PRECLINICAL STUDY OF SIDDHA DRUG BOSANA KUDORI MATHIRAI FOR IT'S ANTI-ULCER, ANTI-SPASMODIC & ANTI DIARRHOEAL ACTIVITIES

Dissertation submitted to

THE TAMILNADU DR. MGR MEDICAL UNIVERSITY

CHENNAI-600032

In partial fulfilment of the requirements

for the award of the degree of

DOCTOR OF MEDICINE (SIDDHA)

BRANCH-II-GUNAPADAM

POST GRADUATE DEPARTMENT OF GUNAPADAM

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GC	-	Gas chromatography
CCD_S	-	Charge coupled devices.
VPC	-	Vapor phase chromatography
GLPC	-	Gas liquid partition chromatography
SPME	-	Solid phase micro extraction
TCD	-	Thermal conductivity detector
FID	-	Flame Ionization detector
CCD	-	Catalytic combustion detector
LD	-	Low dose

Mg	Milligram
Kg	Kilogram
LD_{50}	Lethal Dose $_{50}$
p.o	peros
ML	Milliliter
%	percentage
R&D	Research and Development
EDTA	Ethylene Diamine Tetra Acetic Acid
M	Male
g%	Gram percentage
g	Gram
NOAEL	No-Observed-Adverse-Effect-Level
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Kg	Kilogram
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p.o	peros
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%	percentage
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The author wants to thank **Dr. Murugesan** Scientific Officer IIT, Chennai for ICP analysis, for helping me to do the test ICP-OES,GAS&FTIR.

I express my sincere thanks to **Dr. N. Chidambaranathan, HOD,** Dept. of Pharmacology, Madurai Kamaraj University, Madurai for their excellent help in pharmacological study and other guidance to do the research work.

I express my sincere thanks to **G. Arihara Sivakumar, Associate Professor,** Dept. of Pharmacology, K.M.C.H. College of pharmacy, Coimbatore. For their excellent help in pharmacological study and other guidance to do the research work

I also acknowledge my thanks to **Aravindh Herbal Labs, Pvt ltd,** Rajapalayam for Physico chemical analysis .

The author is greatful to the **Mrs.Poongkodi, M.L.I.S., M.phil., Librarian** Govt. Siddha Medical College,Palayamkottai and Library Assistants for help in literary collections.

I am also thankful to all my college staffs for their kind co-operation for my study. I should express my gratefulness to **All My Classmates** and **PG. Gunapaaam students** for lending their helping hands whenever needed during the course of the study.

I owe my loving thanks to my **Father and My Husband** without their encouragement and understanding it would have been impossible for me to finish this work.

Finally, I wishes my thanks to **Maharaja DTP Services,** Palayamkottai for their marvelous work in completing this dissertation.

I would like to thank **The Tamilnadu Dr.MGR Medical University** for giving permission to carry out my dissertation work.

ABBREVIATIONS

BKM	-	Bosana Kudori Mathirai
CPCSEA	-	Committee for the purpose of control and supervision of experimental animals.
DC	-	Differential Count
EDTA	-	Ethylene Diamine Tetra Aceticacid
ESR	-	Erythrocyte Sedimentation Rate
FTIR	-	Fourier transform infrared spectroscopy
Hb	-	Haemoglobin
IAEC	-	Institutional Animal Ethical Committee.
ICP-OES	-	Inductively coupled plasma optical emission spectrometry
XRD	-	X-Ray powder Diffraction
Ig E	-	Immunoglobulin E
LDH	-	Lactate Dehydrogenase
MCV	-	Mean Corpuscular Volume
OECD	-	Organisation for Economic Co-operation and Development
PCV	-	Packed Cell Volume.
PGE	-	Prostaglandin E
RBC	-	Red Blood Corpuscles
SEM	-	Scanning electron microscope
TLC	-	Thin Layer Chromatograph
GC	-	Gas chromatography
CCD_S	-	Charge coupled devices.
VPC	-	Vapor phase chromatography
GLPC	-	Gas liquid partition chromatography
SPME	-	Solid phase micro extraction
TCD	-	Thermal conductivity detector
FID	-	Flame Ionization detector
CCD	-	Catalytic combustion detector
LD	-	Low dose

Mg	Milligram
Kg	Kilogram
LD_{50}	Lethal Dose $_{50}$
p.o	peros
ML	Milliliter
%	percentage
R&D	Research and Development
EDTA	Ethylene Diamine Tetra Acetic Acid
M	Male
g%	Gram percentage
g	Gram
NOAEL	No-Observed-Adverse-Effect-Level
MLD	Minimum Lethal Dose
MTD	Maximum Tolerated Dose
OECD	Organisation of Economic Co-operation and Development
CPCSEA	Committee for the Purpose of Control and Supervision of Experiments on Animals

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INTRODUCTION

"SIDDHA SYSTEM OF MEDICINE" is ancient medical system prevailed in southern parts of India.

This system is put forward by great siddhars. Who were ancient scholars in spirituality medicine and alchemy.

Siddha is a first system to emphasis. Health as the perfect state of physical, psycological, social and spiritual component of human being.

Thirumoolar a great saint said that,

"One than cure physical ailment is medicine
One that cure psychological ailment is medicine
One that prevent ailment is medicine
One that bestows immortality is medicine"

In siddha medicine are prepared after so many complicated process and after proper purification of plants, minerals, animal products and even metals are also used in the pharmacological process.

So many special drugs and preparation methods are said in the classical text books of siddha which will care wide range of disease from simple fever to cancer.

Siddha system has developed a rich and unique treasure of drug knowledge in which use of plants and minerals is very much advocated. The minerals are not used directly. But the minerals are after purification it convert in to a form that is made bio available to the body.

Mathiraigal (Tablets) are known to be effective in very small doses, usually a few milligrams. It expiry in many months. In siddha text mathiraigal are expiring upto 1 year.

Inthuppu, Inji, chukku, milagu, seeragam, perungayam both promising formulations are being successfully prescribed by siddha and ayurvedic physicians without any side effects since centuries.

Inthuppu which chemically known as sodiam chloride. It is a cheapest drug but more valuble drug for abdominal disorders. If also promotes digestion and it stimulate the functional activity of the stomach.

Inji, chukku, miliagu, perungayam, seeragam both are easily available drugs. These drugs are increases the digestive enzymes and cures Indigestion.

As per literature evidence from anubogavaidya pramma ragasium **"Bosana kudori mathirai"** is indicated especially for **Gunmam, Akni mantham, kiragani vagaigal** and also. So many siddha physicians are using this drug for varied spectrum of diseases.

AIM AND OBJECTIVES

Scope of the Study :

The Scope of the study is to do a scientific review to validate the safety and efficacy of "Bosanakudori Mathirai" for anti-ulcer, anti-spasmodic and anti diarrhoeal activities.

Objectives :

The following methodology was adopted to evaluate the safety and efficacy of the test drug.

- To collect the literature systematically from siddha texts as well as modern science.
- To standardize the preparation of drug according to classical siddha literature.
- To subject the drug to physico chemical and Chemical analysis.
- To detect the elements present in the drug by instrumental analysis.
- To study the acute and subacute toxicity profile of Bosanakudori Mathirai according to OECD guideline.
- To determine the pharmacological activity of Bosanakudori Mathirai.
- To analyse all the above study results to validate the advantage of Bosanakudori Mathirai.

REVIEW OF LITERATURE

3.1 Chukku (Zingiber officinale)

3.1.1 Gunapadam Aspect

Other names:

"மகத்தான வர்க்கமென்றும் நாகராதி என்றும்
மடவான வீயமென்றதற்குப் பேரு
அகத்தான வகநாதமென்றும் பேரு
அருளினோம் வலதமென்றதற்குப் பேரு
வகத்தான வேர்க்கொம் பென்றும் பேரு
வசனித் தோம் மஞ்சத்த மென்றும் பேரு
நகத்தான நாக்கு மென்றும் பேரு
நளிமையாய் சொல்லிவிட்டேன் சுக்கின் பேரே"

- Varkkam
- Naagaraathi
- Veeyam
- Vaganaatham
- Valatham
- Verkombu
- Manjatham
- Naakku

- ***Panja Kaaviya Nigandu***

"சுக்குனுடப் பேர்தனையே சொல்லக்கேளு
சுண்டியாங்கால் வீதம் விசுவமாகும்
முக்குனுட நாகமாம் பேஷமுமாகும்
முக்கிறமாங் கற்பத்திரஞ் சிறுங்கின் பேர்
நக்கினுட தாத்திரிபஞ் சாதமுமாகும்
சாங்கமா யுறப்புமாங் கசப்புமாகும்
பக்கினுடத் திரிதோஷ மானியாகும்
பரிபாஷை நாமமெல்லாஞ் சுக்குக்காமே"

- ***Bogar Nigandu 1200***

Sundi, Kealveetham, Visuvam, Naagam, Besham, Thaathiri Panjathagam, Urappu, Kasappu, Tridhoshamaani.

- Veeram
- Singi
- Naagamal
- Sow baakiyam
- Sundi

- ***TV Samba Sivampillai Agarathi***

Other Names

"Arukkan, Athagam, Aarthragam, Ubagullam, Ularntha inji, Kadupathira, Chukku, Sundi, Sondi, Sowpannam, Sowvarnam, Navasuru, Nagaram, Manavushatham, Vichvabeshjam, Vidamoodiya amirtham, Verkombu

- Gunapadam Mooligai Vaguppu

Properties

Part Used	-	Rhizome
Taste	-	Pungent (Kaarpu)
Character	-	Veppam
Class	-	Pungent (Kaarpu)

Actions:

- Stimulant
- Stomachic
- Carminative
- Sialogogue

General characters:

"சூலை மந்தம் நெஞ்செரிப்பு தோடமேப் பம்மழலை
மூலம் இரைப்பிருமல் மூக்குநீர் - வாலகப
தோடமதி சாரஞ் தொடர்வாத குன்மநீர்த்
தோடம் ஆமம் போக்குஞ் சுக்கு"

-Agasthiyar Gunavagadam

It cures Indigestion, Heart burn, Veppam, Anal Diseases, Bronchial Asthma, Kaasam, Sinusitis, Vaadha Ulcer.

“வாதப் பிணிவயி றூதற் செவிவாய்
வலிதலை வலிகுலை வலியிரு விழிநீர்
சீத த் தொடுவரி பேதிப் பலரோ
சிகமலி முகமுக முகமிடி கபமார்
சீதச் சுரம்விரி பேதச் சுரநோய்
தெறிபடுமெனமொழி குவர்புவி தனிலே
ஈதுக் குதவுமி தீதுக் குதவா
தெனும்விதி யிலைநவ சுறுகுண முனவே”

- ***Theraiyar Gunavagadam***

It cures abdominal distension, ear pain, Vaadha diseases, Facial Diseases, Head diseases, Disentery, Anaemia, Kabha Seetha suram.

Characters:

“ சுக்குறுஞ் சிறப்பைக் கூறின் சுவைகரு லகுவேஸ்நிக்த
மிக்குறு முஷ்ணம் வீரியம் வியன்பாக மதுரமாகுந்
தக்கிலாக் கபமே வீக்கந் தவிர்த்திடும் பண்னும்பிந்தம்
புக்குழல் சுவாசங் காசம் புயைம்ஸ்லீ பதத்தினோடு”

It cures Kabham, Swelling, Pitham, **BronchialAsthma**, Kaasam

- ***Pathartha Panja guna Manjari***

Purification:

- Removal of the upper skin to get purified chukku. It cures constipation.
- Chukku - 1 part
- Sunnampukkal - 2 Part. Mixed together and kept for 1 samam then wash thoroughly and dried then to remove the upper skin.

Types:

- Maachukku
- Kalchukku

The former is succulent and large the later is fibrous and small.

Therapeutic uses:

- Chukku kali is used externally on the forehead it cures Headache, and on neck it cures throat pain and on eyebrows it cures long sight.
- Chukku and Karkandu take 1 varagan each mixed together and powder it, morning and evening can be given with tender coconut milk it cures chest pain and dyspnoea.

“ ஏற்ற சுக்கோ டரத்தை யெருக்கம் வேர்
நாற்ற நொச்சிக் கொழுந்துட னாற்றமுஞ்
சேர்த்து நீருழக் காக்கித் தினங்கொளில்
மாற்ற லாஞ்சன்னி வாத சுரங்களே”

- ***Theran Venba***

Description:

- Chukku
- Arathai
- Erukkanver
- Nochi Kolundu
- Perumarapattai

All theses take equal quantity mixed with some water, boiled well and shrinks. It cures sanni, Vadha fever.

“நன்றான வேர்க்கொம்பு நற்சா ரடைவேரு
மொன்றாய் கியாழமிட் டுண்டுவிடில் - அன்றேதான்
ஓடுமே மந்த மொழியாமல் நாடகன்று
காடுதனிற் போகி விடுங் காண்”

Description

- Chukku
- Saradai ver take equal quantiy make kudineer it cures indigestion

Chukku karpam:

“சுக்கினைப் பொடி செய்து சூரணமாக்கி
யிக்கிரத் திலுண்டிட வயிற் றெரிவுபோங்
குடிநீர்செய்ததைக்குடி நிதநன்மையாம்”

Description

Chukku Powder can be mixed with sugar care juice taken in the morning it cures Gastric burning. Chukku Kudineer is good for health.

