoppered flask. Added 100 ml of distilled alcohol (approximately 95%) 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 dessicator and weighed. Repeated the experiment twice, and taken the average value. The percentage of alcohol soluble extractive was calculated by the formula given below.

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

4. Solubility test:

A. A little amount of the sample was taken in a clean, dry test tube and then shaken well with distilled water.

B. A little amount of the sample was taken in a clean, dry test tube and then shaken well with con. Hcl and Con. H_2SO_4. Sparingly soluble character of the sample indicates the presence of Silicate.

5.2.3 CHEMICAL ANALYSIS OF *POONEERU CHUNNAM* :[44]

The chemical analysis of *Pooneeru Chunnam* was carried out in Bio chemistry lab, National Institute of Siddha.

S.No.	EXPERIMENT	OBSERVATION	INFERENCE
1.	**Physical Appearance of extract**	Dark brown in colour	
2.	**Test for Silicate** A 500mg of the sample was shaken well with distilled water.	Sparingly soluble	**Presence of Silicate**
3.	**Action of Heat** A 500mg of the sample was taken in a dry test tube and heated gently at first and then strong.	No White fumes evolved.	Absence of Carbonate
4.	**Flame Test** A 500mg 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.	Bluish green flame	**Presence of copper**
5.	**Ash Test** A filter paper was soaked into a mixture of extract and dil. cobalt nitrate solution and introduced into the Bunsen flame and ignited.	No Appearance of yellow colour flame	Absence of sodium

ESTIMATION OF ACID AND BASIC RADICALS

Preparation of Extract:

5gm of sample was taken in a 250ml clean beaker and added with 50ml of distilled water. Then it was boiled well for about 10 minutes. Then it was cooled and filtered in a 100ml volumetric flask and made up to 100ml with distilled water. This preparation was used for the qualitative analysis of acidic/basic radicals and biochemical constituents in it.

S.No	EXPERIMENT	OBSERVATION	INFERENCE
	I. Test For Acid Radicals		
1.	**Test For Sulphate** 2ml of the above prepared extract was taken in a test tube to this added 2ml of 4% dil ammonium oxalate solution	Cloudy appearance present	**Presence of Sulphate**
2.	**Test For Chloride** 2ml of the above prepared extract was added with 2ml of dil-HCl until the effervescence ceases off.	No Cloudy appearance was formed	Absence of Chloride
3.	**Test For Phosphate** 2ml of the extract was treated with 2ml of dil.ammonium molybdate solution and 2ml of Con.HNo3	No Cloudy appearance was evolved.	Absence of Phosphate
4.	**Test For Carbonate** 2ml of the extract was treated with 2ml dil. magnesium sulphate solution.	Cloudy appearance was evolved.	**Presence of carbonate**
5.	**Test For Nitrate** 1gm of the extract was heated with copper turning and concentrated H2SO4 and viewed the test tube vertically down.	No Brown gas was evolved	Absence of nitrate
6.	**Test For Sulphide** 1gm of the extract was treated with 2ml of Con. HCL	No rotten egg smelling gas was evolved	Absence of Sulphide
7.	**Test For Fluoride & Oxalate** 2ml of extract was added with 2ml of dil. Acetic acid and 2ml dil. calcium chloride solution and heated**.**	No cloudy appearance.	Absence of fluoride and oxalate
8.	**Test For Nitrite** 3drops of the extract was placed on a filter paper, on that-2 drops of dil.acetic acid and 2 drops of dil. Benzidine solution were placed.	No characteristic changes were noted.	Absence of nitrite

9.	**Test For Borate** 2 Pinches (50mg) of the extract was made into paste by using dil.sulphuric acid and alcohol (95%) and introduced into the blue flame.	No Appearance of bluish green colour.	Absence of borate
II. Test For Basic Radicals			
1.	**Test For Lead** 2ml of the extract was added with 2ml of dil. potassium iodine solution.	No Yellow precipitate was obtained	Absence of lead
2.	**Test For Copper** One pinch (25mg) of extract was made into paste with Con. HCl in a watch glass and introduced into the non-luminuous part of the flame.	No blue colour appeared	Absence of copper
3.	**Test For Aluminium** To the 2ml of extract dil. sodium hydroxide was added in 5 drops to excess.	No yellow Colour appeared	Absence of Aluminium
4.	**Test For Iron** a. To the 2ml of extract, added 2ml of dil. ammonium solution. b. To the 2ml of extract 2ml thiocyanate solution and 2ml of con.HNO3 were added.	Mild Red colour appeared	**Presence of Iron**
5.	**Test For Zinc** To 2ml of the extract dil. sodium hydroxide solution was added in 5 drops to excess and dil. Ammonium chloride was added.	No White precipitate was formed	Absence of Zinc
6.	**Test For Calcium** 2ml of the extract was added with 2ml of 4% dil.ammonium oxalate solution	Cloudy appearance and white precipitate was formed	**Presence of calcium**

7.	**Test For Magnesium** To 2ml of extract dil. sodium hydroxide solution was added in 5 drops to excess.	No White precipitate was obtained	Absence of magnesium
8.	**Test For Ammonium** To 2ml of extract 1 ml of Nessler's reagent and excess of dil.sodium hydroxide solution were added.	No Brown colour appeared	Absence of ammonium
9.	**Test For Potassium** A pinch (25mg) of extract was treated with 2ml of dil. sodium nitrite solution and then treated with 2ml of dil. cobalt nitrate in 30% dil. glacial acetic acid.	No Yellow precipitate was obtained	Absence of potassium
10.	**Test For Sodium** 2 pinches (50mg) of the extract was made into paste by using HCl and introduced into the blue flame of Bunsen burner.	No yellow colour flame evolved.	Absence of sodium
11.	**Test For Mercury** 2ml of the extract was treated with 2ml of dil. sodium hydroxide solution.	No Yellow precipitate was obtained	Absence of Mercury
12.	**Test For Arsenic** 2ml of the extract was treated with 2ml of dil. sodium hydroxide solution.	No Brownish red precipitate was obtained	Absence of arsenic
III. Miscellaneous			
1.	**Test For Starch** 2ml of extract was treated with weak dil.Iodine solution	No Blue colour developed	Absence of starch
2.	**Test For Reducing Sugar** 5ml of Benedict's qualitative solution was taken in a test tube and allowed to boil for 2 minutes and added 8 to 10 drops of the extract and again boil it for 2 minutes. The colour changes were noted.	No Brick red colour is developed	Absence of reducing sugar

3.	**Test For Alkaloids** a) 2ml of the extract was treated with 2ml of dil.potassium lodide solution. b) 2ml of the extract was treated with 2ml of dil.picric acid. c) 2ml of the extract was treated with 2ml of dil.phosphotungstic acid.	No Yellow colour developed	Absence of of Alkaloid
4	**Test For Tannic Acid** 2ml of extract was treated with 2ml of dil. ferric chloride solution	No Blue-black precipitate was obtained	Absence of Tannic acid
5	**Test For Unsaturated Compound** To the 2ml of extract, 2ml of dil. Potassium permanganate solution was added.	Potassium permanganate was not decolourised	Absence of unsaturated compound
6	**Test For Amino Acid** 2 drops of the extract was placed on a filter paper and dried well. 20ml of Burette reagent was added**.**	No Violet colour appeared	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 developed No Red colour developed No Violet colour developed No Blue colour developed.	Absence of quinole pinephrine and pyrocatechol. Absence of Antipyrine, Aliphatic amino acid meconic acid. Apomorphine salicylate and Resorcinol were absent Morphine, Phenol cresol hydrouinone were Absent

5.2.4 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)[45), (46)]

Instrument details:

Model : Perkin Elmer- Spectrum one: FT-IR Spectroscopy

Scan Range : MIR 450-4000 cm-1

Resolution : 4cm-1

Principle:

Fourier Transform Infrared Spectroscopy (FTIR) is an analytical technique used to identify mainly organic materials. FTIR analysis results in absorption spectra which provide information about the chemical bonds and molecular structure of a material. The FTIR spectrum is equivalent to the "fingerprint" of the material and can be compared with cataloged FTIR spectra to identify the material.

Fig No : 9 Fourier Transform Infrared Spectroscopy (FTIR)