Chukkukarpam:

“காலமே யிஞ்சியுண்ணக் காட்டினார் சூத்திரத்தில்
மாலையதி லேகடுக்காய் மத்தியானஞ் சுக்கருந்த
சூலமே தேகமடா சுக்கிலத்தைக் கட்டிவிடும்
ஞாலமே லுனதுவிந்து நற்றேங்காய் போலாமே”

- ***Thiruvalluvanaayanarkarpam – 300***

-

"சீந்தி நீர்க் கண்டம் தெறிசுக்குத் தேனளவாய்
மோந்தபின் யார்க்கும் தலைக்குலத்தில் - காந்தி
மலைக்குத்து மால்யானை வள்ளுவர் முப்பாலாற்
தலைக்குத்துத் தீர்வு சாத்தற்கு"

-Thiruvalluva mallai., Thaamodharanar

Chukku kudineer:

"சுக்கு கடுக்காய் நிலவேம்பு சுகமாய் வேப்பந் தோல்சீந்தில்
கைக்கும் புடலம் கொடியது வுங்கதிக்கு மொவ்வொன்றோர் கழஞ்சு
யொக்க நறுக்கி யிருநாழி யுழக்காய் - காய்ச்சிக் குடிப்பீரேல்
தக்க விடமுஞ் சுரம் பலவுந் தான்விட்டோடுந் தப்பாதே"

Description

- Chukku
- Kadukkai
- Nilavembu
- Vepanthol
- Seenthil
- Peipudal take each 1 *Kalanji* make the kudineer it cures Toxic fever.
- Chukku powder and cow's milk mixed together and drink for proper appetite.
- Chukku is also chewed for tooth ache
- Small piece of chukku is inserted in the ear it cures ear pain, Kabha diseases.
- Chukku is dried and applied for joint pains.
- Chukku Kudineer 80ml is drinked daily for 2 (or) 3 times it cures stomach pain vomiting indigestion abdominal distension.
- Chukku is chewed to cure Throat pain and sore throat.
- Chukku is grinded with mother's milk and applied on the forehead it cures headache.
- Chukku I part is soaked in Kayanthakarai juice for one day and Elam 1 part is soaked in Inji Juice for one day, then mix these together and dried in sunlight for 3 days and powder act. 1-2gram powder is given in morning and evening with ghee for good sperm motility.
- Chukku, kadukkai, vazhuluvai all these powdered and given for 1 mandalam with Honey it gives good voice articulation for singer's.

- Chukku, Kadukkai, vengaram, Cheenagaram take equal quantity mixed with sugar, honey and grinded with lemon juice and applied on the tongue for uncontrolled vomiting.

SIDDHA FORMULATIONS

1. **Chukku legium**

   Dosage : Punnaikai alavu

   Indication : Discentry, Piles, Diarrhoea, Indigestion.

2. **Verkombu Chooranam**

   Dosage : Verugadi alavu

   Indication : Peptic ulcer, Indigestion.

3. **Pirandai Legium**

   Dosage : Punnaikai alavu

   Indication : Piles, Peptic ulcer, Vomitting.

4. **Sundai vatral Thiravagam**

   Dosage : 5 drops

   Indication : Discentry, Diarrhoea.

5. **Sivathai Chooranam**

   Dosage : Varagan (4 gram)

   Indication : Piles, Peptic ulcer, Constipation.

6. **Sundai vathal Chooranam**

   Dosage : Verugadi alavu

   Indication : Discentry, Diarrhoea.

7. **Komiya Legium**

   Dosage : 3 Kasu eadai

   Indication : Pepticulcer, Obesity.

8. Puliyarai Kirutham

Dosage : 1 Spoon

Indication : Discentry, Diarrhoea.

9. Asvaganthathi chooranam

Dosage : Verugadi alavu

Indication : Infertility, Ghonerrhoea, loss of appitite, Peptic ulcer.

10. Nilavagai Chooranam

Dosage : Verugadi alavu

Indication : Constipation, Piles, Peptic ulcer, Flatulence.

11. Naraththai legium

Dosage : Punnai kai alavu

Indication : Peptic ulcer, Anorexia,Flatulence, Indigestion.

12. Thalisathi Vadagam

Dosage : Pakku alavu

Indication : Discentry, Diarrhoea.

13. Thaier Sundi Chooranam

Dosage : Thirigadi

Indication : Indigestion, Diarrhoea.

3.1.2 BOTANICAL ASPECTS

CHUKKU (ZINGIBER OFFICINALE)

Taxanamical Classification:

Zingiber officinale (Roscoe)

Kingdom	:	Plantae-Plants
Subkingdom	:	Tracheobionta-Vascular plants
Superdivision	:	Spermatophyta-Seed plants
Division	:	Magnoliophyta-Flowering plants
Class	:	Liliopsida-Monocotyledons
Subclass	:	Zingiberidae
Order	:	Zingiberales
Family	:	Zingiberaceae - Ginger family
Genus	:	Zingiber P. Mill. - Ginger
Species	:	Zingiber officinale Roscoe - Garden ginger

Description:

Ginger has a perennial rhizome or stem which creeps and increases in size underground. Roots grow from the bottom of the rhizome and shoots from the upper surface.

In the spring it sends up from its rhizome a green reed-like stalk about 2 feet high, with narrow lanceolate leaves. These leaves die back after the growing season. The flowering stalk rises directly from the rhizome with the leaves and consists of an oblong spike with scalloped green bracts. From each bract one or more white or yellowish-green flowers is produced, blooming for several days. The underground rhizome is the source of commercial "ginger root".

Geographic Distribution:

Ginger is said to be a native of China and India. It is cultivated in West Indies, Jamaica, Africa.

Native Legends and Names:

Ginger was introduced into the Americas after the discovery of that country by the Spaniards. Francisco de Mendosa transplanted it from the East Indies into Spain, where Spanish-Americans cultivated it vigorously, so that in 1547 they exported 22,053 cwt.

Indigenous Practices:

Ginger is now cultivated in great quantities in Jamaica and comes in United States dried and preserved.

The root from the West Indies is considered the best. Also imported from Africa, there are several varieties known in commerce. Jamaica or White African is a light-brown color with short rhizome, very pungent. Cochin has a very short rhizome, coated red-grey color. "Coated or Uncoated" is the trade term for rhizome with peel on or skinned. Green Ginger is the immature undried rhizome. Preserved Ginger is made by steeping the root in hot syrup and then crystallizing it. Ratoon is uncultivated Ginger.

3.1.3 LATERAL RESEARCH:

Abstract

Ginger (*Zingiber officinale* Roscoe, Zingiberacae) is a medicinal plant that has been widely used in Chinese, Ayurvedic and Tibb-Unani herbal medicines all over the world, since antiquity, for a wide array of unrelated ailments that include arthritis, rheumatism, sprains, muscular aches, pains, sore throats, cramps, constipation, indigestion, vomiting, hypertension, dementia, fever, infectious diseases and helminthiasis.

Currently, there is a renewed interest in ginger, and several scientific investigations aimed at isolation and identification of active constituents of ginger, scientific verification of its pharmacological actions and of its constituents, and verification of the basis of the use of ginger in some of several diseases and conditions.

This article aims at reviewing the most salient recent reports on these investigations.

The main pharmacological actions of ginger and compounds isolated therefrom include immuno-modulatory, anti-tumorigenic, anti-inflammatory, anti-apoptotic, anti-hyperglycemic, anti-lipidemic and anti-emetic actions. Ginger is a strong anti-oxidant substance and may either mitigate or prevent generation of free radicals. It is considered a safe herbal medicine with only few and insignificant adverse/side effects.

More studies are required in animals and humans on the kinetics of ginger and its constituents and on the effects of their consumption over a long period of time.

3.2 PERUNGAYAM (FERULA ASAFOETIDA)

3.2.1 Gunapadam Aspect

Other Names:

“அருளினோம் சோமநாதி என்றும் பேரு
ஆடகண்டகாரி யாதி யென்றும் பேரு
எருளினோம் இந்தத்தூளி என்றும் பேரு
இறங்குராமென்ற தற்குப் பேருண்டாச்சு
உருளினோம் உக்கித மென்றிதற்குப் பேரு
உசிதமுள்ள குந்தக மென்றதற்குப் பேரு
குருளினோம் குட்டமென்றதற்குப் பேரு
கூறினோம் பெருங்காய ஆதீதப் பேரே”

- Somanathi
- Aada Kandakari
- Aathi
- Indhuli
- Kooraam
- Ukkitham
- Kunthagam
- Kuttam

- ***Agathiyar Yemathathuvam Ennum Panjakaviya Nigandu***

"ஹிங்கு ராமடமத் யுக்ரமிருஞ்சூப தூபனந்தான்
பொங்குஜந் துக்ன மோடு பூதநாசனமு மாகும்
மங்கிடா பாஹ்லீக மருவிய வகூடகந்தம்
துங்கமார் ஜரணத் தானுஞ் சொல் பெருங்காயத்தின் பேரு”

- Nigandu Rathnagaram

- Hingu
- Raamadam
- Athyukram
- Soobathubanam
- Janthuknam
- Boothanasa

- Paagiiligam
- Vaguda Kandham
- Jaranam

"வல்லீகமே யிரணம்
சந்துநாசம் பூத நாசமிங்கு - கந்தியத்தி
யாக்கிரமி ராமடம் பெருங்காயமாம்"

- Valligam
- Ranam
- Santhunasam
- boothanasam
- Ingu
- Kanthi
- Athiyakkram
- Ramadam

Ferula asafoetida:

Perungayam is a latex Gum obtained from a perennial Plant. the latex is usually collected in earthern pots and dried. It is either packaged in solid blocks or powdered and sold. Asafoetida is popularly called perungayam in Tamil. It occurs in places such as parasigam, Ofganistan, Kashmir & Punjab.

Perungayam is one of the Trithoda Sama Porul. It balances the pulses digestion Perungayam is one of the constituents of Trigayam. Trigayam is

- Perungayam
- Milagu
- Vengayam

Perungayam was used by Indians for more than Hundred Years in food products and curing diseases

Part's Used

Gum Resin

Properties :

Colour	-	Yellow
Odour	-	Garlic Odour
Taste	-	It is bitter in taste Soluble in alcohol
Suvai	-	bitter
Character	-	Veppam
Class	-	Pungent

Vernacular Names

Tamil	-	Perungayam
English	-	asafoetida
Sanskrit	-	Hingu, balhika
Hindi	-	Hing
Bengali	-	Hing
Marathi	-	Hing
Guajarati	-	Hing
Kannadam	-	Hing
Telugu	-	Inguva
Malayalam	-	Perungayam
Oriya	-	Hengu
Kashmir	-	Yang
Bombay	-	Hing
Persian	-	Anguzeh,
Burma	-	Shinka, Singu
Malay	-	Hingu
Arabic	-	Typib

Actions:

- Stimulant
- Carminative
- Antispasmodic
- expectorant
- Laxative
- Anthelmintic
- Diuretic
- Aphrodisiac
- Emmenagogue
- Nervine tonic
- sedative
- Digestive

Purification

- "வசலைவிலை சாறுவிட்டு வன்பெருங் காயத்தை
- நனையவொரு சாமமரை நன்றாம்"
- It sook in the Lotus leaves water For one Hours.
- Fried if until it lost itshumidity.

General Characters

"தந்தவே தந்தமூலத் தெழும்பிணி
சருவகாளம் விருச்சிகங் கீடம்மா
மந்தம் வாதம் உதராவர்த்தம் அல்குல் நோய்
மார்பணங்கட்ட குன்மம் மகோதரம்
உந்துகெர்ப்பத்தின் வித்திரஞ் சூலைச்சூர்
உதிரப்பூச்சி சிலேத்து மத்துறும் வலி
வந்த மெய்க்கடுப் போடிவை முற்றுமே
மாயு நாறு நற் காயங் கிடைக்கினே"

It cures teeth diseases, snake bites, ***vatha diseases***, gastric ulcers, Ascites, *Kaba diseases,* body pain *Eraippu*.

-Gunapadam Mooligai Vagupu

It cures abdominal distension, indigestion, gastric ulcer, increased vadha, ear diseases

It cures vadha disease, 8 types of gastric ulcer, vaginal diseases, uterine problems. Kaba diseases, toothe disordes, Ascites, Chest Pain.

"அஷ்ட குன்மங்கள் அணுகாது ஆகமத்தில்
ஒட்டிய வாயுதிரட்சி யோடுமே முட்டவே
அருங்காயம் புட்டியாம் ஆயிழையீர் கேளீர்
பெருங்காய முண்டு குணம் பேசு"

- ***Agathiyar Kunavagadam***

Perumgayam and Ulunthu Powder can be mixed and inhales it cures *Eraippu,* Indigestion.

Therapeutic uses

- It in valuable remedy for hysteria and nerve disorder of women and children flatulence, flatulent colic and spasmodic affection of bowal especially when connected with hysterive, in faistion and emotional states. Nerve palpibations, lypochondriasis and other affection due to hysteria in the spasmodic, and the obstinate cougtis of childhood remaining after advanced stages of whooping cough, pheumonia and bronchitis of children, and the chronic bronchitis and asthuma of adults.
- Asafoetida is useful as an an thelmintic for roural worms in children enema is an effectual means of semoraj thnead-worms from reetun and lower bowel.
- In dental earies a mixture of opivm and asafetida is placed in hollow tooth to rehiene the ache.
- In diarrhea and early stage of cholera a pill. consisting of asafetida camphor and black papper grain each and opium ¼ grain is of great value.