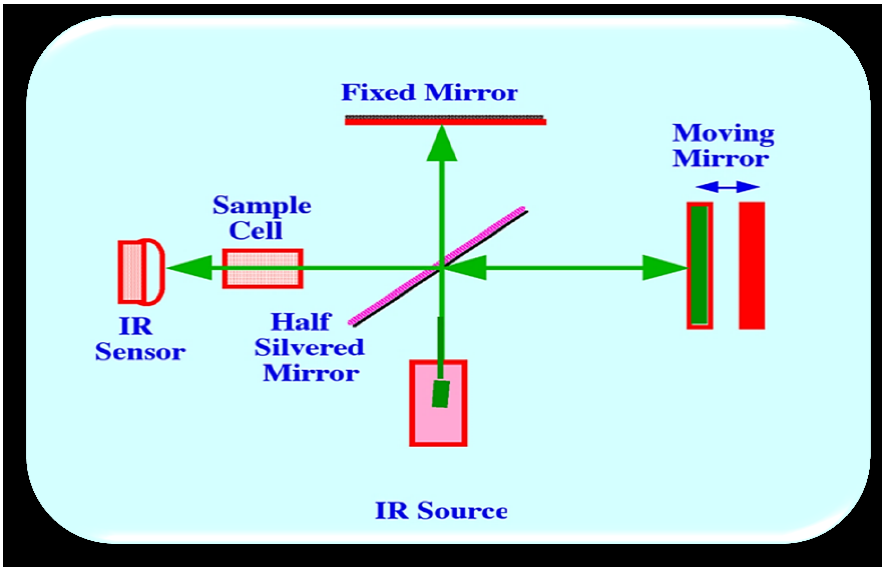


Fig No: 10 FTIR Mechanism

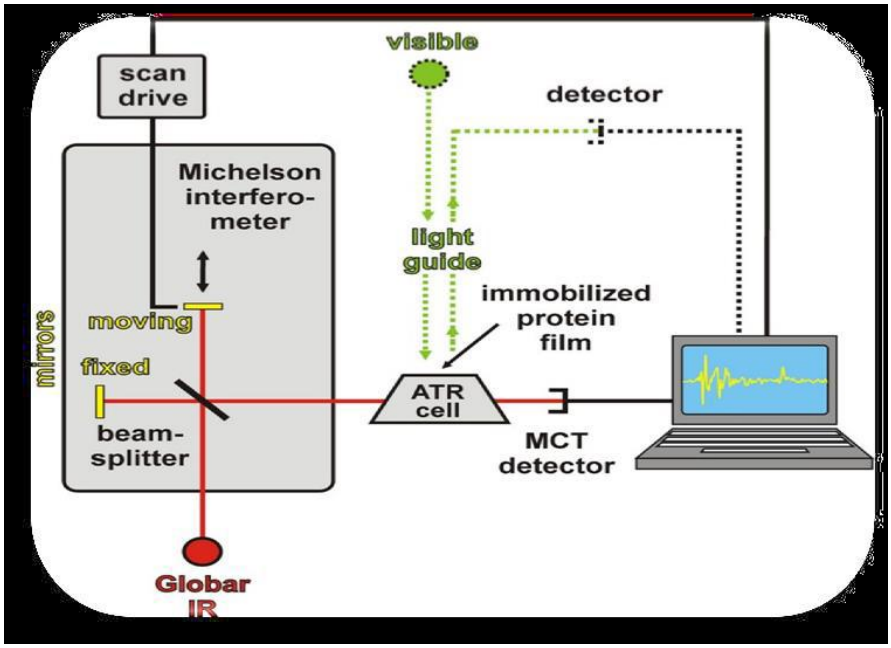


Fig No: 11 Mechanism of FTIR analyzer

Fourier Transforms Infrared Spectroscopy analytical capabilities:

- Identifies chemical bond functional groups by the absorption of infrared radiation which excites vibrational modes in the bond
- Especially capable of identifying the chemical bonds of organic materials
- Detects and Identifies organic contaminants
- Identifies water, phosphates, sulphates, nitrates, nitrites, and ammonium ion.
- Detection limits vary greatly, but are sometimes <1013 bonds/cm3 or sometimes sub monolayer
- Useful with solids, liquids, or gases.

Applications:

- Infrared spectrum is useful in identifying the functional groups like -OH, -CN, -CO, -CH, -NH2, etc. Also quantitative estimation is possible in certain cases for chemicals, pharmaceuticals, petroleum products, etc. Resins from industries, water and rubber samples can be analyzed.

Sample preparation method:

FT-IR spectra were recorded at SAIF, IIT Madras, India. The Perkin Elmer Spectrum One Fourier Transform Infrared (FTIR) Spectrometer was used to derive the FT IR Spectra of *Pooneeru Chunnam* 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. The samples were 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. FT- IR Spectra were used to determine the presence of the functional groups and bands in the *Pooneeru Chunnam*.

5.2.5 ATOMIC ABSORPTION SPECTROPHOTOMETRIC ANALYSIS (AAS):[47],[48]

Atomic absorption spectroscopy (AAS) and atomic emission spectroscopy (**AES**) is a spectroanalytical procedure for the quantitative determination of chemical elements using the absorption of optical radiation (light) by free atoms in the gaseous state. Atomic absorption spectroscopy is based on absorption of light by free metallic ions.

Principle:

The technique makes use of the atomic absorption spectrum of a sample in order to assess the concentration of specific analytes within it. It requires standards with known analyte content to establish the relation between the measured absorbance and the analyte concentration and relies therefore on the Beer-Lambert Law.

Fig No: 12 Atomic absorption spectroscopy

Instrumentation:

In order to analyze a sample for its atomic constituents, it has to be atomized. The atomizers most commonly used nowadays are flames and electrothermal (graphite tube) atomizers. The atoms should then be irradiated by optical radiation, and the radiation source could be an element-specific line radiation source or a continuum radiation source. The radiation then passes through a monochromator in order to separate the element-specific radiation from any other radiation emitted by the radiation source, which is finally measured by a detector.

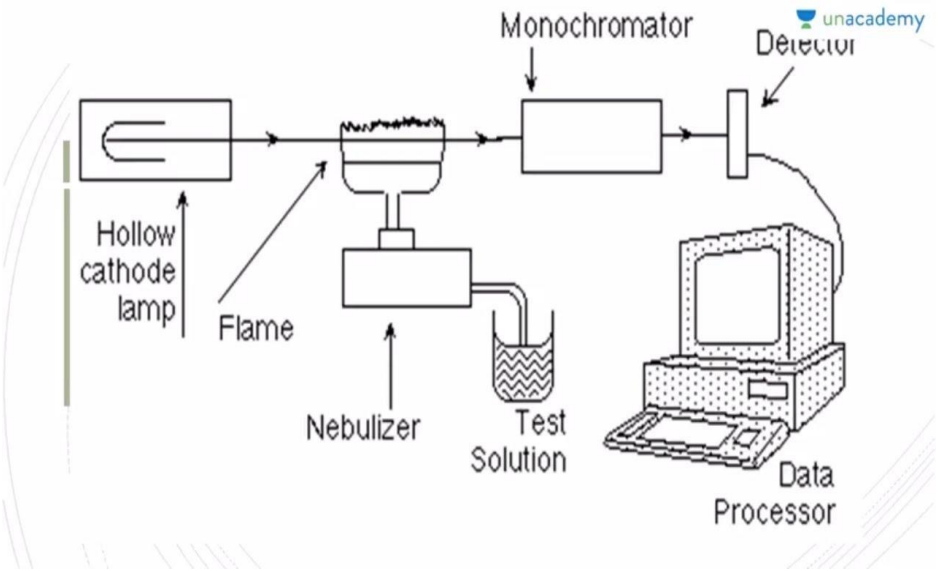


Fig No: 13 Instrumentation of AAS

The **main advantages of** AAS are given below:

- High sample throughput
- Easy to use
- High precision
- Inexpensive technique

The main **disadvantages of** AAS are as follows:

- only solutions can be analyzed
- less sensitivity compared to graphite furnace
- relatively large sample quantities are required (1-3 ml)
- problems with refractory elements

Sample Preparation for AAS Analysis:

As per the standard preparation of solution for the AAS, usually solution of 50 ml was prepared, in the proportion 1:25:25 ratio i.e, 1gm of sample were digested in 25ml conc. HCL and 25 ml of double distilled water and kept overnight and filtered the solution by whatman filter paper, 50ml of prepared solution was added with 950 ml of double distilled water, finally 1000 ml solution was prepared of moisture content was calculated with reference to the air dried drug.

5.2.6 X-RAY FLUORESCENCE (XRF):[49]

X-Ray Fluorescence is a lab-based technique used for bulk chemical analysis of rock, mineral, sediment, and fluid samples and the emission of characteristic "secondary" (or fluorescent) X-rays from a material that has been excited by being bombarded with high-energy X-rays or gamma rays.. The technique depends on the fundamental principles of x-ray interactions with solid materials, similar to XRD analysis. XRF analysis is one of the most commonly used techniques for major and trace element analysis, and chemical analysis, particularly in the investigation of metals, glass, ceramics and building materials, and for research in geochemistry, forensic science due to the relative ease and low cost of sample preparation.

As the d value of the diffracting crystal is known, the detector and diffracting crystal can be moved (using the goniometer) through an angle q so that only X-rays with a specific wavelength arrive at the detectors. Therefore, the X-ray detector can be "tuned" to measure only the X-rays produced by fluorescence of atoms of one element; the intensity of this radiation is proportional to the abundance of that element within the sample.

Fig No: 14 X-ray Fluorescence

Advantages:

1. Relatively simple, cheap and quick analyses
2. Accurate analyses of a range of elements
3. "Dry" method and therefore requires minimal sample preparation (for trace element analysis).
4. Few consumables.

XRF Limits

Since XRF measurements rely on quantity, there are limits on the measurements. The normal quantitative limit is 10 to 20 ppm (parts per million), usually the minimum particles required for an accurate reading.

XRF also can’t be used to determine Beryllium content, which is a distinct disadvantage when measuring alloys or other materials that might contain Beryllium.

5.2.7 THIN LAYER CHROMATOGRAPHY (TLC) :[50],[51]

Principle of TLC :

Thin layer chromatography uses a thin glass plate coated with either aluminum oxide or silica gel as the solid phase. The mobile phase is a solvent chosen according to the properties of the components in the mixture. The principle of TLC is the distribution of a compound between a solid fixed phase (the thin layer) applied to a glass or plastic plate and a liquid mobile phase (eluting solvent) that is moving over the solid phase. A small amount of a compound or mixture is applied to a starting point just above the bottom of TLC plate.

The plate is then developed in the developing chamber that has a shallow pool of solvent just below the level at which the sample was applied. The solvent is drawn up through the particles on the plate through the capillary action, and as the solvent moves over the mixture each compound will either remain with the solid phase or dissolve in the solvent and move up the plate. Whether the compound moves up the plate or stays behind depend on the physical properties of that individual compound and thus depend on its molecular structure, especially functional groups. The solubility rule "Like Dissolves Like" is followed. The more similar the physical properties of the compound to the mobile phase, the longer it will stay in the mobile phase. The mobile phase will carry the most soluble compounds the furthest up the TLC plate. The compounds that are less soluble in the mobile phase and have a higher affinity to the particles on the TLC plate will stay behind.

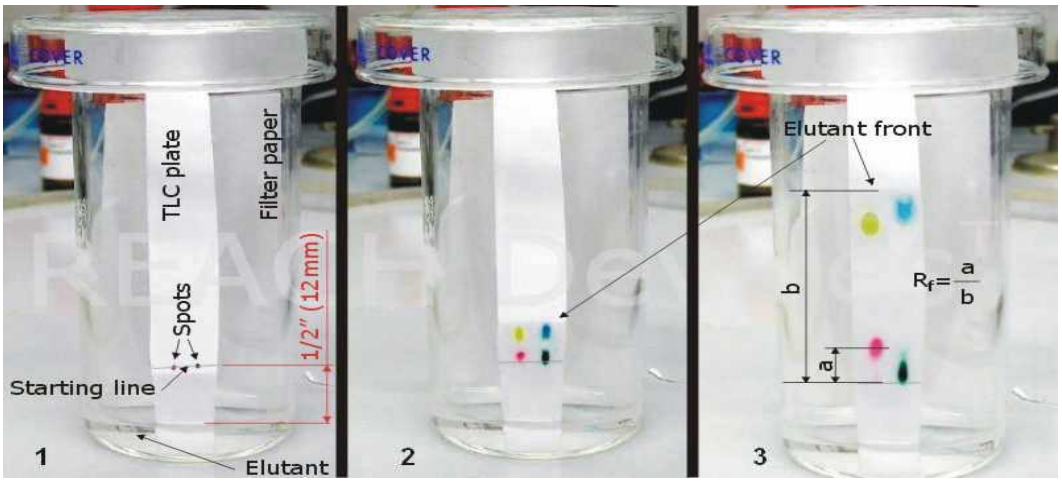


Fig No: 15 Thin Layer Chromatography

$$Rf = \frac{\text{Distance travelled by the component}}{\text{Distance travelled by the solvent}}$$

Applications:

TLC is widely used separation technique used in life sciences and chemistry studies. It is used to isolate and analyses mixture of compounds, and used in biochemical analysis, separation of inorganic ions, quantitative analysis and analysis of components of food stuffs.

TLC has applications in different field of study such as in the pharmaceutical industry, insecticides and pesticides industry, medicine and other industries.

Advantages [52]

- Very less equipment is used. It is very simple method. It is also sensitive method.
- The components are separated in very little time as the components will elute out very quickly.
- The components present in the sample can be separated and recovered out easily by scratching the powdery coating on the plate and hence quantitative separation of spots or zones are possible.
- With TLC, it is possible to visualize the components by UV light.
- Isolation of preparative quantities can be accomplished with thicker layers of adsorbent.

Disadvantages

- In this method the plate length is limited and hence separation takes place only upto certain length.
- The separation takes place in an open system or in open condition and hence there are chances that sample may be affected by the humidity and temperature.

6.1. EVALUATION OF ANTI-SPASMODIC ACTIVITY OF *POONEERU CHUNNAM* IN SWISS ALBINO MICE.[53],[54],[55]

Aim

To study the Anti-spasmodic activity of *Pooneeru Chunnam* in swiss albino mice by Charcoal meal test method.

Materials and Methods:

Test Substance	:	***Pooneeru Chunnam***
Animal Source	:	Sathyabama Institute of Science and Technology, Chennai.
Animals	:	Swiss Albino Mice (Male-12, Female-12)
Age	:	6-8 weeks.
Body Weight	:	25-30gms.
Acclimatization	:	7 Days prior to dosing.
Veterinary Examination	:	Prior and at the end of the acclimation
Identification of Animals Marking	:	By Cage Number, Animal Number and Individual by Picric Acid
Diet	:	Pellet Food
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^{o}c
Relative Humidity	:	Between 30% And 70%
Air Changes	:	10 To 15 per Hour
Dark and Light Cycle	:	12:12

Selection of animals:

Healthy Swiss albino mice (25-30gms) of both sexes were used for this study with the approval of the Institutional Animal Ethical Committee of Sathyabama University.

(IAEC approved No: SU/CLATR/IAEC/XIII/135/2019).

The animals kept in plastic cages and maintained at 24-28^{o}C. All the mice were housed individually with free access to food, water and libitum. They were feed 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 experiments. 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 to legislation on welfare.

The experimental protocol

Animal grouping:

Both sex of adult swiss albino mice weighing (25-30gms) were used in this study. Mice were divided into 4 groups, consisting of six animals for each group.

Group I : Received Charcoal meal alone (control)

Group II : Received *Pooneeru Chunnam* (50mg/kg orally)+ charcoal

Group III : Received *Pooneeru Chunnam* (150mg/kg orally)+ charcoal

Group IV : Received standard drug Loperamide (2mg/kg orally)+ charcoal.

Swiss albino mice were divided into four groups, consisting six mice for each groups. Group I (Vehicle control) received honey, Group II received 50 mg/kg of *Pooneeru Chunnam* and Group III received 150 mg/kg of *Pooneeru Chunnam* respectively. Group IV (standard group) received 2mg/kg of Loperamide p.o,.

Procedure

Antispasmodic effect of the test compounds will be assessed by measurement of small intestine transit following oral administration of a charcoal meal. Charcoal transit test will be utilized for measuring the percentage of spasmolytic potential of the trial drug. Movement of charcoal meal in the intestine will be assessed. For this test drug will be administered orally at two dose level for test group along with loperamide (2 mg/kg) will be given orally to mice for standard group and 30 min later 0.5 ml of charcoal meal containing 3% charcoal plus 5% tragacanth suspension will be administered orally for all the above groups. Forty five minutes after charcoal meal administration, each animal will be sacrificed and distance of charcoal movement in the small intestine was measured using a ruler.

Statistical analysis:

Data were expressed as Mean ± SEM. Significance was assessed by "t" test or ANOVA followed by Dunnet's test. The minimum level of significance was fixed at

*P < 0.05, **P < 0.01, ***P < 0.001.

6.2.EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF *POONEERU CHUNNAM* IN WISTAR ALBINO RATS

Aim:

To study the Anti-inflammatory effect of *Pooneeru Chunnam* in Wistar albino rats by Carrageenan-inducedt paw edema method.

Materials and Methods:

Test Substance	**:**	***Pooneeru Chunnam***
Animal Source	**:**	TANUVAS, Madhavaram, Chennai.
Animals	**:**	Wistar Albino Rats (Male -12, Female -12)
Age	**:**	6-8 weeks
Body Weight	**:**	140-160gm.
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 (140- 160g) of both sexes were used for this study with the approval of the Institutional Animal Ethics Committee and obtained from NIS IAEC. (IAEC approved no: NIS/IAEC-V/09082017/06).

The animals kept in polypropylene cages and maintained at 24-28°C. All the rats were housed individually with free access to food, water ad libitum. They were fed with standard diet and kept in well ventilated animal house and they also maintained with alternative dark-light cycle of 12hrs throughout the studies. Rats were allowed an acclimatization period of 14 days before actual experiments. The rats were closely observed for any infection and if they shown any signs of infection they were excluded from the study. The animal experiment was performed with accordance to legislation on welfare.

The experimental protocol

Animal grouping:

Both sex of Adult wistar Albino rats weighing (140-160g) were used in this study. Rats were divided into 4 groups, consisting of six animals for each group.

Group I **:** Vehicle control - received only honey (1 ml) orally

Group II **:** Received Standard drug Indomethacin (10mg/kg orally)

Group III **:** Received *Pooneeru Chunnam* (50 mg/kg orally) + Honey

Group IV **:** Received *Pooneeru Chunnam* (140 mg/kg orally) +Honey

Acute anti inflammatory effect was evaluated by carrageenan-induced hind paw edema method. Carrageenan was administrated by sub-plantar injection of 0.1 ml freshly prepared 1% suspension in right hind paw in rats. Group II animals were pretreated with standard drug Indomethacin, 10mg/kg body weight and Group III, and IV of animals were pre treated with , *Pooneeru Chunnam* 50 mg/kg and 140 mg/kg respectively at 1hr before eliciting paw edema. Rat‘s paw volume was measured initially and then 1, 2, 3 hrs after the carrageenan injection by using plethysmographic method.

The edema inhibitory activity was calculated according to the following formula-

Edema (%) inhibition = (1-D/C)x 100

Where,

D-represents the percentage difference in increased paw volume after the administration of test drugs to the rats.

C-represents the percentage difference of increased volume in the control groups.

Stastistcal analysis

All the results were reported as Mean ± SD. They were further analyzed using one way analysis of variables (ANOVA) followed by Dunnet‘s test. Test for significance is

*P < 0.05, **P < 0.01, ***P < 0.001.

6.3. EVALUATION OF ANALGESIC ACTIVITY OF *POONEERU CHUNNAM* IN SWISS ALBINO MICE

Aim

To study the Analgesic Activity effect of *Pooneeru Chunnam* in Swiss albino mice by Eddy's Hot plate method.