SIDDHA FORMULATIONS:

1. **Asta Chooranam**

   Dosage : Verugadi alavu

   Indication : Peptic ulcer, abdomiral disorders.

2. **Saman Chooranam**

   Dosage : Verugadi alavu

   Indication : Indigestion, Peptic ulcer, Flatulence, Disentry.

3. **Thapalavana Chooranam**

   Dosage : Verugadi alavu

   Indication : Disentry, Diarrhoea, Piles, Peptic ulcer.

4. **Narathankai Kaduku**

   Dosage : 1 gram

   Indication : Abdominal pain, Peptic ulcer.

5. Karun Kozhi Chooranam

Dosage : Verugadi alavu

Indication : Peptic ulcer, Diarrhoea.

6. Perungaya legiam

Dosage : 1 Kalangu (5 gram)

Indication : Arthritis, Peptic ulcer.

7. Perungaya Chooranam

Dosage : Verugadi alavu

Indication : Peptic ulcer.

8. Perungaya Thiravagam

Dosage : 5 drops

Indication : Diarrhea, Discentry.

9. Kabada Mathirai

Dosage : Ilanthai Vithai alavu

Indication : abdominal disorders, Discentry, Diarrhoea, Amibic discentry.

10. Akginimuga Chooranam

Dosage : Verugadi alavu

Indication : Discentry, Diarrhoea.

3.2.2 BOTANICAL ASPECTS

PERUNGAYAM (FERULA ASAFOETIDA)

Taxanomical classification

According to Bentham and Hooker Classification "Perungayam" is classified as follows.

Kingdom	-	Plant
Division	-	Phanerogams
Class	-	Dicotyledons
Sub-Class	-	Polypetalae
Series	-	Calyciflorae
Order	-	Umbellales
Family	-	Umbelliferae
Genus	-	Ferula
Species	-	Asafoetida

Botanical Description

Petiole

About 9 inches, triangula – cylindrical solid, with a short, membranous, intra-petiolar ligula at the base, the rachis laterally compressed double winged along the top with the narrow decurrent bases of the leaflets.

Flower

Flowers polygamous the fertile umbels large, solitary, terminating the lateral branches, the male much smaller, very dense, globular, clustered at the ends of peduncles, flowers are 10-20 in the main and 5-6 is partial umbels.

Calyx

Calyx teeth very slightly marked.

Corolla

Petals oblong – ovate acute, entire – pale yellow.

Androecium

Filaments are as long as the petals

Gynoecium

Styles, long, spreading, deciduous, Stylopod, prominent, cupped with a sinuous (or) lobed margin.

"Mass" asafoetida is the common commercial form, uniform in mass.

"Paste" form contains extraneous matter.

Since pure asafoetida is not preferred due to its strong flavour, it is mixed with starch and sold as compounded asafoetida mostly in bricket form. It is also available in free flowing (power form) or in tablet forms.

Volatile Oil

The odour and stimulant property of asafoetida are due to this oil which may be obtained by distilling asafoetida with water or alcohol. It contains several sulphide of ferulyl, two terpenes which yield sesquiterpene and blue coloured oil.

Investigations by Bauman (1929) have shown that about 50% of the resin consist of resene and volatile oil. Part of the resene, asaresene A was obtained in crystalline form. The drug also contains about 1.3% of free ferulic acid and about 16% of very unstable ester of Ferulic acid with asaresinol.

The resin portion consists chiefly of asaresinotannol, free of combined with ferulic acid.

Umbelirerone present in the combine state.

Oil of asafoetida is obtained by the stream distillation of the gum resion.

The Physico – Chemical properties of the oil are as follows.

Sp. Gravity - 1.493 – 1.515

Sulphur Content - 15.3 – 29%

Pinene, Another Terpene present.

The disagreeable odour of the oil is reported mainly to the disulphide.

Gum Contains

- Glucuronic Acid
- Galactose
- Arabinose
- Rhamnose and Protein

Chemical Structure:

Asafoetida

Phyto Chemicals	**Plant Part**
2- Butymethy - disulfide	Gum
2- Butylmethyl – tetra Sulfide	Gum
2 – Butylmethyl - trisulfide	Gum
Allylpropyl – Sulfide	Essential Oil
Alpha – Pinene	Gum
Asaresiontannol	Gum
Bassorin	Gum
Beta-Pinene	Essential Oil
Butyl Propenyl – disulfide	Essential Oil
Cadinene	Essential Oil
Diallyl – Sulfide	Gum
Fernesferol	Gum
Ferulic – acid	Essential Oil
Foetidin	Gum
Galautose	Gum
Glucuronic – acid	Gum
L – Arabinose	Gum
Propenyl – Sulfide	Gum
Rhamnose	Gum
Sec – butyl- Propenyl – disalfide,	Resin, Exudates
Umbell iferone	Essential Oil
Valeric – Acid	Essential Oil

3.2.3 Lateral research

According to the Chinese,European, Iranian and Indian traditional medicines,oleo gum resin of Ferula assa-foetida (asafoetida) has therapeutic effects on different kinds of disease.Some of these effects are related to the disease of nervous system such as hysteresis and convulsion.In recent studies,some anti-epileptic and neuroprotective roles were also considered for it and we examined its possible role on treatment of peropheral neuropathy.

Material and methods

In vitro studies were carried out to identyfy the response of isolated sciatic nerves to different concentrations of oleo gum resin of asafoetida solved in looks solution .Then ,in vivo studies were conducted to evaluate its effect in amelioration of peripheral neuropathy in mice.Peripheral neuropathy was induced by intraperiotoneal injection of high doses of pyridoxine in adult Balb/c male mice.Tail flick tests were performed to identify the incidence of neuropathy in animals.After 10 days treatment with asafoetida,the efficiency of treatment was assessed by behavirol,electrophysiological and histological studies.

3.3 INJI (ZINGIBER OFFICINALE)

3.3.1. GUNAPADAM ASPECT

Other names

கனமான வறுகாத வல்லமென்றும்
கருவான ஆதரமாத்துவாக மென்றும்
மெதமான மேளாதி யென்றும் பேரு
மேகக் கருவாதி யென்றுமிதற்கு பேரு
மதமான மகரமென்றிதற்குப் பேரு
மானாகி மூலியென்றிதற்கு பேரு
நரமான நரம்பென்று மிதக்குப் பேரு
நாம்சொன்னோம் இஞ்சியின் நல்லப்பேரே

- **Panjakaviya nigandu**

- Varukkatha vallam
- Arththaragam
- Melathi
- Megakaruvathi
- Makaram
- Managi mooli
- Narambu

Other Names

Iilakkottai. Allam, Narumaruppu mathil. Arthragam

- ***Gunapadam Mooligai Vaguppu***

Properties

Part used	-	Rhigome of fresh ginger
Taste	-	Pungent (kaarpu)
Character	-	Veppam
Class	-	Pungent (Kaarpu)
Action	-	Carminative Stomachi Sialogogue Digestive Stimulant.

Vernacular Names

Tamil	-	Inji
Sans	-	Srongarera, sring a beram
kujarath	-	Adu
mn	-	Ala, Alen
Telungu	-	Allam
Can	-	Hashi - Shunti
Kon	-	Alen
Duk	-	Adrak, Ada, Adi

General Characters:

இஞ்சிக் கிழங்குக்கு இருமல் ஐயம் ஓக்காளம்
வஞ்சிக்குஞ் சன்னிசுரம் வன்பேதி - விஞ்சுகின்ற
சூலையறும் வாதம்போந் துண்டாத தீபனமாம்
வேலையுறுங் கண்ணாய் விளம்பு.

It cures cough, Iyam, sanni, fever, diarrhoea, vatham and soolai.

Purification:

Removal of the outer layer to get purified

Therapeutic uses

- Equal part of ginger juice, lemon juice and rock - salt, well mixed together or eual part of ginger and rock - salt should be taken just before meals. It clean the togue and throat, Increases he Appetite and produces an agreeable sensation.
- Ginger juice rubbed on and around the navel is said to cure all kinds of diarrhea.
- Ginger juice and onion mixed together and given relieves nausia, vomitting.
- Ginger juice mixed with sugarcandy and given twice daily is a good remedy for diabetes (Both types)
- Relaxed sore thoat, hoorseness and loss of voice are some times benefits by chew icy a piece of glug ee so as to produced a copious flow of saliva.
- Take, thola of giyantic swallow - work (mudur) roots and pestle well in a mortar to be made into pills of the sine of black peper. In Choler cases administer this pills with lake - warm water.

SIDDHA FORMULATIONS

1. **Injji Lagium**

   Dosage : Pakka alavu

   Indication : Peptic ulcer, Rhomatic Arthritis, Vomiting, Indigestion.

2. **Kandathri Chooranam**

   Dosage : Verugadi alavu

   Indication : Anorexia, Indigestion, Diarrhoea.

3. **Lagu vilvathi legium**

   Dosage : Pakka alavu

   Indication : vomiting, anorexia, Indigation.

4. **Aaththirathi legium**

   Dosage : Pakka alavu

   Indication : Indigation, Diarrhoea.

5. **Kanda Sarkarai Legium**

   Dosage : Pakka alavu

   Indication : Diarrhoea, Abdominal disfunction, vomiting.

6. **Vilvathi legium**

   Dosage : Pakka alavu

   Indication : Indigation, Diarrhoea, Anorexia

7. **Sathaveri Nei**

   Dosage : 1 Spoon

   Indication : Seria mantham, Flatulence, Diarrhoea.

8. **Kukkuda Legium**

   Dosage : 3kalanju (`15 gram)

   Indication : piles, peptic ulcer, Hernia

9. Inji chooranam

Dosage : Verugadi alavu.

Indication : Diarrhoea discertry.

10. Inji thiravagam

Dosage : 5 drops

Indication : Indigation, Diarrhoea.

11. Kadukkai Legium

Dosage : Punnaikai alavu

Indication : Piles, abdominal disorders, peptic ulcer.

3.3.2 BOTANICAL ASPECTS

INJI (ZINGIBER OFFICINALE)

Taxanamical Classification:

Zingiber officinale (Roscoe)

Kingdom	:	Plantae-Plants
Subkingdom	:	Tracheobionta-Vascular plants
Superdivision	:	Spermatophyta-Seed plants
Division	:	Magnoliophyta-Flowering plants
Class	:	Liliopsida-Monocotyledons
Subclass	:	Zingiberidae
Order	:	Zingiberales
Family	:	Zingiberaceae - Ginger family
Genus	:	Zingiber P. Mill. - Ginger
Species	:	Zingiber officinale Roscoe - Garden ginger

Description:

Ginger has a perennial rhizome or stem which creeps and increases in size underground. Roots grow from the bottom of the rhizome and shoots from the upper surface.

In the spring it sends up from its rhizome a green reed-like stalk about 2 feet high, with narrow lanceolate leaves. These leaves die back after the growing season. The flowering stalk rises directly from the rhizome with the leaves and consists of an oblong spike with scalloped green bracts. From each bract one or more white or yellowish-green flowers is produced, blooming for several days. The underground rhizome is the source of commercial "ginger root".

Geographic Distribution:

Ginger is said to be a native of China and India. It is cultivated in West Indies, Jamaica, Africa.

Native Legends and Names:

Ginger was introduced into the Americas after the discovery of that country by the Spaniards. Francisco de Mendosa transplanted it from the East Indies into Spain, where Spanish-Americans cultivated it vigorously, so that in 1547 they exported 22,053 cwt.

Indigenous Practices:

Ginger is now cultivated in great quantities in Jamaica and comes in United States dried and preserved.

The root from the West Indies is considered the best. Also imported from Africa, there are several varieties known in commerce. Jamaica or White African is a light-brown color with short rhizome, very pungent. Cochin has a very short rhizome, coated red-grey color. "Coated or Uncoated" is the trade term for rhizome with peel on or skinned. Green Ginger is the immature undried rhizome. Preserved Ginger is made by steeping the root in hot syrup and then crystallizing it. Ratoon is uncultivated Ginger.

3.3.3 LATERAL RESEARCH:

Abstract

Ginger (*Zingiber officinale* Roscoe, Zingiberacae) is a medicinal plant that has been widely used in Chinese, Ayurvedic and Tibb-Unani herbal medicines all over the world, since antiquity, for a wide array of unrelated ailments that include arthritis, rheumatism, sprains, muscular aches, pains, sore throats, cramps, constipation, indigestion, vomiting, hypertension, dementia, fever, infectious diseases and helminthiasis.

Currently, there is a renewed interest in ginger, and several scientific investigations aimed at isolation and identification of active constituents of ginger, scientific verification of its pharmacological actions and of its constituents, and verification of the basis of the use of ginger in some of several diseases and conditions.

This article aims at reviewing the most salient recent reports on these investigations.