Materials and Methods:

Test Substance	**:**	***Pooneeru Chunnam***
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 – 25 gm.
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 12 per hour
Dark and light cycle	**:**	12 : 12 hours

Selection of Experimental animals:

The experiment protocol was submitted and approved by Institutional Animal Ethical Committee of NIS, **(IAEC approved No: NIS/IAEC–V/09082017/06)**. Swiss albino mice (20-25 gm) of approximate same age were employed in this investigation.

The animals kept in polypropylene cages and maintained at 24-28°C. All the mice were housed individually with free access to food, water ad 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 shown any signs of infection they were excluded from the study. The animal experiment was performed with accordance to 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, pons, hind 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 Physical – using tail compressions.

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 analgesic 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 response to painful thermal stimuli that was a direct indicator of nociceptive threshold. Jumping represents a more elaborated response, with latency and encompasses an emotional component of escaping.

Experimental design

The animals were divided into 4 groups, consisting of six animals for each group.

Group I : Vehicle control (Honey- 1 ml)

Group II : Standard drug - Pentazocine (5mg/kg/i.p)

Group III : *Pooneeru Chunnam* (50 mg/kg) + Honey.

Group IV : *Pooneeru Chunnam* (150 mg/kg) +Honey.

Experiment Method:

Eddy's Hot plate method.

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 times of the animals were then noted at 0, 30, 60,120 and 180 minutes 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.

7.RESULTS

Many studies have been carried out to bring the efficacy and potency of the drug ***Pooneeru Chunnam***. The study includes literary collections, organoleptic character, physicochemical analysis, pharmacological and analytical studies. The drug *Pooneeru Chunnam* has been selected from the text ***"Anubhoga Vaithiya Navanitham".***

- Botanical aspect explains the active principle and medicinal uses of the plants.
- Gunapadam review brings the effectiveness of the drug in the management of dysmenorrhea.
- The pharmacological review explains about the evaluation of Anti spasmodic, Anti inflammatory, Analgesic activities.

5. STANDARDIZATION OF THE DRUG

5.1 STANDARDIZATION OF THE DRUG *POONEERU CHUNNAM* AS PER SIDDHA CLASSICAL LITERATURE:

Siddhars used these following standardization methods to ensure the safety and efficacy of the *chunnam*. It shows the effectiveness of the drug.

Table. No. 1 Results of Siddha Standardization

S.NO	Parameter	Results of *Pooneeru chunnam*	Interpretation
1.	Floating on Water	Floats on water	Lightness of drug.
2.	Finger Print Test	Impinged in the furrow of fingers	Indicates fine particles of powder.
3.	Luster	Lusterless	Change of specific character of raw material after incineration
4.	Taste	No specific taste, Mild irritation is felt	Change of specific character of raw material after incineration

Interpretation:

1. **Floating on water:**

The test drug which was float on water has less specific gravity. Thus *Pooneeru chunnam* possesses specific gravity less than the water.

2. **Finger print test**:

Only the particles which are in micro fine size can enter into the furrows of the finger print. Finger print test indicates the presence of micro fine particles in *Pooneeru chunnam.*

3. **Lusterless & taste:**

Pooneeru chunnam is lusterless and tasteless because there is no free metal present.

5.2 STANDARDIZATION OF THE DRUG *POONEERU CHUNNAM* BY USING MODERN TECHNIQUES:

Traditional remedies is advantageous, it does suffer some limitations. The main limitation is the lack of standardization of raw materials, of processing methods and of 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 analyzing it through various studies. Following tables and charts are the results of physicochemical and chemical analysis. Physical characterization and estimation of basic and acidic radicals have been done and tabulated. Pharmacological activity and analytical studies of the drug were derived. Its result has been tabulated below.

5.2.1 ORGANOLEPTIC CHARACTERS:

Table No. 2. Organoleptic characters of *Pooneeru Chunnam*

S.NO	Parameters	Results
1	Colour	Ash coloured
2	Odour	Odourless
3	Taste	Tasteless

5.2.2 PHYSICOCHEMICAL ANALYSIS:

Table.No.3. Physicochemical characterization of *Pooneeru Chunnam*

S.No.	**Parameters**	**Percentage**
1	Loss on drying	4.82%
2.a	Total ash value	0%
2.b	Acid insoluble ash	0%
3.a	Water soluble extractive	17.54%
3.b	Alcohol soluble extractive	0.76%
4.	Solubility	Soluble in acids (Hcl and H2So4)

Interpretation

The stability of a drug and its shelf–life is reliant on moisture content. Determination of moisture (Loss on drying) in a drug is one of the important tests in pharmaceutical analysis. The analytical parameters like total Ash value, Acid insoluble ash value, Loss on drying values are helping us to interpret the digestion and solubility capacity of the drug.

Physico-chemical analysis of *Pooneeru Chunnam* showed that Loss on drying (LOD) is 4.82% which shows that low moisture content present in the prepared medicine. Increased

moisture content is the issue for instability of a drug and lesser shelf life of a drug. Since *Pooneeru Chunnam* was well prepared, it could get maximum stability and better shelf life. Longer shelf life i.e., 100 years for *Chunnam* mentioned in Siddha literature is justified from the above observation.

By the above results, the trial drug shows that total ash value was found to be 0% whereas the acid insoluble ash is 0% respectively. The value of total ash in the formulation is low because of the absence of inorganic ingredients. Total ash value used to estimate the inorganic material such as silicate, carbonates, oxalates and phosphates.

The water soluble extractive values indicate the presence of sugar, acids. The alcohol soluble extractive values indicate the presence of polar constituents like phenols, alkaloids, steroids, glycosides, flavonoids. Water soluble extractive and alcohol soluble extractive values of this formulation were 17.54% and 0.76% respectively.

5.2.3 CHEMICAL ANALYSIS OF *POONEERU CHUNNAM*

Table No:4 Chemical analysis of *Pooneeru Chunnam*

S.NO	Parameter	Results
1	Test for silicate	Present
2	Action on heat	-
3	Flame test	Bluish green flame is not present that indicates the absence of copper
4	Ash test	-

Table.No.5. Results of acidic radical studies of *Pooneeru Chunnam*

S.NO	**Parameter**	**Observation**	**Result**
1	Test for Sulphate	A white precipitate insoluble in con. HCL is obtained	Positive
2	Test for Chloride	-	Negative
3	Test For Phosphate	-	Negative
4	Test For Carbonate	Cloudy appearance was evolved.	Positive
5	Test For Nitrate	-	Negative
6	Test for Sulphide	-	Negative
7	Test For Fluoride & oxalate	-	Negative
8	Test For Nitrite	-	Negative
9	Test for Borate	-	Negative

Table No:6 Results of basic radicals studies of *Pooneeru Chunnam*

S.NO	Parameter	Observation	Result
1	Test For lead	-	Negative
2	Test for copper	-	Negative
3	Test for Aluminium	Characteristic changes	Positive
4	Test for Iron	Red colour appear	Positive
5	Test for Zinc	White precipitate is formed	Positive
6	Test for Calcium	-	Negative
7	Test for Magnesium	White precipitate is obtained	Positive
8	Test for Ammonium	-	Negative
9	Test for Potassium	Yellowish precipitate is obtained	Positive
10	Test for Sodium	-	Negative
11	Test for Mercury	-	Negative
12	Test for Arsenic	-	Negative

Table No:7 Miscellaneous compounds of *Pooneeru Chunnam*

S.NO	Parameter	Observation	Result
1	Test for starch	Blue colour developed	Positive
2	Test for reducing sugar	-	Negative
3	Test for alkaloids	Yellow colour developed	positive
4	Test for tannic acid	-	Negative
5	Test for unsaturated compound	-	Negative
6	Test for amino acid	-	Negative

The result of preliminary chemical analysis reveals that the trail drug *Pooneeru chunnam* has **Silicate, Sulphate, Carbonate, Aliminium, Iron, Zinc, magnesium, potassium, starch and alkaloids.**

5.2.4 FTIR Analysis:

Fourier Transform Infra- Red Spectroscopy (FTIR) analysis results in absorption spectra provide information about the functional group and molecular structure of a material.