The main pharmacological actions of ginger and compounds isolated therefrom include immuno-modulatory, anti-tumorigenic, anti-inflammatory, anti-apoptotic, anti-hyperglycemic, anti-lipidemic and anti-emetic actions. Ginger is a strong anti-oxidant substance and may either mitigate or prevent generation of free radicals. It is considered a safe herbal medicine with only few and insignificant adverse/side effects.

More studies are required in animals and humans on the kinetics of ginger and its constituents and on the effects of their consumption over a long period of time.

3.4 INTHUPPU (SODIUM CHLORIDE IMPURA)

3.4.1 GUNAPADAM ASPECT

Other Names

படியான வாணி மாக்கசமென்றும் பேரு
பரிவான சைந்தலவணமென்றும் பேரு
சடியான சந்திலகமென்றும் பேறு
சார்வான நாகநெய்யென்றும் பேரு
வெடியான வித்தகமென்றும் பேரு
விளங்கினோ மதிலவணமென்றும் பேரு
கடியான கடி மாசமென்றும் பேரு
காரகஞ்சாரக இந்துப்பினிடப் பேரே"

- Panjakaviya Nigandu

- Vaani
- Maaksasm
- Saindhalavanam
- Santhilagam
- Naaganei
- Vithagam
- Mathilavanam
- Kadimaasam
- Kaara kanjaragam

'இந்துப்பின் பேர்தனையே இயம்பக்கேளு
எழிலான செந்தூரத்தஞ் சயந்தவமாகும்
சந்திரனான முப்பதாம் தணிந்த மதிக்கூர்மை
தயங்காத வேளை தனிற்ற ளர்ந்த பனிக்கூர்மை
மந்திரம் போல் வளர்ந்த மரியுப்புச் சீலாலம்
மதியென்ற பூரத்தின் மித்ருவாகும்
மந்திரம் போல் பாண்டத்தில் அமைந்த அப்பு
ஆச்சர்ய மிந்துப்புப் பேருமாமே"

- Bogar Nigandu

- Sindhuratham
- Sainthavam
- Chandhiravuppu

- Mathikoormai
- Panikoormai
- Mathivauppu
- Silalam
- Poorathin Mithru
- Paandathirku Samaitha Vuppu

Vernacular Names :

Tamil	-	Indhuppu Sainthavum
English	-	Rock Salt, Halite
Hindi	-	Khanji namka, Saindhava, Lahori namak
Mar	-	Mitha
Gujarat	-	Mitha
Bengali	-	Nimok, Num

Weight:

- 2-10 Pound

Colour

- Dirty brown Colour - Outer Surface
- White - Inner Surface

Synthetic Preparation

12000 liters of sea water is heated in a new vessel the salt in taken 310kg of this salt is taken in a base thickened vessel and heated (Kaadakkini) until the salt melts.

Vediuppu	-	5 *Palam* (175g)
Seenakkaaram	-	5 *Palam* (175g)
Pooneeru	-	3 *Palam* (105gm)

When these are powdered melted and cooled it looks like dimand. Pachai Karpooram dies in this.

Magnesaci Sulphas : (Seemai Indhuppu)

According to modern chemical theory the magnesaci Sulphas is prepared from the heated kariuppu Solution which was taken from sea water or it can

be prepared by mixing calcium carbonate of magnet with sulphuric acid this salt looks like needle, white in colour and salty in test. It is soluble in water and insoluble in alcohol.

Purification

"இந்துப்புக் காடி இசையத் தெளித்து வெய்யில்
அந்தத்தான் சுத்தியதாம் அல்லாது – நந்தாமல்
ஆட்டுநீர் விட்டே ஆடுகின்றார் சுத்தியதாய்
தோட்டமுற நல்லதுவாம் செப்பு"

Purification

- Soaked in Vinegar 3 days and dried in sunlight.
- Smashed with Goat's urine (or) vinegar for 72 minutes and dried in sunlight.

Actions:

- Purgative (*Malakari*)
- Carminative (*AgatuvaiVagatri*)
- Diuretic (*SiruneerPerukki*)
- Appitiser (*Pasitheeththundi*)
- In purgative action it is best cream of tartaric

Dosage

- 1-2 *Varagan* (4.2 to 8.4 gm) *Purgative*
- 4-5 *Varagan* (16.8 to 21gm) *laxative*

Rarely it is given in separate form specially it is given with *kakkaratan* Seed powder.

General Characteries

"அட்டகுன்ம மந்தம் அசிக்க்கரஞ் சூர் சீது பித்தந்
துட்ட வையம் நாடிப்புண் டோடங்கள் - கெட்டமலக்
கட்டுவிட விந்தையக் காமியநோய் வன்கரப்பான்
விட்டுவிட விந்துப்பை விள்"

"சென்னிக்கண்ணா பற்றுர் செவிகவுள்கண் டம்பகநோய்
சந்தியா சங்காசந் தாகமிரைப் - புன்னிரத்த
மூலஞ் சிலந்திநளி மூடிமநஞ் சூதை வலி
சூலஞ் சிதையுமிந்தாந் சொல்"

It cures 8 types it gastric ulcer, Indigestions, *Asirkkaram*, *Kabapitham*, *three dhosam,Swasam,* bleeding piles, vaadha Kaduppu, Pain, Head disease, eye disease, tongue disease, Vaginal disease, check disease, Spider bite, scorpio bite, Rat bite.

- Gunapadam Thaathu Jeeva Vagupu

Therapeutic uses:

- To Cure body sprain Rock Salt pasted and applied externally
- It can be heated and used for formation it cure pain and swelling
- It is dissolved in hot water to induce vomiting.

SIDDHA FORMULATIONS

1. **Thadima Mathi Chooranam**

   Dosage : Verugadi alavu

   Indication : Abdominal distention, diarrhea, Flatulence.

2. **Sarthulathi Chooranam**

   Dosage : Verugadi alavu

   Indication : Peptic ulcer, diarrhoea.

3. **Nagarathi Chooranam**

   Dosage : Verugadi alavu

   Indication : Diarrhoea, Peptic ulcer.

4. **Bavana Kadukkai**

   Dosage : Sundai alavu

   Indication : Peptic ulcer, Diarrhoea, piles, abdomiral diseases.

5. **Sanga thiravagam**

   Dosage : 1 Drop

   Indication : Peptic ulcer, Gastritis

6. **Panjalavana Chooranam**

   Dosage : Kasu alavu

   Indication : Peptic ulcer

7. Sarapenthira kuligai

Dosage : Kundrimani alavu

Indication : Peptic ulcer

8. Vediuppu Thiravagam

Dosage : 1 – 3 drops

Indication : Peptic ulcer

9. Kunma Kudori Melugu

Dosage : Sundai alavu

Indication : Indigestion, Peptic ulcer

10. Inhuppu Chooranam

Dosage : 1 Thola

Indication : Indigestion, Vomiting

11. Vaivadagam Chooranam

Dosage : 1 – 2 Varagan

Indication : abdominal distention, stomach pain, constipation.

3.4.2 CHEMICAL ASPECT

INTHUPPU (Sodii Chloridum Impura)

Vernacular Names :

Sans	:	Saindhava
Eng	:	Rock – Salt
Arab	:	Mil-he-tabazand
Pers	:	Namake sang
Hind	:	Sendhalon, Sodhalon
Duk	:	Sondanimak
Guj	:	Sindhaluna
Tel	:	Saindhalavanam
Tamil	:	Indu-uppu
Can, Kan & Mah	:	Sendhurlavana
Mal	:	Intu-uppu
Ger	:	Natrium Chloricum

Identification

1	Colour	-	Clear or white also clear blue nipples with a large juck, purple, pink yellow and grey
2.	Crystal habit	-	Predominantly cubes and in Massive sedimentary beds but also granular fibrous and compact.
3.	Crystal System	-	Isometric 4/m bar 32/m
4.	Cleverage	-	Perfect in three directions in cubes
5.	Mohs Scale Hardness	-	2 – 2.5
6.	Luster	-	Glassy
7.	Refractive Index	-	1.544
8.	Streak	-	White
9.	Specific Gravity	-	2.1
10.	Wonsity	-	2.1 – 2.6 g/cc
11	Solubility	-	In Water
12.	Other Characters	-	Salty Flavour

Impurities:

Gypsum	-	CaS04
Sylvite	-	KCL

Properties:

Rock Salt is plastic and flows slowly under great pressure.

General Properties:

Name	-	Sodium Chloride Impura
Chemical Formula	-	Nacl
Appearance	-	White or Clear Solid

Physical Property

Molecular Weight (Nacl)	-	58.4428
A tomic Weight (NA+)	-	22.98768 (39.337%)
A tomic Weight (Cl-)	-	35.4527 (60.663%)
Viscosity	-	10^{18} Poises at 18^0
		10^{17} Poises at 80^0
Bulk density	-	1.154 (7216^3)
Angle of repose	-	32^0
Melting Point	-	1.465^0C (2.669 ^{0}F)
Hardness	-	2.5
Critical Humidity at 20 ^{0}C	-	75.3%
PH of aqueous solutions	-	Neutral
Head of fusion	-	1.35 g cal/gm

Ionic Bonds

Na [(Ne)3 S1) + [(NE)3 S2 3 P5]

Na + $[(Ne)^+ Cl^-$ [(Ne) 3 S2 3 P6]

Content of Nacl

1 gm of Nacl Contains

0.3934 gm of Sodium (Na+)

0.6066 gm of Chlorine (Cl-)

Safety:

Ingestion	-	Dangerous in large quantities
Inhalation	-	May cause irritation
Skin	-	May Cause irritation
Eyes	-	May Cause irritation
Radio Active	-	Don't have Radio active

Crystal Structure

Nacl form crystal with cubic symmetry. In this the large chloride ion are arranged in a cubes close packing white the small sodium ions fill the octahedeal gaps between them. Each ion surrounded by six of the other kind this same basic structure is found many other minerals and is known as the Halite Structure.

Solubility:

Solubility of Nacl in Variour Solvents (1gm Nacl/100g of solvent at 25^0C) cubes. It is brownish white externally and white internally. It has a pure saline taste and burns with a yellow fame.

H20	-	36
Liquid Ammonia	-	3.02
Methanol	-	1.4
Formic acid	-	5.2

Action:

In small does it is highly carminative stomachic and digestive. It promotes the appetite and assists digestion and assimilation. In large doses (1 to 2 drachms) it is cathartic, in still larger doses (4 to 8 dramchms) it is emetic. Rock salt possesses stronger purgative properties than cream ofbut like this it is not a satisfactory cathartic given alone. Combined with other purgatives it is equal it not superior to it.

Salt for Human nutrition

Human nutrition is a major market for salt because salt is an essential component of the human diet.

Sodium

Major extra cellular (The serum of human blood contains 5.5 parts/1000 by weight of Nacl) electrolyte responsible for regulating water balance, PH and Osmotic pressure.

Chloride

Essential to good Health

It preserves acid base balance in the body aids, potassium absorption, supplies the essence of digestive stomach acid, and enhances the ability of the blood to carry car-bondi-oxide from respiring tissue to the lungs.

Flavour Enhance:

Salt is commonly used as a flavors enhancer for food and has been identified as one of the basic tastes.

Unfortunately, the excess amount of salt intake where the required in take is much lower causes elevated levels of blood pressure, increased risks of heart attack and strock.

Source:

Found in nature in extensive beds. Mostly associated with clay and calcium sulphate, To obtain it, holes are dug into these rocks which soon become filled -up with salt water, the water evaporated and the salt is left ready for use.

Uses:

It is given in dyspepsia and other abdominal disorders. To rouse digestion weakened by diarrhoea- rock- salt and yavakshar (alkali – potassium carbonas impura) are given, in convalescenc. When heated it is used to painful, swollen and such other parts. Rock salt with warm water is used as an emetic. A compound powder called vadavanal churna containing rock salt, long pepper, pipli, cubebs, chitrak, ginger and myrobalans in equal parts, mixed and made into a powder is used in anorexia. Flatulence and biliousness. Dose in 5 to 15 grains two or three times a day with water.

Synthetic preparation

A medicinal salt called Nariekelakshara is highly recommended in chakradatta as valuable in the form of dyspepsia which is attended with pain two or three hours after meals. It is thus prepared.

Take a coconut – fruit full of water, make a hole in it and - till the coconut with rock-salt and dissolve it in its water. Then close the opening, cover the nut with a layer of clay and roast it in a pit of fire. The salt thus roasted is given with the addition of long pepper. Dose is about a quarter tola. A powder made of rock salt 10 grains Kaladana 1 drachm and day ginger 10 grains is a good laxative, in a single dose. As a digestive, a compound powder made of rock salt, chebulic myrobalan. Cmblic myrobalan and long pepper is equal parts is recommended in doses of 10 grains twice a day. A powder containing pancha lavana 5 Parts, impure oxide of iron 5 parts and emblic myrobalan 4 parts is useful in doses of 10 grains in despepsia, conjested liver etc. A medicated oil named salpa Masha Taila is used as an application in rheumatism, contracted knee joint, shift shoulder as joint etc.,

Formation :

It is typically formed by the evaporation for salty water (such as sea water) which contain Na^+ and Cl – icons.

One finds Rock salt deposits ringing at dry lake bed, island marginal sea and inclosed bay and estuaries in arid regions of world.

3.4.3 LATERAL RESEARCH

ABSTRACT

Each and every system of medicine has its own exclusive specialities. Selection of drugs based on the chemical composition and action is the common entity followed all over the world. An important method of selection of drugs based on the tastes, characteristics, effects and ultimate taste has been followed in India's holistic indigenous medical science called Siddha science. According to Siddha science, all the things in the universe both inside and outside the body are made up of five basic elements namely space, air, fire, water and soil in balanced proportion. The basic motto of Siddha science is, "Food itself is medicine and medicine itself is food". The six tastes of food materials are also composed of these basic elements. Intake of all these six tastes at right proportion in our diet maintains the physiological homeostasis in our body. Excessive intake of a particular taste or avoiding some other taste leads to alteration. Such an alteration in this balanced proportion leads to ailments. Giving a drug that balances this alteration is the treatment method of Siddha science. Hence most of the drugs in Siddha science are the ingredients of our food we take in our regular diet. This paper deals with this advanced and excellent method of treatment. This paper includes various topics – nature of the body, humours of the body, assessing the patient, diagnosis, the six tastes ofthe drugs, nature of drugs and selection of drugs.

3.5. SEERAGAM (CUMINUM CYMINUM)

3.5.1. GUNAPADAM ASPECT

Other Names:

சீரகத்தின் பேர்தனையே செப்பக்கேளு
செயலான துத்தார பலமுமாகும்
மேரகத்தின் மெத்திபஞ் சியமசாரியாகி
மிடுக்கான உபகும்பபீசம்
காரகத்தின் காளிகா சுரபஞ்சியாகுங்
கடிசான குஞ்சிகா பிறக்கி விகாயாகும்
தாரகத்தின் பித்த நாசனியுமாகுந்
தளுக்கான சீரகத்தின் நாமமாமே.

- Bogar Nigandu 1200

- Thuththara palam
- Meththibam
- Siyamasaavi
- Kasara Pakujsi
- Kujjka prith
- vithaya
- pithanasini

Asai, Narseeri, Bosana kudori, Methiyam, seeri, ubagumbapeesam.

-Gunapadam Mooligai Vaguppu

Properties:

Taste	:	Pungent, sweet
Character	:	Cool
Class	:	Sweet
Used Part	:	Seeds
Action	:	Carminative
		Stimulant
		Stomachic
		Astringent
		Digestive
		Antispasmodic
		Antidysentric
		Diuretic

Purification:

"சீராமிரண்டுஞ் சிறந்த நாடத்திலிட்டு
வாராயடுப்பு மேல் வறு"

- It is roasted in low flame and taken.

General Characters:

"வாந்தி யருசிகுன்மம் வாய்நோய்பீ லிகம் இரைப்
போந்திருமல் கல்லடைப்பி லாஞ்சனம் உட்-சேர்ந்த கம்மல்
ஆசனகு டாரியெனும் அந்தக் கிரகணியும்
போசனகு டாரி யுண்ணப் போம்".

"வாய்வொடு நாசிநோய் வன்பித்தஞ் சேராது
காயம் நெகிழாது கண் குளிருஞ் - தூயமலர்க்
காரளகப் பெண்மயிலே கைகண்ட தித்தனையுஞ்
சீரகத்தை நீதினமுந் தின்".

It cures vomiting, Anorexia, peptic ulcer, oral and liver disorders. cough, renal stone, worm infestation diarrhea, Nasal disorders, vadham and pitha diseases.

"சீரகத்தன்மை தேரின் திகழ்வோடு வேநீஷம்
நேர்சமங் கருவேபாகம் நிகழ்வாதங் கிருமிகுன்மம்
கூரிய வயிறுப்பல் தகாள்ளதி சாரம் யாவும்
வேறருந் தீபனம்பின் வாயன்பாசனத்தை யாக்கும்"

It cures ulcers, Flatulence, worm infestitation, Diarrhoea, vadlan and it also Increases Appetite.

Therapeutic uses:

- Cumin seeds are aromatic and spicy, extensively used as condiment. In digenous medicine. Cumin seeds have long been considered stimulant and carminative, stomachic astringent and useful in diarrhea and dyspepsia.
- Seeds are also cooling in effect and there fore form on ingredient of most prescriptions for gonorrheal.
- The seeds powder mixed with honey, salt and clarified butter are applied to scorpion bite.
- Seeds mixed lemon juice are administered for nausea in pregnant females.

- The seeds take internally shortly after child birth increases secretions of milk.
- A quantity of seeds lightly smeared with ghee put into pipe and smoked relieven hiccough.
- Cumin oil can be readily converted artificially into thymol. Thymol is used an anthelmintic against hookworm infection and also antiseptic forming part of many propritory preparations

SIDDHA FORMULATIONS :

1. **Thippili Rasayanam**

   Dosage : Nellikai alavu

   Indication : Peptic ulcer, Vomiting, Asthma, Anorexia.

2. **Thippiliyathi legium**

   Dosage : Pakka alavu

   Indication : Peptic ulcer, Gonerrhoea, anorexia, Indigestion.

3. **Sowbakiyasundi legium**

   Dosage : Pakka alavu

   Indication : Anorexia, Akni mantham, Bleeding, peptic ulcer.

4. **Seraga podi**

   Dosage : Kalanju alavu (5 gram)

   Indication : Peptic ulcer, Gonerrhoea, anorexia, Indigestion.

5. **Aruvagai Chooranam**

   Dosage : Verugadi alavu

   Indication : Peptic ulcer, chest pain, Anorexia.

6. **Thibakgini Chooranam**

   Dosage : Verugadi alavu

   Indication : peptic ulcer, Ingunial hernia.

7. Kadugu legium

Dosage : Punnaikai alavu

Indication : Piles, Indigestion, Peptic ulcer, Warminvestation.

8. Panjathibakini legium

Dosage : Pakka alavu

Indication : Diarrhoea, Discentry, Vomiting, abdominal disfunction.

9. Vajirathi Legium

Dosage : Pakka alavu

Indication : Peptic ulcer, Internal piles, external piles.

10. Panjathibakini Chooranam

Dosage : Verugadi alavu

Indication : Peptic ulcer, Indigestion, anorexia

11. Kesari Legium

Dosage : 1 – 2 Varagan

Indication : Peptic ulcer, Anorexia, Abdominal pain, Diarrhoea.

12. Sarabangavilvathi legium

Dosage : 1 Varagan

Indication : Peptic ulcer, Discentry.

13. Mokkirattai Chooranam

Dosage : Thandrikkai alavu

Indication : Peptic ulcer, Vomiting, Abdominal disorders.

3.5.2. BOTANICAL ASPECTS

Seeragam (Cuminum cyminum)

Taxonomical classification:

Kingdom	:	Plant
Class	:	Dicotyledonae
Subclass	:	Polypetalae
Family	:	Umbelliferae
Genus	:	Cuminum
Species	:	Cyminum
Botanical Name:		Cuminum cyminum, Linn

Vernacular Names:

Tamil	-	Seeragam	German	-	Kreuzkummel
Bengali	-	Jira	Burma	-	ziya
English	-	Cumin	Arabic	-	Kamuna
Hindi	-	Zira	Gujarathi	-	Jiru
Marathi	-	Jiraghi	Syria	-	Kemun
Telugu	-	Jiraka			
Urdu	-	Jirah			

Identification of the Family:

Leaves have sheathing bases. Stems hollow. Inflorescence umbels. The involucres of bracts stand out prominently, G (2). Ovary inferior, two chambered with one pendulous anatropous ovule in each loculus. Fruit cremocarp and odoriferous.

Identification of the plant:

The slender annular herb about 1 feet height with a much branched angular or striated stems bearing 2 or 3 particle linear leaves twice or thrice 3 partite, ultimate segments filiform. Umbels compound, rays few, bracts and bracteoles, several, linear, rigid, calyx-teeth small, subulate, unequal. Petals oblong or obovate, emarginate, white often unequal fruits cylindrical.

Description:

Habitat:

It is cultivated throughout the temperate, sub-tropical regions like in India, Persia and Afghanistan.

Habit:

A small slender,erect and annual herb about with the much branched angular and striated stem.

Leaves:

2 or 3 ,bipinnate,dissected,ultimate segments linear,filiform,sheaths white margined.

Inflorescence:

In compound umbels.

Flowers:

Bisexual, regular, actinomorphic epigynous, white in terminal leaf opposed, few rayed.

Calyx:

Calyx teeth small on the inferior ovary

Corolla:

5 petals at various sizes, free, yellow in color

Androecium:

5 stamens, free

Gynoecium:

Bicarpellary, syncarpous pistil, inferior ovary, 2 chambers, one ovule in each chamber.

Fruit:

The fruits are greyish about ¼ inch long, tapering towards both base and apex and compressed laterally with ridges covered by pupillose hairs. The hairs may be absent in some forms .

Chemical Constituents:

The chief constituent is cumaldenyde C_{10} H_{12} O (P-iso-propyl benzaldehycte) which forms nearly 20 to 40% of the oil. Besides the aldehyde, the oil contains P-cymene, Pinene, dipentene, cumene, cuminic alchohol, B-phellandrene and –terpenol.

Seeds analysis carbohydrates 36.3%, moisture 11.9, protein 18.7 fibre 12, calcium 1.08, phosphorous 0.49%, Iron 31mg/100gms, Vitamin A-870 I.u/100g and vitamin C 3mg/100g.

Therapeutic uses:

Cumin seeds are aromatic and spicy, extensively used as condiment. In digenous medicine, cumin seeds hare long been considered stimulant and carminative, stomachic, astringent and useful in diarrhoea and dyspepsia.

3.5.3. LATERAL RESEARCH

Abstract

The effect of the fruit essential oil of *Cuminum cyminum* Linn. (Apiaceae) (*syn. Cuminum odorum* Salisb) on the epileptiform activity induced by pentylenetetrazol (PTZ) was evaluated, using intracellular technique. The results demonstrated that extracellular application of the essential oil of *Cuminum cyminum* (1% and 3%) dramatically decreased the frequency of spontaneous activity induced by PTZ in a time and concentration dependent manner. In addition it showed protection against pentylenetetrazol-induced epileptic activity by increasing the duration, decreasing the amplitude of after hyperpolarization potential (AHP) following the action potential, the peak of action potential, and inhibition of the firing rate. These membrane effects suggest cellular mechanisms by which the essential oil of *Cuminum cyminum* can inhibit the PTZ-induced epileptic activity.

3.6. MILAGU *(Piper nigrum)*

3.6.1. Gunapadam Aspect:

Other Names:

மிளகினுடப் பேர்தனையே விளம்பக்கேளு
முதிர்ந்து நின்ற திரை போக்கி மரிசியாகும்
வளகினுட வலசமுமா தீட்சணமாகும்
மகத்தானது வன்மாஞ் சியாமமாகுஞ்
குளகினுட மூஷ்ணமாம் சத்துவ நேஷங்
கோலக மாஞ்சரதுந் தனியுமாகும்
வளகினுட வாதத்தை யறுக்குகின்ற
மகத்தான மிளகுக்கு நாமமாமே

- *Bogar Nigandu 1200*

- Thiraipokki
- Marisi
- Valasam
- Thetsanam
- Thuranam
- Seyamam
- Mooshnam
- Sathuvanesam
- Kolagam
- Sarathunthini
- Vadha Arukki

Kalinai, Kari, Kayam, Therangal, kolagam, meriyal, sarumabantham, rallisam, masam, kurumelagu, malaiyali.

-Gunapadam Mooligai Vaguppu

சொல்லியதோர் அருட்டனென்றும் இதற்குப் பேரு
சொற்பெரிய மதங்கன் என்றும் பேருண்டாகும்
அல்லிய தோர் மலைத்திருக்க னென்றும் பேரு
அஷ்டமாசாதி யென்றும் இதற்குப் பேரு
கல்லியதோர் கத்திரிச னென்றும் பேரு
கருத்துரட னென்றும் நேர்வளந்தா னென்றும்
மல்லயதோர் கெந்தக னென்றிதற்குப் பேரு
வசனித்தோம் மிளகினிட அதீதப் பேரே.

- Panchakaviya Nigandu

- Arutan
- Mathengan
- Malaithirukkan
- Astamasathi
- Kathirisan
- Karvuthurudan
- Nerrvalandan
- Kanthakan

Properties:

Taste	:	Pitter, Pungent
Character	:	Veppam
Class	:	Pangent
used part	:	Seed
Action	:	

- Acrid
- Carminative
- Stimulant
- Antiperiodic
- Resolvent
- Rubefacient
- Antivatha
- Atidode.

Vernacular Names:

Tamil	:	Milagu
Eng	:	Pepper
Jehegu	:	Miriyalu
Malayam	:	Kurumilagu
Kannadam	:	Menasy
Sanskrit	:	Maricha
Hindi	:	Kali mirich
Persian	:	Filfliaisiah
Bengoli	:	Glomorich, Morich, Kalamorich
Gujarathi	:	Kalimori
Urdu	:	Fulfil Sioyah, Kalimirich

Purification:

"…. மாமிளகின் வார்மோரைப் படிய
உளறவிட்டுத் தானுலர்த்தி ஒட்டிலிள வறுப்பு
தேற வறுக்க சுத்தியாகும்"

Soak in the sour butter milk for 3 hours and dried.