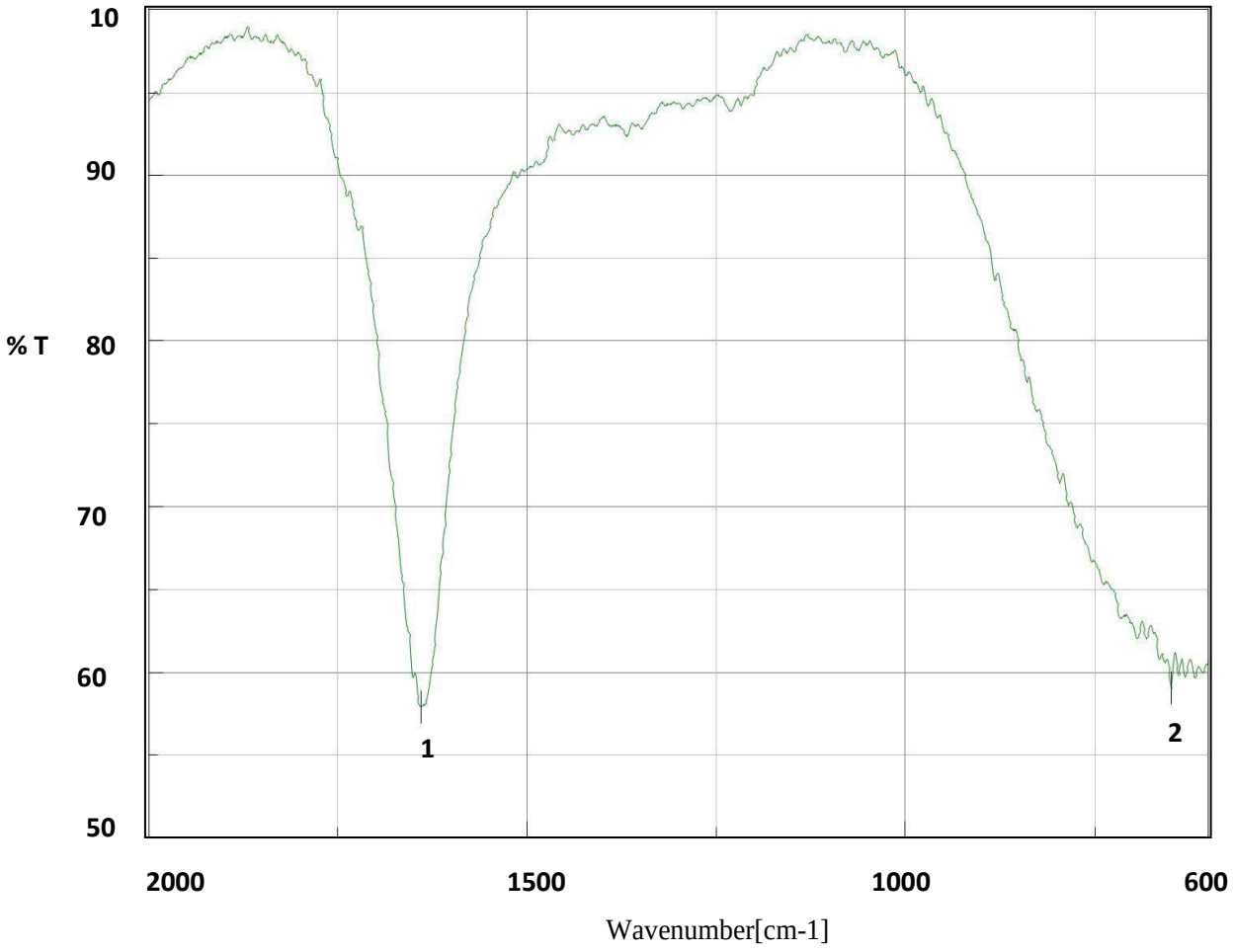


Table No: 8 Vibrational and Functional group of *Pooneeru Chunnam*

S.NO	Wave number (cm-1)	Intensity	Vibrational modes of ***POONEERU CHUNNAM*** **in IR region.**	Functional group
1	1640	57.8616	C=C Stretching,	Alkene
2	648	59.0142	C-Br stretching,	Halo compound

Interpretation:

In the FTIR Spectra analysis, *Pooneeru Chunnam* sample exhibits the peak value as shown in Table , at the wave number of 1640, 648 having C=C Stretch, C-Br Stretch.

This indicates the presence of some organic functional groups such as alkane, halo compound.

5.2.5 Heavy Metal Analysis

Table 9. Heavy Metal Analysis of *Pooneeru Chunnam*

S.NO	Test Parameters	Results	Unit
1	Arsenic (as As)	**BLQ (LOQ : 0.01)**	mg/ kg
2	Mercury (as Hg)	**BLQ (LOQ : 0.01)**	mg/ kg
3	Lead (as Pb)	**BLQ (LOQ : 0.08)**	mg/ kg
4	Cadmium (as Cd)	**BLQ (LOQ : 0.1)**	mg/ kg

Note : BLQ: Below Limit of Quantification; **LOQ :** Limit of Quantification.

Interpretation:

From the above results, the heavy metals such as Arsenic, Mercury, Lead and Cadmium were observed in BLQ. Hence the safety of the drug *Pooneeru Chunnam* is ensured for clinical use.

5.2.6 X-Ray Fluorescence (XRF) :

Table no 10. Oxide form of elements in *Pooneeru Chunnam*

Element in oxide form

Formula	Concentration (%)
Na_2O	42.96
CaO	21.42
Cl	9.12
SO_3	8.32
MgO	8.14
SiO_2	3.49
K_2O	2.32
Fe_2O_3	1.55
PbO	1.11
Al_2O_3	0.75
MOO_3	0.27
P_2O_5	0.22
TiO_2	0.13
Ru	0.13
ZnO	0.09
MnO	0.09
SrO	0.04
CuO	99 PPM

Table No:11 Element form of *Pooneeru Chunnam*

Element form

Formula	Concentration (%)
Na	39.93
Ca	23.96
Cl	14.43
Mg	6.44
S	5.10
K	2.75
Si	2.21
Fe	1.74
Pb	1.54
Al	0.47
MO	0.26
P	0.23
Ti	0.20
Sr	0.06
Zn	0.06
Ru	0.04
Cu	98 PPM

Interpretation:

XRF analysis, it is observed that so many elements are present in *Pooneeru Chunnam*. Some of the elements such as sodium, calcium, chloride and manganese are playing a major therapeutic role in *Pooneeru Chunnam.* Level of oxide form of sodium (Na_2O) was found to be higher (42.96%) in *Pooneeru Chunnam* when compared to elemental form (39.93%). Similar trend was noted in the case of sulphur, potassium, silicon, iron, lead, aluminium, molybdenum, phosphorus, titanium, strontium zinc and ruthenium.

5.2.7 THIN LAYER CHROMATOGRAPHY (TLC) :

Interpretation:

TLC was carried out with three different solvents, and it was found that there was no spot found in the plate at 366nm and 254nm. This indicates that the sample given does not contain any organic compounds or phytochemicals. Thus, the *Pooneeru Chunnam* given is purely inorganic substance without organic impurities.

(NOTE: Any herbal substance burnt above 100ºC will be devoid of phytochemical)

6. PHARMACOLOGICAL STUDIES

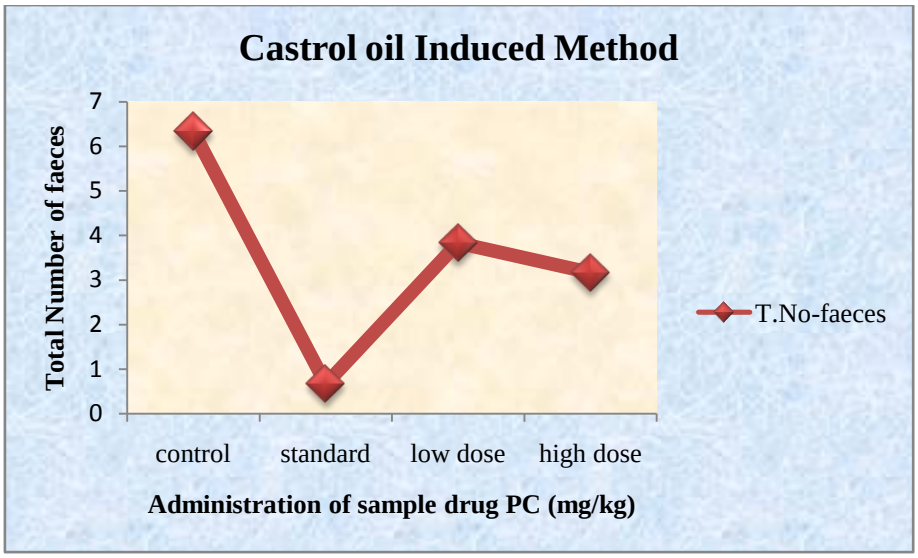
6.1 EVALUATION OF ANTI-SPASMODIC ACTIVITY OF POONEERU CHUNNAM USING CASTOR OIL-INDUCED INTESTINAL MOTILITY IN MICE

Effect of PC on castor oil-induced Intestinal Motility in Mice

Table no 12 . Effect of PC in intestinal motility

Castor oil Control	Onset of Defecation (min)	Mean number of Defecation in 4 hrs	Total number of Wet faeces	% Inhibition
Mean	**32.33**	**7.667**	**6.333**	
Std. Deviation	**3.615**	**1.862**	**1.506**	**0**
Std. Error	**1.476**	**0.7601**	**0.6146**	
Low Dose PC	**Onset of Defecation (min)**	**Mean number of Defecation in 4 hrs**	**Total number of Wet faeces**	**% Inhibition**
Mean	**53.33**	**6.167**	**3.833**	**39.68253968**
Std. Deviation	**4.082**	**1.941**	**1.472**	
Std. Error	**1.667**	**0.7923**	**0.6009**	
High dose PC	**Onset of Defecation (min)**	**Mean number of Defecation in 4 hrs**	**Total number of Wet faeces**	**% Inhibition**
Mean	**78.17**	**5.167**	**3.167**	**49.84**
Std. Deviation	**7.083**	**1.472**	**1.472**	
Std. Error	**2.892**	**0.6009**	**0.589**	
STD -Loperamide	**Onset of Defecation (min)**	**Mean number of Defecation in 4 hrs**	**Total number of Wet faeces**	**% Inhibition**
Mean	**191.7**	**1.5**	**0.6667**	**89.52**
Std. Deviation	**23.71**	**0.8367**	**0.8165**	
Std. Error	**9.68**	**0.3416**	**0.3333**	

Chart No: 1 Effect of *Pooneeru Chunnam* by Castrol oil induced intestinal motility in mice



Effect of PC on gastrointestinal transit and Peristalsis index (%)