General Characters:

"சீதசுரம் பாண்டு சிலேஷ்மங் கிராணிமூலம்
வாதம் அருசிபித்தம் மாமூலம் ஓதுசன்னி
யாசம் அபஸ்மாரம் அடன்மேகம் காசம் இவை
நாசம் கறிமிளகி னால்"

"கோணுகின்ற பங்கவலி குய்யரோ கம்வாதம்
சோணிதங்க ழுத்திற்குள் தோன்றும் நோய் - காணரிய
காதுநோய் மாதர் குன்மங் காமாலை மந்தம் என்றீர்
ஏதுநோய் காய் இருக்கில் ஈங்கு.

It cures the Malarial Fever, Anemia, diarrhoea, piles, ulcer, Flattulance, Anorexia, Diabeties, cough, hemeplegia, vaginal disease, Neck and Nasal disorders, Jaundice, pitham, vatham, vedhasonitha Noi, and Sanni.

Therapeutic uses

- It is prescribed in cholareae, dyspepsia, flatulance, aliments.
- An infusion of black paper Forms a useful stimulant gargle in relaxed sore - throat and hoarseness dependent thereon and in tootheache also.
- Piperine is given with much benefit in aguee, gonorrhoea, haemorrhoids etc in doses of 3 to 10 grains.
- In Intermittent fever black pepper in doses of about a drachm is recommended to be given with the juice of leaves of ocimum sanctum or jeucas linifoha.
- The drug is also used in scorpion bite.

SIDDHA FORMULATIONS

1. **Milagu thiravagam**

   Dosage : Kasu eadai

   Indication : Peptic ulcer, Indigestion, Anorexia

2. **Milagu Legium**

   Dosage : Punnai kai alavu

   Indication : Peptic ulcer, Vaivu, Diarrhoea.

3. **Karuvepilai vadagam**

   Dosage : Illanthai vithai alavu

   Indication : Anorexia, Diarrhoea, Discentry.

4. **Kanthaga Chooranam**

   Dosage : Verugadi alavu

   Indication : Peptic ulcer, Indigestion, Constipation.

5. **Sowbagiya sundi Chooranam**

   Dosage : Verugadi alavu

   Indication : Peptic ulcer, Indigestion, Diarrhoea.

6. Pirandai Vadagam

Dosage : I Manthai vithai alavu

Indication : Peptic ulcer, Vomitting, Anorexia

7. Thirikadgu Kirutham

Dosage : 1 Spoon

Indication : Piles, Diarrhoea.

8. Pirandai Chooranam

Dosage : Verugadi alavu

Indication : Peptic ulcer, Vomitting, piles, Abdominel, disorders.

9. Thirikadugu thiravagam

Dosage : 5 Drops

Indication : Peptic ulcer, Bronchial Asthma.

10. Muppirandai Chooranam

Dosage : Verugadi alavu

Indication : Peptic ulcer, Indigestion, Piles.

3.6.2. BOTANICAL ASPECTS

MILAGU (PIPER NIGRUM)

Taxonomical Classification:

Kingdom	:	Plantae – Plants
Subkingdom	:	Tracheobionta – Vascular plants
Superdivision	:	Spermatophyta – Seed plants
Division	:	Magnoliophyta – Flowering plants
Class	:	Magnoliopsida – Dicotyledons
Subclass	:	Magnoliidae
Order	:	Piperales
Family	:	Piperaceae – Pepper family
Genus	:	Piper *L.* – pepper
Species	:	Piper nigrum *L.* – black pepper

Common name:

Black pepper, white pepper, green pepper, peppercorn, Madagascar pepper (English); pippali (Sanskrit); kali mirch (Hindi, Urdu); milagu (Tamil)

Synonym:

Muldera multinervis Miq.

Habitat:

Montane tropical evergreen forest.

Geography and distribution

Black pepper is native to the Western Ghats of Kerala State in India, where it grows wild in the mountains.

It is cultivated all over the tropics as a commercial crop. Vietnam, Indonesia, Brazil and India are the major producers.

DESCRIPTION

Overview:

A climber that grows to a height or length of 10 m or more. Once the main stem is established it grows many side shoots to create a bushy column.

The plants form short roots, called adventitious roots, which connect to surrounding supports.

Leaves:

Almond-shaped, tapering towards the tip, dark green and shiny above, paler green below, arranged alternately on the stems.

Flowers:

Borne in clusters along flowering stalks known as spikes. 50–150 whitish to yellow-green flowers are produced on a spike.

Fruits:

Round, berry-like, up to 6 mm in diameter, green at first but turning red as they ripen, each containing a single seed. 50–60 fruits are borne on each spike.

Fruits are picked when green and immature to produce green pepper; when fully grown but still green and shiny to produce black pepper; and when slightly riper to produce white pepper (for which the fruits are also soaked to remove the fleshy outer layer).

Other pepper plants

Other plant species are also known as pepper or peppercorns and are used in a similar way as black pepper, for example, Indian long pepper, *Piper longum*, which has a milder flavour than black pepper. It is native from Assam to Burma, and is a cultivated crop in the drier regions of India.

Pink pepper is obtained from *Schinus terebinthifolia* (Brazilian pepper tree). It grows as a tree, is in a different plant family, Anacardiaceae (cashew family), from black and long pepper and is native to South and Central America. Its pinkish-red fruits often enter European markets where it is used as a black pepper-like flavouring.

Sichuan pepper, a common spice used in Asian cuisine, is obtained from *Zanthoxylum* species (citrus family, Rutaceae).

Peppercorns should not be confused with chilli peppers (*Capsicum* species) such as *Capsicum annuum*(potato family, Solanaceae).

Uses

Food

The fruits of *Piper nigrum* are used to make black pepper. This hotly pungent spice is one of the earliest known and most widely used spices in the world today. It is used as flavouring, particularly for savoury foods, meat dishes, sauces and snack foods. It is also used as a table condiment.

Black pepper, white pepper and green peppercorns are all produced from *Piper nigrum* fruits, but are harvested at different times and are processed differently.

India is a key producer of black pepper and exports much of what is grown. Peppercorns from Malabar and Tellicherry in Kerala, India, are particularly prized for their flavour and pungency.

Black pepper is also used to produce pepper oil and oleoresin, which are frequently used in the production of convenience foods and sometimes also for perfumery.

Of lesser importance is the use of preserved immature green pepper or fresh pepper fruits, which are eaten more like a vegetable.

Traditional medicine

Black peppercorns

Black peppercorns feature as remedies in Ayurveda, Siddha and Unani medicine in South Asia. They are most frequently used as an appetizer and to treat problems associated with the digestive system, particularly to eradicate parasitic worms. Some traditional uses of black pepper are supported by scientific evidence.

In Siddha medicine, black pepper has been used to aid digestion, improve appetite, treat coughs, colds, breathing and heart problems, colic, diabetes, anaemia and piles. Stomach ailments such as dyspepsia, flatulence, constipation and diarrhoea are all treated with black pepper, which may be mixed with other substances such as castor oil, cow's urine or ghee.

Black pepper has been prepared in tablet form as a remedy for cholera and syphilis, sometimes combined with other substances. It has also been used in tooth

powder for toothache, and an infusion of black pepper has been suggested as a remedy for sore throat and hoarseness. Black pepper may be chewed to reduce throat inflammation.

Externally, it has been applied as a paste to boils and to treat hair loss and some skin diseases. Oil of pepper is reputed to alleviate itching. A mixture of sesame oil and powdered black pepper has been recommended for application to areas affected by paralysis. A mixture of black pepper and honey is regarded as a remedy for night blindness. Black pepper has been given by inhalation to comatose patients. It is also believed to be useful against hepatitis, urinary and reproductive disorders. In Ayurveda and Siddha medicine, a paste made using white pepper is applied to treat some eye diseases.

Phytochemical constitutents

Black pepper contains about 3% essential oil, whose aroma is dominated (max. 80%) by monoterpenes hydrocarbons: sabinene, beta-pinene, limonene, furthermore terpinene, alpha-pinene, myrcene, delta3-carene and monoterpene derivatives (borneol, carvone, carvacrol, 1,8-cineol, linalool). Sesquiterpenes make up about 20% of the essential oil: beta-caryophyllene, humulene,beta-bisabolone and caryophyllene oxide and ketone. Phenylether (eugenol, myristicin, safrole) are found in traces. Loss of monoterpenes due to bad storage conditions (especially for ground pepper) should be avoided.

Pharmacological activity

Constituents:

Black pepper has been found to contain piperine, alkamides, piptigrine, wisanine, dipiperamide D, and dipiperamide E.

Acetylcholinesterase inhibitory activity:

In an *in vitro* study, an extract of *Piper nigrum* L. seeds showed 50-65% inhibitory activity on acetylcholinesterase.

Antibacterial effects:

In an *in vitro* study using 12 different genera of bacterial populations isolated from the oral cavity of 200 individuals, an aqueous decoction of black pepper (*Piper nigrum* L.) exhibited 75% antibacterial activity as compared to aqueous decoction of

bay leaf (53.4%) and aqueous decoction of aniseed (18.1%), at the concentration of 10mL/disc.

Anti-inflammatory effects:

Based on animal study, a polyherbal formulation (Aller-7/NR-A2) containing extracts from seven medicinal plants including *Phyllanthus emblica*, *Terminalia chebula*, *Terminalia bellerica*, *Albizia lebbeck*, *Piper nigrum*, *Zingiber officinale*, and *Piper longum* demonstrated 31.3% inhibition against carrageenan-induced acute inflammation in Wistar Albino rats, while ibuprofen (50 mg/kg orally) exerted 68.1% inhibition. Aller-7 also exhibited a dose-dependent (150-350mg/kg) anti-inflammatory effect against Freund's adjuvant-induced arthritis in Wistar Albino rats; an approximately 63% inhibitory effect was observed at a dose of 350mg/kg.

Antilarval activity:

Piptigrine, isolated from the dried ground seeds of *Piper nigrum* Linn., exhibited toxicity of 15.0ppm against fourth instar larvae of *Aedes aegypti* Liston.

Antioxidant effects:

Based on animal study, a polyherbal formulation (Aller-7/NR-A2) containing extracts from seven medicinal plants including *Phyllanthus emblica*, *Terminalia chebula*, *Terminalia bellerica*, *Albizia lebbeck*, *Piper nigrum*, *Zingiber officinale*, and *Piper longum*exhibited concentration-dependent scavenging activities toward biochemically generated hydroxyl radicals (IC_{50} 741.73mcg/mL); superoxide anion (IC_{50} 24.65mcg/mL by phenazine methosulfate-nicotinamide adenine dinucleotide [PMS-NADH] assay and IC_{50}4.27mcg/mL by riboflavin/nitroblue tetrazolium [NBT] light assay), nitric oxide (IC_{50} 16.34mcg/mL); 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical (IC_{50} 5.62mcg/mL); and 2,2-azinobis-ethyl-benzothiozoline-sulphonic acid diammonium salt (ABTS) radical (IC_{50} 7.35mcg/mL). Aller-7 inhibited free radical-induced hemolysis in the concentration range of 20-80mcg/mL. Aller-7 also significantly inhibited nitric oxide release from lipopolysaccharide-stimulated murine macrophages.

Cytochrome P (CYP) 450 effects:

In *in vitro* studies, constituents isolated from *Piper nigrum*, including piperine and dipiperamides D and E, potently inhibited some CYP450 metabolic pathways, including CYP2D6 and CYP3A4.

Gastrointestinal effects:

In a clinical study of intestinal peristalsis in 16 healthy volunteers, consumption of 1.5g of black pepper in capsules increased the orocecal transit time from 90 ± 51 minutes to 122 ± 88 minutes (p=0.09). In an *in vitro* study, piperine inhibited digoxin and cyclosporine A transport in Caco-2 cells with IC_{50} values of 15.5 and 74.1mcM, respectively. The bactericidal and anti-adhesive properties of black pepper have also been investigated against *Helicobacter pylori*, however, aqueous extracts did not show bactericidal effect on any of the isolates.

Neural effects:

In an *in vitro* study using whole-cell patch-clamp electrophysiology, piperine, a pungent alkaloid found in black pepper, had similar agonist effects on the human vanilloid receptor TRPV1 as capsaicin. However, piperine could induce greater receptor desensitization and exhibit a greater efficacy than capsaicin.

3.6.3.LATERAL RESEARCH :

Abstract:

Black pepper (Piper nigrum L.) native of south India popularly known as" king of spices". Pepper is mostly used in the curry recipes as masalas and also as ingredient in the prescriptions of folk medicine, Ayurveda and traditional medicinal systems. The spicy tang of pepper is due to the presence of piperamides which are the pungent bioactive alkaloids accumulate in the skin and seeds of the fruit. Among them piperine is the major chemical constituent responsible for the bitter taste of the black pepper. In the present study piperine was evaluated for its antimicrobial activity against Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Alternaria alternata, Aspergillus niger, Aspergillus flavus and Fusarium oxysporum. The antibacterial activity was measured by agar well diffusion method and antifungal activity by poisoned food technique. Piperine showed antimicrobial activity against all tested bacteria with zone of inhibition ranged from 8-18mm. maximum zone of inhibition was against Gram positive bacteria Staphylococcus aureus (18mm) and minimum against Gram negative bacteria Escherichia coli (8mm). Piperine showed maximum antifungal activity towards Fusarium oxysporum (14mm) and very least effect against Aspergillus niger (38mm). The results showed significant activity of piperine and suggesting its use as natural antimicrobial agent.