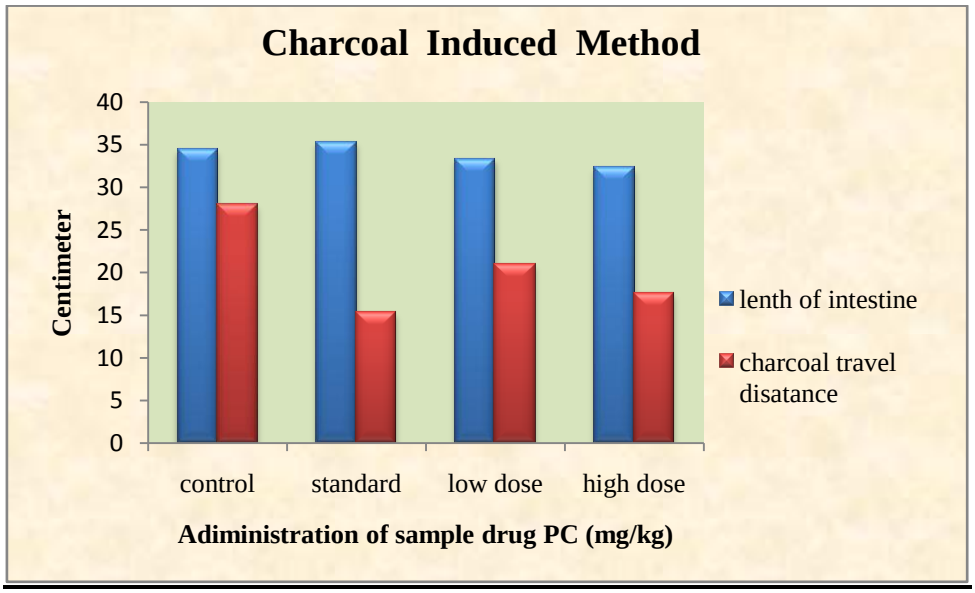
Using charcoal meal in Mice

Table no. 13. Anti Spasmodic activity of *Pooneeru Chunnam*

Charcoal meal control	Length of intestine (cm)	Distance travelled by charcoal (cm)	Peristalsis index (%)
Mean	**34.5**	**28**	**81.34**
Std. Deviation	**3.332**	**2.366**	**4.401**
Std. Error	**1.36**	**0.9661**	**1.797**
Low Dose PC	**Length of intestine (cm)**	**Distance travelled by charcoal (cm)**	**Peristalsis index (%)**
Mean	**33.33**	**21**	**62.85**
Std. Deviation	**4.082**	**3.286**	**4.364**
Std. Error	**1.667**	**1.342**	**1.781**
High dose PC	**Length of intestine (cm)**	**Distance travelled by charcoal (cm)**	**Peristalsis index (%)**
Mean	**32.33**	**17.67**	**54.73**
Std. Deviation	**2.503**	**1.966**	**5.785**

Std. Error	**1.022**	**0.8028**	**2.362**
STD – Loperamide	**Length of intestine (cm)**	**Distance travelled by charcoal (cm)**	**Peristalsis index (%)**
Mean	**35.33**	**15.33**	**42.94**
Std. Deviation	**3.077**	**4.082**	**8.567**
Std. Error	**1.256**	**1.667**	**3.498**

Chart no: 2 Anti spasmodic activity of *Pooneeru Chunnam* by charcoal induced method.



Observation and Inference

It was observed from the present investigation that onset of defecation was shortly induced in castor oil treated group with the mean time of 32.33 ± 1.47 mins. Treatment with trial drug PCat both the dose level of 50 and 150 mg/kg has shown significant delay in the onset of defecation time to the maximum of 78.17 ± 2.89 mins. There was huge delay in defecation time were observed in standard drug treated group with the mean time of 191.7 ± 9.68 mins

The mean number of wet fecal pellet in the Castrol oil group was 6.33 ±0.6, while in the trial drug (PC) treated group this value was found to be 3.83 ±0.60 and 3.16 ± 0.58 in a dose dependent manner. Standard drug loperamide significantly reduced the wet fecal pellet count of about 0.6 ± 0.33.

The total number of defecation observed in the castor oil control group was found to be 7.66 ± 0.76, while in the trial drug (PC) treated group it was found to be 6.16 ±0.79 and 5.16 ± 0.60 when compare to that of the standard drug loperamide with frequency of 1.5 ± 0.34.

Percentage protection offered by the test drug PC at the dose of 50 mg/kg against castor oil induced purgation was found to be 39.68 %, whereas treatment with PC at the dose of 150 mg/kg it was found to be 49.84 % when compared with standard loperamide with the percentage protection of 89.52 %.

Peristalsis was measured as index of gastro intestinal motility. Peristalsis index of charcoal meal control group was found to be 81.34 %. Further treatment with PC at both the dose level of 50 and 150 mg/kg exhibit significant reduction in peristalsis index up to 57.78% when compared with standard loperamide with the lowest PI of 42.94%.

There was significant reduction in distance travelled by the charcoal plug was observed in PC treated group with the transit distance of 17.67 ± 0.80 cm when compare to that of the control with distance of 28 ± 0.96cm. Further standard drug shown prominent decrease in transit distance of 15.3 ± 1.66 cm

Based on the data's obtained from the present investigation it was concluded that the trial drug PC exhibited significant anti – Spasmodic activity on gastrointestinal transit using charcoal meal and in castor induced purgation model.

6.2 EVALUATION OF ANTI- INFLAMMATORY ACTIVITY IF *POONEERU CHUNNAM* IN WISTAR ALBINO RATS

Table No. 14: Effect of *Pooneeru Chunnam* on carrageenan induced paw edema method

Groups	Paw edema volume (ml)			
	0hr	1hr	2hr	3hr
Group I-Control	1.76±0.13	2.21±0.14	2.53±0.16	2.78±0.14
Group II Indomethacin 10mg/kg	1.23±0.62	1.1±0.8	1.0±0.59	0.86±0.32
Group III PC 50mg/kg	1.26±0.61	1.20±0.66	1.18±0.51	1.15±0.64
GroupIV PC140mg/kg	1.45±0.65	1.15±0.78	1.10±0.66	0.98±0.49

Chart No: 3 Anti-inflammatory activity of *Pooneeru Chunnam* by Carrageenan Induced paw edema method

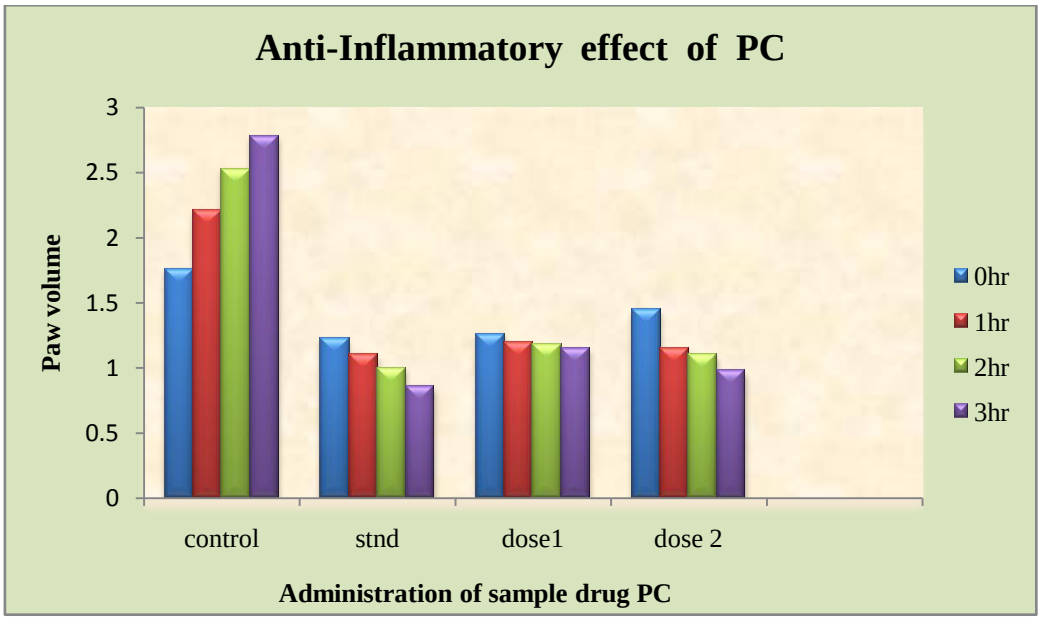
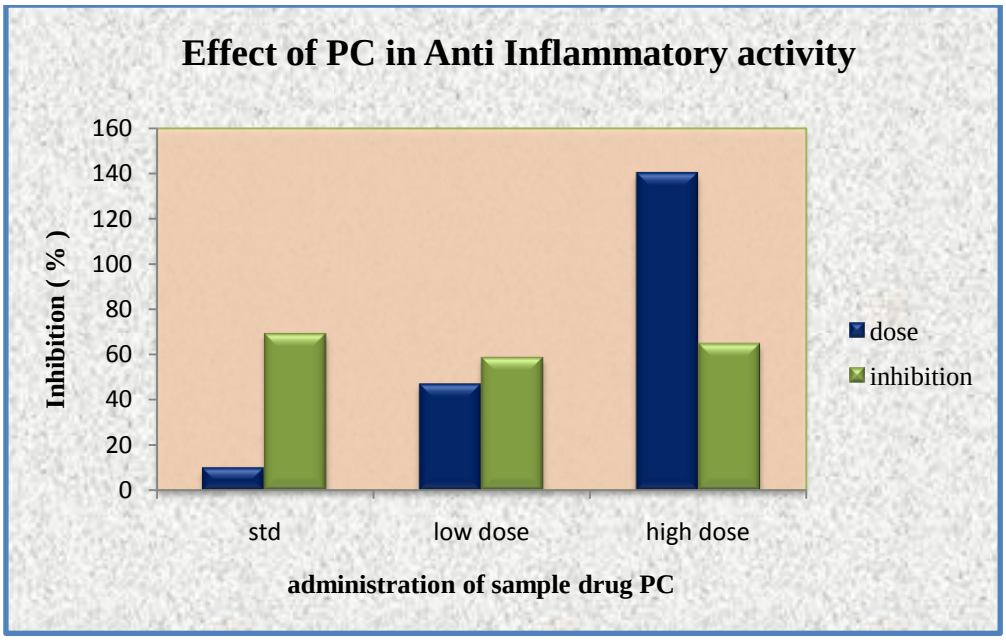


Table No 15: Effect of *Pooneeru Chunnam* on inhibition of paw edema method

Groups	% of Inhibition of paw edema			
	0hr	1hr	2hr	3hr
Indomethacin 10mg/kg	30.11	50.2	60.47	69.06
PC 50mg/kg	28.4	45.7	53.35	58.63
PC140mg/kg	17.61	47.96	56.52	64.74

Chart No 4: Anti- inflammatory activity of *Pooneeru Chunnam.*



Result of Anti-Inflammatory activity

The effect of *Pooneeru Chunnam* on carrageenan-induced rat paw edema at different hours of study was compared to that of control for the evaluation of anti-inflammatory activity on the basis of percent inhibition of paw edema volume.

Pooneeru Chunnam at 50 mg/kg dose showed significant anti-inflammatory activity ($P < 0.01$) at 3rd hour when compared to control group. At 140 mg/kg the drug showed significant result ($P<0.01$) at 3rd hour when compared to control group.

Effect of *Pooneeru Chunnam* on inhibition of paw edema method with low dose 50 mg/kg found to be 58.63% and high dose 140 mg/kg found to be 64.74 % when compared to standard group has 69.06% of inhibition. Among the result, *Pooneeru Chunnam* has better significant at 140mg/kg when compared to standard group.

Conclusion

Thus it is concluded that administration of *Pooneeru Chunnam* at the dose of 140 mg/kg exhibits significant ($p<0.01$) anti-inflammatory activity in Wistar albino rats when compared with control group and its percentage of inhibition is 64.74% when compared with standard group.

6.3 EVALUATION OF ANALGESIC ACTIVITY OF *POONEERU CHUNNAM* IN SWISS ALBINO MICE

Table No. 16: Analgesic activity of *Pooneeru Chunnam* in Swiss albino mice

Groups	Treatment	Reaction time in sec			
		0min	**60min**	**90min**	120min
I	Control	2.5±0.54	2.5±0.54	2.1±0.75	2.3±1.03
II	Pentazocine (5mg/kg)	2.6±0.81	6.5±0.98	6.9±0.75	7.5±1.04
III	Low dose (50mg/kg)	2.5±1.04	4.4±1.16	4.6±0.81	5.2±0.89
IV	High dose (150mg/kg)	2.5±1.04	5±0.89	5.5±1.04	6.5±1.21

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 *Pooneeru Chunnam* by Eddy's hot plate method.

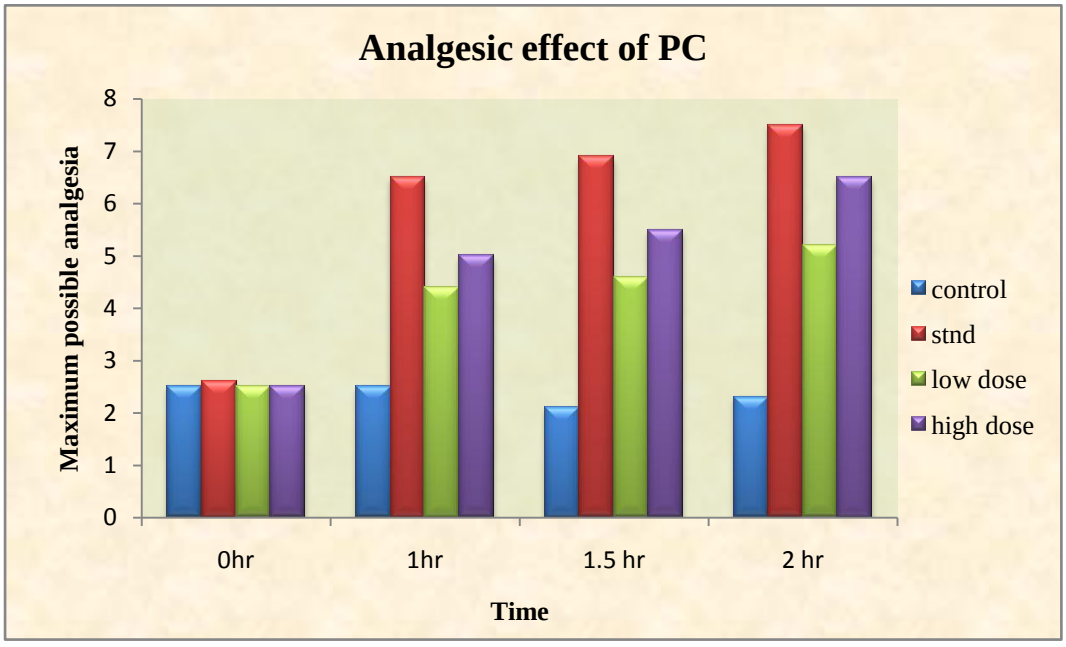
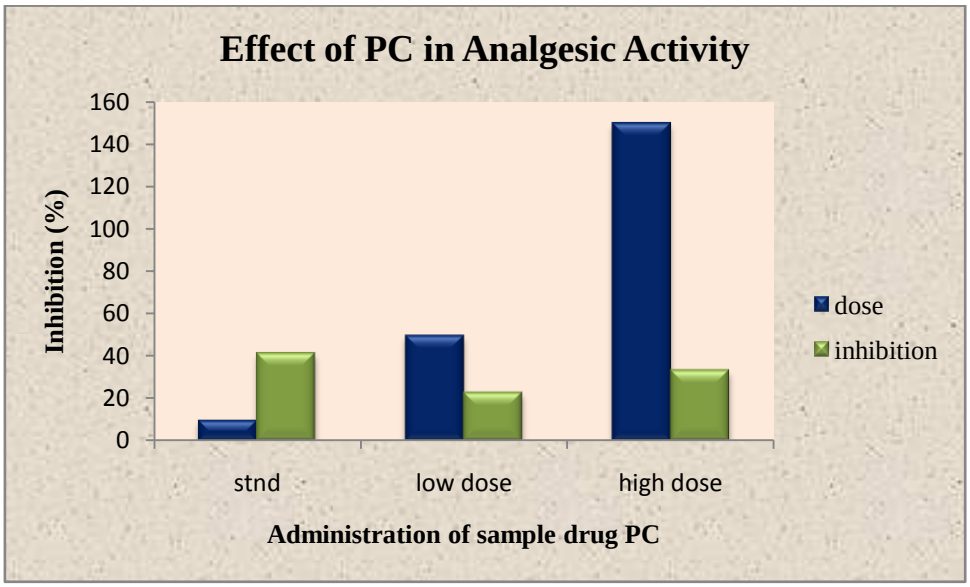


Table No. 17: Analgesic activity of *Pooneeru Chunnam* in Swiss albino mice

Groups	% of Inhibition of analgesic			
	0hr	1hr	1.5 hr	2hr
Pentazocine (5mg/kg)	0.8	32	37.2	40.94
Low dose PC (50mg/kg)	0	15.2	19.37	22.83
High dosePC (150mg/kg)	0	20	26.35	33.07

Chart No : 6 Effect of *Pooneeru Chunnam* in Analgesic activity.



Result of Analgesic activity of *Pooneeru Chunnam* in Swiss albino mice

Analgesic activity was carried out by Eddy's Hot plate method. *Pooneeru Chunnam* at dose of 50 mg/kg showed analgesic activity with the statistical significance of ($P<0.01$) at 120 min when compared to control group. At 150 mg/kg, the drug showed analgesic activity with significance ($P<0.01$) at 120mins when compared to the control group.

Effect of *Pooneeru Chunnam* on inhibition of Eddy's Hot plate method with low dose 50 mg/kg found to be 22.83% and high dose 150 mg/kg found to be 33.07 % when compared to standard group has 69.06% of inhibition. Among the result, *Pooneeru Chunnam* has better significant at 150mg/kg when compared to standard group.

Conclusion

Thus it is concluded that administration of *Pooneeru Chunnam* at the dose of 150 mg/kg exhibits highly significant ($p<0.01$) analgesic activity in Swiss albino mice when compared with control group and its percentage of inhibition is 33.07% when compared with standard group.

8. DISCUSSION

The trial drug *Pooneeru Chunnam* was selected from the text "*Anuboga Vaithiya Navaneetham* part-3" for screening the pharmacological effect of Anti- spasmodic, Anti inflammatory and Analgesic activities.

The drug was prepared as per the procedure and subjected to various studies to reveal potency and effectiveness against the disease.

Pooneeeru Chunnam had been subjected to various studies and it confirms the literature evidences. Literary collections, physicochemical studies, chemical analysis and pharmacological studies were done to prove the Anti- spasmodic, Anti inflammatory and Analgesic activities of *Pooneeru Chunnam.*

Literary review about the ingredients of *Pooneeru Chunnam* from various text books gave hope about its activity. The studies strongly substantiated textual references and as discussed below.

Literary collections:

Literary collections include drug review, which consist Botanical aspect, Gunapadam aspect, and pharmacological reviews which supported this study.