3.7. ELUMICHAI (Citrus limon)

3.7.1. GUNAPADAM ASPECT

Other names

செப்பினதோர் தேசிநீர் கூதழச்சாறென்றும்
சிறுகிளியின் பழச்சாறென்றும் பேரு
நெப்பின தோர் நிம்பவளச்சாறென்றும் பேரு
நோவாலி மாதரசி என்றும் பேரு
உப்பினதோர் உபனோரஞ்சகனென்றும் பேரு
உடலிபித்து முறிமாத ரென்றும் பேரு
சிப்பிபெறும் பேசும் கனிமாத ரென்றும் பேரு
செயலான எலுமிச்சம் பழத்தின் பேரு

- Pancha kaviya Niganda

- Thesi neer
- Koodhala saram
- Siru kiliyan palazhacharu
- Nimpavalasaru
- Madharasi[(52)]

ஏற்கும் சதா பலம் சம்பீரமெலு மிச்சைக்கே
தாக்குதபித்த சத்தியுடன் தாகம்போம் நோக்கில்
கபவாத உற்பணத்தைக் காட்டியே நிற்கும்
அபவாதம் என்றே அறி

***Agasthiyar Vaithya Chindamani Venba* – 4000**

- Sadha balam
- Kambeeram

Part Used:

Leaf, fruit, fruit juice, oil.

Properties of Fruit:

Taste	**:**	Sour
Character	**:**	Veppam (hot)
Class	**:**	Karpu (pungent)
Action	**:**	Kulirchi undakki (refrigerant)

Action:

- Thermogenic
- Carminative
- Stimulant
- Digestive
- Laxative
- Anthelmintic
- Antiseptic
- Antiscorbutic
- Mosquito repellent

General character

தாகம் குநகநோய் தாழாச் சிலிபத நோய்

வேகங்கொள் உன்மாதம் போங் கட்டுவாதித்தொழிலில்
மன்னெலுமிச் சங்கனியை வாழ்த்து

Lemon fruit is useful in the treatment of thirst, paronychia, filariasis, *unmatham*, *pitham*, eye diseases, ear ache, vomiting.

சதாபலக் கனிகாய் சமூலமு முணவே
நிதானமாய்ப் பயித்திய நிந்தைநோ யகலுமே

When roots, leaves, fruits of lemon are used it cures diseases arising due to *thee kutram* and *veri noi*.

Therapeutic uses

- Lemon juice taken along with honey strengthens *thadhukkal* (humour).
- In case of paronychia, the affected finger is placed in the hole made in a lemon fruit.

Cuminseed are roasted with honey.To this lemon juice is added and a *kudineer* is made.it is used to cure uncontrolled vomiting, diarrhea produced by purgatives like Croton tiglium.

In men about the age of 70, in whom development of cataract is in its initial stage, when few drops of lemon juice are used as eye drops in early morning ,it gradually dissolves the cataract and the eye sight gets clear by and by.

In poisoning with croton seeds, castor seeds, physic nut and roots of tapioca plant, 4-5 ounce of lemon juice diluted with equal quantity of plain water, when taken relieves purging, vomitting, and other symptoms.It is an antidote which should always be first tried.

Juice of the fruit in the dose of 4-6 drachms is employed as a useful refrigerant drink in small –pox,measeles,scarlatina and other form of fever where there is a very hot dry skin and much thirst.It can be taken in cases of haemorrhage from the lungs,stomach ,bowels,uterus,kidney,and other internal organs.

SIDDHA FORMULATION

1.Visha Mooshti Thylam:

Usage : Taken internally and applied over the body before bathing.

Indication: Thimir vatham, Sandhu vatham, Asthi vatham, Sarvanga vatham.

2.Sura kalikkam

Usage : Eye drop

Indication : Fever

3.Sutha Vallathi Ennai:

Usage : Given internally, used as nasal drop and applied all over the body.

Indication : Muga vatham (facial palsy), Anda vatham, Pitham, Neela kasam, Sinusitis

4.Nelli Vadagam:

Dosage : Size of a kottai pakku

Indication : Pandu (anaemia), Kamalai (jaundice), Irumal (cough), Ezhai, Sogai(dropsy), Thagam (thirst), Headache.

5. Vallarai kridha:

Dosage : 3 Varagan

Indication : Megam 20, Grandhi 8, Kuttam 18, Mega sooli

6. Ponnankanni kridham:

Dosage	:	2 Varagan
Indication	:	Cures UTI, Neer kaduppu, TB

7. Sadhapala sarpath:

Dosage	:	1.5 -2 palam
Indication	:	Improves digestion, Decreases thirst.

8.Veleluthu Vennai:

Usage	:	Eye drops for 40 days
Indication	:	Presbyopia

3.7.2.BOTANICAL ASPECT

LEMON (Citrus limon)

Taxonamical Classification

Kingdom	:	Plantae
Order	:	Sapindales
Family	:	Rutaceae
Genus	:	Citrus
Species	:	limon

Vernacular Names

English	-	The Lemon of India
Hindhi	-	Jambiri nimbu
Sanskrit	-	Jambir
Gujarat	-	Goddiya

Habitat

Originated in Asia and is now grown commercially world wide in tropical ,semi-tropical and warm temperate countries including the Mediterranean region.Usually found in area which has a height of 4000 foot.

Habit:

Lemon is a spinous shrub or tree.It grows to a height of about 3 to 6 m .Leaf perfectly joined to the petiole.Petiole is narrowly winged.Flowers bisexual or male.Petals white ,tinged purple.Stamens 20 to 30

Part Used:

Fruit, fruit peel, seeds, leaves.

Fruit:

Fruit is oblong or rounded mamillate,yellow when ripe;pulp abundant and strongly acidic and bitter. Rind is filled with a sweet essence.

Composition of Lemon:

Lemon is rich in vitamin C,but also contains good quantity of potassium,calcium and phosphorus.It contains small quantities of iron,copper,magnesium and vit A ,as well as several B group vitamins.Lemon also produces its own essential oil which plays a major role in its medicinal properties.It also contain carotenoids and coumarins.

Lemon Peel Oil consists mainly of terpenes, particularly limonene, also gamma terpinene and beta-phellandrene. There are small amounts of sesquiterpenes and aldehydes. Among the aliphatic aldehydes are n-octyl aldehyde, n-nonyl aldehyde, and citral.

Action of Fruit:

Fruit is a refrigierant; its juice is antiscorbutic, due to the presence of citric acid anthelmintic, antiseptic, carminative, digestive, laxative, stimulant, stomachic, thermogenic. Lemon possesses very powerful antioxidant property. It is a powerful antibiotic and antiseptic.

Chelating Effect of Lemon:

Modified citrus pectin is a soluble fiber found in the peel and pulp of citrus and is most well known for its ability to help lower cholesterol levels. More recently, there has been a growing interest in its ability to help gently and safely remove toxic heavy metals such as mercury, lead, and cadmium from the body.

Mercury is a heavy metal that is a growing environmental concern. It is emitted into the air from burning coal or fuel or even by volcanoes. According to the EPA nearly all fish and shellfish contain traces of mercury, with larger fish such as King mackerel, shark, and swordfish containing the greatest amounts. Young children and unborn babies are at the greatest risk from mercury toxicity because it impacts their developing nervous system and can lead to learning disabilities.

A 2000 NHANES study found that 8% of 16-49 year old women had blood mercury concentrations greater than the baseline amount associated with health problems. In the last few years the growing threat of toxic toys has made headlines with lead and even more toxic cadmium being found in our children's toys. Symptoms of chronic heavy metal exposure can mimic other diseases and be difficult to pinpoint. Allergies, inflammation, memory, and nervous system disorders can be symptoms. Some heavy metals can increase the risk of certain cancers.

The standard treatment to remove heavy metals from the body is chelation therapy. This involves the use of a chelating agent, such as DMSA or EDTA, given via IV or taken orally. They bind with the metal and then it can be excreted from the body.

Pectin is thought to bind and then excrete heavy metals through the gut. A specific brand of modified citrus pectin has been shown to also significantly increase

urinary excretion of arsenic, lead, and cadmium in healthy adults with a normal toxic load.

More recently a preliminary clinical trial was conducted to determine modified citrus pectin's ability to reduce the body's toxic load of mercury. After four months of 15 grams/day of modified citrus pectin they found a significant decrease in total body mercury burden for all 5 study participants. There was a mean average decrease of just over 69% with no side effects reported.

This supplement has also been studied in children with high lead levels. After receiving 15g/day for 28 days the children showed a dramatic decrease in lead levels. The researchers noted the potential of this supplement as a safe and effective gentle chelating agent and that further studies to confirm its benefits are justified.

In addition to heavy metal removal, modified citrus pectin has shown potential in early studies to help reduce the size and growth of tumors and reduce the risk of metastasis of breast, colon, and prostate cancer. This simple supplement derived from citrus certainly appears to be very promising.

Consumption of Lemon:

Daily intake of lemon is excellent for health, but effects differ depending on if they are taken with or between meals.With meals,it improves digestion and facilitates the work of digestive organs including the liver, pancreas, etc.. Between meals it essentially has a cleaning action.

Daily consumption of lemon juice can make huge difference in the appearance of skin.It acts as an anti aging remedy and can remove blackheads and wrinkles.It will be of the most effective remedies to reduce belly fat and obesity.

Lemon juice, fresh, canned, concentrated and frozen, or dehydrated and powdered, is primarily used for lemonade, in carbonated beverages, or other drinks.

It is also used for making pies and tarts, as a flavoring for cakes, cookies, cake icings, puddings, sherbet, confectionery, preserves and pharmaceutical products. A few drops of lemon juice, added to cream before whipping, gives stability to the whipped cream.

Lemon peel is the source of lemon oil, pectin and citric acid. Lemon oil, often with terpenes and sesquiterpenes removed, is added to frozen or otherwise processed lemon juice to enrich the flavor. It is much employed as a flavoring for hard candies.

3.7.3. LATERAL RESEARCH

Researchers conducted a study to look at the connection between ozone and asthma and other lung disorders. It was found that the higher incidence of asthma in urban areas is due in large part to the absence of natural "ozone scavengers" produced by plants.

"Ozone in the outer atmosphere is essential for life on earth because it absorbs the destructive ultraviolet radiation emitted by the sun. But on earth, it is a dangerous component of air pollution. Numerous studies have shown exposure to ozone, even at low levels, induces airway inflammation and lung injury in humans and animals.

Ozone scavengers are substances that devour ozone. Researchers believed these substances could be the key to preventing asthma. They conducted experiments exposing rats with induced asthma-like symptoms to limonene. Limonene is the main component in citrus oil. The lung function of the rats showed that limonene inhalation prevented asthmatic symptoms. However more research in both animals and humans is currently underway to look at citrus oils and their use in treating asthma.

3.8. DISEASE REVIEW

3.8.1 SIDDHA ASPECT OF THE DISEASE

DEFINITION

Ulcer is characterised by indigestion, heat burn, vomiting, malaise, weightloss and depression

During the course of the disease the patient suffering from severe colicky pain leans forward due to intolerated pain and hence it has the name gunmam in siddha literature.

AETIOLOGY (Noi varum vazhi)

“கயமான குடலி னுள்ளே
கல்லுமி நெல்லு மாமே
கல்லொரு மயிரா யுள்ள
கசடது குடலிற் பற்றி
வல்லபாங் கதுவா யன்னஞ்
செரியாத மாசி னாலே
மெல்லிய கிருமி கொண்டு
குன்மநோய் மருவுங் காணே”

- Pararasa Sekaram

It is considered that the disease may develop due to the following factors,

- The food particles that increases vayu
- The food particles that contain soil, husk, stones, dust hair, puddy husk
- Consumption of spring water, stagnated water, and the water mixed with lime stone.
- Consumption of coconut milk and the products which upsets proper digestion.
- Stress, repeated fastings, depression may aggravate the disease
- Practise of violent Yogic exercises.

Due to the above resons the Gastro intertinal tract is inflammed resulting in severe colicky pain and produces the disease ulcer.

Prodromal symptoms

- Anorexia
- Nausea
- Vomiting
- Abdominal tenderness
- Belehing

Type of ulcer (Gunmam)

1. Acoording to Yugi muni the disease is classified into eight types.

"செய்யவே அட்டகுன்மஞ் செயலைக் கேளாய்
செயலான வாதகுன்மம் சூலை குன்மம்
எய்யவே பித்தகுன்ம மாகும்
ஏலான வலிகுன்மம் சத்திகுன்மம்
தையவே சன்னிகுன்மஞ் சிலேட்டும குன்மம்
சாகசமாம் குன்மமது எட்ட தாகும்
கொய்யவே யிதனுடைய குணங்க ளெல்லாம்
குறிப்பறிந் தொவ்வொன்றாய் கூர்ந்து பாரே"

- Yugi Chinthamani

The types of gunmam said by yuhi muni are listed as follows.

- Vayu kunmam
- Vatha kunmam
- Pitha kunmam
- Eri kunmam
- Vali kunmam
- Sathi kunmam
- Sanni kunmam
- Iya kunmam

There are many types of kunmam described by various siddhars in various literatures. Allthough there is many different views in the classification of kunmam. Most of the siddhars are following the same ideas.