Physico- chemical analysis:

- The trial drug *Pooneeru Chunnam* showed that the loss on drying (LOD) was 4.82% which reveals the low moisture content present in the prepared medicine. Low moisture content- drug could get maximum stability and better shelf life.
- The trial drug shows that total ash value was found to be 0% whereas the acid insoluble ash is also 0%. The value of total ash in the formulation is nullified because of the absence of inorganic ingredients.

Chemical analysis:

Chemical analysis of the drug *Pooneeru chunnam* revealed the presence of Silicate, Sulphate, Carbonate, Aliminium, Iron, Zinc, magnesium, potassium, starch and alkaloids**.**

Instrumental analysis:

The standardization of the drug was evaluated by chemical characterization with functional analysis, Characterization and Identification of Crystalline materials , heavy metal analysis and determine the purity of a substance by FTIR, XRF, AAS and TLC respectively.

- The FTIR results showed the presence of the wave number of 1640, 648 having C=C Stretch, C-Br Stretch.This indicates the presence of some organic functional groups such as alkane, halo compound.
- The heavy metals such as Arsenic, Mercury, Lead and Cadmium were observed in BLQ. Hence the safety of the drug *Pooneeru Chunnam* is ensured for clinical use.
- XRF analysis, it is observed that so many elements are present in *Pooneeru Chunnam*. Some of the elements such as sodium, calcium, chloride and manganese are playing a major therapeutic role in *Pooneeru Chunnam.*
- In TLC analysis, it indicates that the sample given does not contain any organic compounds or phytochemicals. Thus, the *Pooneeru Chunnam* given is purely inorganic substance without organic impurities.

Pharmacological studies:

The pharmacological activities like Anti- spasmodic, Anti- inflammatory and Analgesic activity of *Pooneeru Chunnam* shown significant effect.

Anti- spasmodic Activity

The anti- spasmodic activity was evaluated using charcoal and castor oil induced intestinal motility in mice. It found to be as percentage of the distance travelled by charcoal plug for each of animal.

There was significant reduction in distance travelled by the charcoal plug was observed in PC treated group with the transit distance of 17.67 ± 0.80 cm when compared to that of the control with distance of 28 ± 0.96cm. Further standard drug shown prominent decrease in transit distance of 15.3 ± 1.66 cm.

Anti – inflammatory activity

The anti- inflammatory activity was evaluated using carrageenan- induced paw edema method in wistar albino rats. *Pooneeru Chunnam* has shown significant ($p<0.01$) anti–inflammatory activity in wistar albino rats when compared with control group and its percentage of inhibition is 64.74% when compared with standard group.

Analgesic activity

The Analgesic activity was evaluated using eddy's hot plate method in swiss albino mice. From the result it is concluded that administration of *Pooneeru Chunnam* at the dose of 150 mg/ kg exhibits significant ($p< 0.01$) analgesic activity in swiss albino mice when compared with control group and its percentage of inhibition is 33.07% when compared with standard group.

` Thus scrutinizing all the above mentioned factors it is concluded that the test drug *Pooneeru Chunnam* has been scientifically validated and it is a safe and a potent Anti-spasmodic drug. It also possesses Anti- inflammatory and Analgesic activity which supports the effective treatment for managing *Soothagavali* (Dysmenorrhea), *Gunmam* (Gastric ulcer) and *Seriyammai* (Indigestion).

9.SUMMARY

- The test drug *Pooneeru Chunnam*, a traditional Siddha formulation was selected from the Siddha literature "*Anuboga Vaithiya Navaneetham* part-3" for its Anti-Spasmodic, Anti- inflammatory and Analgesic activities.
- The test drug was prepared as per the procedures mentioned in Siddha literature. All the ingredients were identified and authenticated by the experts.
- Review of Literature in various categories was carried out. Siddha aspect, botanical aspect and pharmacological review were discussed about the drug and the disease.
- The drug was subjected to analysis such as physicochemical, chemical, instrumental and pharmacological analysis.
- Chemical analysis of the drug *Pooneeru Chunnam* revealed the presence of Silicate, Sulphate, Carbonate, Aliminium, Iron, Zinc, magnesium, potassium, starch and alkaloids.
- Identification of functional groups was engaged by using Fourier Transform Infra-Red Spectroscophy (FTIR).
- Heavy metal analysis was engaged by using Atomic absorption spectrophotometric analysis of heavy metals(AAS)
- Characterization and Identification of Crystalline materials by X-Ray Fluorescence (XRF)
- Monitor the progress of a reaction, determine the purity of a substance and to allow more accurate quantitative analysis by using Thin- layer chromatography Analysis (TLC)
- The instrumental analysis report reveals that the heavy metals like Lead, Cadmium, Arsenic and Mercury were found in below the desirable limit.
- Pharmacological studies were done. It revealed that the drug *Pooneeru Chunnam* possess Anti- Spasmodic, Anti- inflammatory and Analgesic activities in animal models.
- From the results and the statistical analysis it was proved that the drug *Pooneeru Chunnam* has
    - Anti- Spasmodic activity
    - Anti- inflammatory activity
    - Analgesic activity.

- This present study suggests that *Pooneeru Chunnam* has remarkable medicinal value in the treatment of Dysmenorrhea. Thus the Siddha formulation *Pooneeru Chunnam* is standardized and validated its efficacy for the management of Dysmenorrhea (*Soothaga vali*) and it would be a great drug of choice.

10.CONCLUSION

From the above analytical studies (i.e, qualitative and quantitative analysis) and pharmacological studies (i.e, Anti-spasmodic, Anti – inflammatory and Analgesic activities), the drug *Pooneeru Chunnam* have Anti- Spasmodic, Anti – inflammatory and Analgesic activities. It was concluded that the *Pooneeru Chunnam* can be used in the management of *Soothagavali* (dysmenorrhea).

11. ANNEXURE

CERTIFICATE

This is to certify that the project title *standardization and pharmacological screening of Pooneeru Chunnam* **has been approved by the IAEC.** Total No. of animal Sanctioned: 24 Rats + 24 Mice (Male or Female)

Approval No: NIS/IAEC-V/09082017/06

Prof. Dr.V.Banumathi	Prof Dr.K.Nachimuthu
Chairman IAEC	**CPCSEA nominee**

Signature with date

V. Banumathi 3/8/2017

Chairman/Member Secretary of IAEC: **CPCSEA nominee:** 3/8/2017

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

Name of the principal Investigator:

Dr.U.Madhunitha,
1st yr PG Scholar,
Department of Gunapadam,
National Institute of Siddha.

Name of the Guide :

Dr.S.Sivakkumar,MD(S)
Lecturer,
Department of Gunapadam,
National Institute of Siddha.

CERTIFICATE

Ministry of AYUSH

NATIONAL INSTITUTE OF SIDDHA

Ministry of AYUSH, Government of India

Tambaram Sanatorium, Chennai - 600 047.

WORKSHOP ON
RESEARCH METHODOLOGY & BIOSTATISTICS

This is to certify that

Dr. U. MADHUNITHA

has participated in the above Workshop held from 16.04.2018 to 20.04.2018 conducted by the Dept. of Noi Naadal, at National Institute of Siddha, Tambaram Sanatorium, Chennai-600 047.

Dr. G.J. Christian
Coordinator
HoD, Dept. of Noi Naadal,
National Institute of Siddha

Prof.Dr.V.Banumathi
Director,
National Institute of Siddha
Chennai – 600 047.

NATIONAL INSTITUTE OF SIDDHA

An Autonomous Body under Ministry of AYUSH
Govt. of India

Workshop on

Laboratory Animal Care and Basic Research Techniques

(12-16 February, 2018)

CERTIFICATE

This is to certify that Dr. U. MADHUNITHA has participated as Trainee / ~~Resource Person~~ / ~~organizing committee member~~ in the workshop on **"Laboratory Animal Care and Basic Research Techniques"** held on 12 - 16 February, 2018 at National Institute of Siddha, Chennai, Tamil Nadu.

Dr. V. Suba
Organizing Secretary

Prof.Dr.V.Banumathi
Director

NATIONAL INSTITUTE OF SIDDHA, CHENNAI – 600047

BOTANICAL CERTIFICATE

Certified that the following plant drugs used in the Siddha formulation **"Pooneeru chunnam"** taken up for Post Graduation Dissertation studies by **Dr.U.Madhunitha** M.D.(S), II year, Department of Gunapadam, 2018, are identified through Visual inspection, Experience, Education & Training, Organoleptic characters, Morphology and Taxonomical methods as

Pergularia daemia (Forssk) Chiov (Asclepiadaceae), Leaf

Oryza sativa Linn. (Poaceae), Seed

MEDICINAL BOTANY * CHENNAI 600 047 * National Institute of Siddha

Certificate No: NISMB3262018

Date: 09-03-18

Authorized Signatory

Dr. D. ARAVIND, M.D.(s),M.Sc.,
Assistant Professor
Department of Medicinal Botany
National Institute of Siddha
Chennai - 600 047. INDIA

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

Accredited with "A" Grade by NAAC

Chennai – 600 119, Tamil Nadu, India

INSTITUTIONAL ANIMAL ETHICS COMMITTEE

CERTIFICATE

This is to certify that the project entitled 'Evaluation of Antispasmodic potential of Siddha formulation Pooneeru Chunnam in Mice' has been **approved.**

IAEC Number: SU/CLATR/IAEC/XIII/135/2019

Name of the P.I: Dr.U.Madhunitha

Animal Sanctioned: *Mus musculus*

Total: Male – 12 ;Female- 12.

Date: 05.01.2019

B. Sheela Rani

Dr. B. SHEELA RANI
CHAIR PERSON

Dr. N. SARAVANAN
CPCSEA MAIN NOMINEE

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