The signs and symptoms explained in eight types of kunmam by yuhi are described as follows.

1. Vazhi kunmam (Vatha kunmam)

The clinical features in *vazhi kunmam* are Anorexia, abdominal tenderness, malaise, headache, myalgia, fainting, constpation. It mostly affects the patient ranges between 20 to 30 years of age, abdominal pain is aggravated after eating and it is preceded by vomiting. Vomiting somewhat reduce the pain. The vomitus contain small amount of blood and it is black. Loss of appetite, eating aggravates vomiting are characterised by vazhi kunmam.

2. Azhal kunmam (Pitha kunmam)

The clinical features in *azhal kunmam* are heart born vomiting. the vomitus contain, mucous and bile. Pain present in micturation and haematuria are common. dizziness, constipation colicky abdominal pain, headache, tiredness. It mostly affect the indiduals ranges from 30 to 50 years of age.

3. Iya kunmam (Kabha kunmam)

The clinical features of *Iya kunmam* are body become emaciated, diziness, vomiting abdominal distension, abdominal pain, hematmesis. It looks like stomach cancer. It mostly affects the old aged.

4. Sanni kunmam (mukutra kunmam)

Abdominal distension, diarhoea, heartburn, belching, breathlessness, diziness.

5. Vayu kunmam (Payure kunmam, solai kunmam)

Vayu kunmam is characterised by Indigestion, undisgested food in vomiting, anorexia, abdominal distension, sweating, bodypain, colicky abdominal pain.

6. Eri kunmam

After few hours of consumption of food there is severe stomach pain, salivation, headache belching, diarrhoea, severe sweating body became emaciated are the clinical features in *eri kunmam*.

7. Sarthi kunmam (vanthi kunmam)

Undigested food in vomiting, diziness, stomach pain, constipation, fatigue, anorexia, numbness, general diabelity are the clinical features seen in *sarthi kunmam.*

8. Vali kunmam

Indigestion, abdominal distension, flatulence depression, anorexia, Insomnia, diarrhoea, pain in the axilla, hippain, fever are the clinical features in *vali kunmam.*

Mukkutra Verupadugal:

"தொடர்வாத பந்தமலாது குன்மம் வராது"

"புண்ணாய் வலிக்கும் பொருமுங் குடலோடிக்
தண்ணா மலத்தை தம்பிக்கப் போக்காது
ஒண்ணான ஆசன முறவெ சுருக்கிடும்
பண்ணார் குளிர்சீதம் பகுத்திடும் வாதமே"

- Thirumoolar

Peptic ulcer disease is caused by increase in vatha. It is due to improper food habits it disturbs the Udanan, Abanan, Samanan. It results in Indigestion, constipation, Abdominal distension and Vomitting.

Pulse:

"வலத்திற் சூலை இடத்திற் குன்மம்"

Vatham, Pitham, Kabha pulses are exaggrated in the right side it is soolai disease and it is exaggrated in the leftside it is *Gunmam.*[(23)]

- Noinadal noi mudhal nadal Vol-II

3.8.2 MODERN ASPECT:

Definition :

The term peptic ulcer refer to an ulcer in the lower oesophagus, stomach or dueodenum, in the jejunum after surgical anastamosis to the stomach or rarely in the ileum adjacent to a meckel's diverticulum ulcers in the stomach or duodenum may be acute or chronic both penetrate the nuscularis mucosal but acute ulcer shows the evidence of brosis, erosions do not penetrate the musularis mucosa.

Causes of gastric ulcer :

- H. Pylori infection
- NSAIDS
- Neoplasm (Carcinoma, Lymphome, Lympho, Sarcoma)
- Stress
- Crohn's disease
- Infections cherpes simplex, cyto megalovinu

Causes of Dueodenal ulcer :

Common cause :

- H.Pylori infection
- NSAIDS

Uncommon Cause :

- Zolinger – Ellison syndrome (aastrinome)
- Hypercalemia
- Granulomatous disease, chron's disease, sarcoidosis
- Neoplasia (carcinoma, lymphoma, leiomyoma)
- Infections (tuberculosin, herpes simplex)

Pathology :

Chronic gastric ulcer is nearly always single 90% are situated on the lesser curve within the antrum or at the junction between emphysema body and antral mucosa.

Chronic duodenal ulcer is usually situated in the first part of the duodenum. Just distal to the junction of pyloric and duodenal mucosa 50% are on the anterior wall more than one peptic ulcer is found in 10-15% of case. Acute ulcer or erosions are frequently multiple and are more widely distributed.[24]

Types of Peptic Ulcer :

1. Acute peptic ulcer
2. Chronic peptic ulcer

Acute peptic ulcer :

Acute peptic ulcer developing after head injury, burns, severe sepsis, surgery (or) trauma are termed stress ulcers. Gastric hyper secretion is the usual cause of acute ulcer, head injury, while the reflex of duodenal contents and mucosal ischemia, may be responsible factors after burns or shock.

Chronic peptic ulcer :

1. Chronic gastric ulcer
2. Chronic duodenal ulcer

Clinical Features :

Duodenal ulcer – Symptoms

1. Epigastric pain
2. Distension of abdomen
3. Anorexia

Pain and when it is persist ant may result in weight loss.

Peristatant vomiting in an ulcer subject usually indicates some degree of gastric out flow obstruction.

Signs :

Pointing sign

1. **Muscle guarding (or) Rigidity :**

   May be present with active ulcer or deeply penetrating ulcer.

2. **Pesistatic waves :**

   Gastric retention due to duodenal ulcer near pyloris. Obstuction due to

   a. Inflammation
   b. Scaring due to surgery

3. **Occult Blood in stools.**

Gastric Ulcer :

1. Epigastric pain
2. Nausea & vomiting
3. Weight loss

Table : 1

Difference between chronic gastric ulcer Chronic duoclenal ulcer

S.No.		Chronic D.U	Chronic G.U
1.	Age	Usually 30.40yr	Usually 40-60yr
2.	Sex	Male dominate	Females dominate
3.	Mechanisam	Excess gastric secrtion due to parietal cell mars.	Nusocal resistance due to deficient mucus prenucosal metaplais.
4.	Blood Group	Usually 'O"	Less common
5.	Stress	Possiby more related	Not so
6.	Site of ulcer	First part of the duodenum in the anterior wall	Within 6cms of the pylonus close to the ceiser cultivate
7.	Site of pain	Epigastric region more towards right side	Epigastic region more towards left side
8.	Onset of pain	1-3hrs after food	Immediately affair intake of food.
9.	Character of pain	Burning	Dull aching of stiching
10.	Radiation of pain	Upwards in characters	Backwards over para vertebral region.
11.	Hamatemesis	Maleane common	Hamatemesis common
12.	Vomitting	Induced	Spontaneous
13.	Abdominal signs	Pointing sign on right side	On the left side

14.	Beriummeal x-ray and screening	Hyper-mobility tender duodenal bulb and deformity of duodenal bulb rarely ulcer crater	Ulcer cratr and niche and opposite wall.
15.	Endoscopy	Duodenoscopy may reveal the ulcer	Gastroscopy may reveal the ulcer

Complication :

Complication of peptic ulcer are

1. Haemorrhage
2. Perfortaion
3. Gastric Outlet Obstruction
4. Cancer.

Differential Diagnosis :

1. Chronic intestinal ameobiasis
2. Chronic Cholecystitis
3. Chronic Appendicate
4. Chronic gastritis
5. Chronic pancreatitis
6. Zollinger Ellison syndrome.

Diagnosis – laboratory findings

Laboratory tests are normal in uncomplicated peptic ulcer disease but are ordered to exclude ulcer complication or confounding disease entities. Anemia may occur with acute blood loss from a bleeding ulcer or less commonly from chronic blood loss. Leukocytosis suggests ulcer penetration or perforation. An elevated serum amylase in a patient with severe epigastric pain suggests ulcer penetration into the pancreas. A fasting serum gastrin level to screen for zollinger Ellison syndrome is obtained in some patients. Because acid inhibition may raise serum gastrin levels.

Endoscopy

Upper endoscopy is the procedure of choice for the diagnosis of duodenal and gastric ulcers. Duodenal ulcers are virtually never malignant and do not require biopsy 3 to 5% of benign appearing gastric ulcers proved to be malignant.

Imaging

Barium upper gastro intestinal series is less sensitive for of detection of ulcers and less accurate for distinguishing benign from malignant ulcers – Abdominal CT Imaging is obtained in patients with suspected complications of peptic ulcer disease. (perforation, penetration or obstruction).

Testing for H, pylori

- Urease test
- fecal antigen assay
- Urea breath test

4. MATERIALS AND METHODS

4.1. Preparation of the Drug

Bosana Kudori Mathirai has been selected from the classical siddha literature Anuboga Vaidhiya Bramma Ragasiyam

Ingredients of the test drug are Chukku, Milagu, Seeragam, Inji, Indhuppu, Perungayam.

Collection of the drugs :

The raw drugs Chukku, Milagu, Seeragam, Inji, Indhuppu, Perungayam are brought from local drug shop in Tirunelveli, Tamilnadu.

Identification and Authentication of drugs.

The raw materials were identified and authenticated by the experts of PG Gunapadam Dept Government Siddha Medical College, Tirunelveli.

The identified raw materials were conserved in the laboratory of PG Gunapadam Government Siddha Medical College, Tirunelveli.

Preparation of the Drug

A.	Chukku (Zingiber officinale)	-	1 Palam (35 gm)
B.	Perungayam (Ferula Asafoetida)	-	1 Palam (35 gm)
C.	Inji (Zingiber officinale)	-	2 Palam (70 gm)
D.	Inthuppu (Sodium Chloride Impura)	-	2 Palam (70 gm)
E.	Seeragam (Cuminum cyminum)	-	3 Palam (105gm)
F.	Milagu (Piper nigrum)	-	3 Palam (105 gm)

Purification Of Drugs :

1. Chukku:

Soak in the limestone water for 3 hrs and remeove the skin and dry it

2.Perungayam:

Fried it until it lost its humidity

3.Inji:

Removed outer hard layer

4.Inthuppu:

Inthuppu soaked in the goat urine for 25 minutes and dried it.

5.Seeragam:

Remove soils and dust particles

6.Milagu :

Soak in the sour butter milk for 3 hours and dried sunlight

Process of preparation:

The purified drugs are rubbed with lemon juie in a stone motor (Kalvam) for 6 hrs. Make it a sized sundaikai Alavu (798 mg) and dride in the shade than kept in Airtight container and labeled.

Indication:

Gunmam, Akni mantham, Vayitru vali, Vayu, Soodhaga vali, Kirakani vagaigal.

Fig : 1 Ingredients of Bosana Kudori Mathirai

Before Purification

Chukku

Perungayam

Inji

Inthuppu

Seeragam

Milagu

Fig : 2 Ingredients of Bosana Kudori Mathirai

After Purification

Chukku

Perungayam

Inji

Inthuppu

Seeragam

Milagu

Fig : 3 Bosana Kudori Mathirai

On Processing

Prepared Drug - Bosana Kudori Mathirai

4.2. STANDARDIZATION OF THE DRUG

The standardization of the drug is essential to exhibit the purity, quality and quantity of the drug. This is basically done by chemical, Physico chemical and instrumental analysis.

4.2.1. PHYSICAL STANDARDIZATION

Testing Physical characterization of Sample:

Colour Examination:

Ten tablets were taken into watch glasses and positioned against white back ground in white tube light. Its colour was observed by naked eye and note in results.

Odour examination:

Ten numbers of tablets were smelled individually. The time interval among two smelling was kept two minutes to overturn the effect of previous smelling. Odour of BKM tablet was noted in results table.

Size examination:

The diameter of ten tablets was measured by Vernier caliper. The mean value of diameter was noted. (Lohar DR-Protocol for testing ASU drugs)

Weight Variation Test:

It was carried out to make sure that, each number of tablets contains the proper amount of drug. The test was carried out by weighing the 20 tablets individually using analytical balance, then the average weight was calculated, and comparing the individual tablet weights to the average. (Sukalyan Sengupta 1988)

The percentage of weight variation is calculated by using this formula.

$$\% \text{ of wt. variation} = \frac{\text{Individual wt.} - \text{Average wt.}}{\text{Average wt.}} \times 100$$

Weight Variation limits of Tables (IP)

Average weight of tablets	Maximum percentage of weight difference allowed
80mg or less	± 10.0
Between 80mg and 250mg	± 7.5
250mg and more	± 5.0

Accepted tablet:

Weight Variation limits of the sample not more than two tablets are outside the percentage limit and no tablet differs by more than two times the percentage limit according to the above table.

Suspected tablet:

Suspected tablet variation was not more than six tablets are outside the percentage limit and no tablet differs by more than two times the percentage limit according to the table.

Rejected tablets:

When a tablet weight variation test results showed rejected tablets mean in that test sample one tablet differs by more than two times the percentage limit according to the table or More than six tablets are outside the percentage limit. (Sukalyan Sengupta, 1988)

Solubility:

A pinch of the sample was taken in a dry test tube and shaken well with distilled water. A little amount of the sample is shaken well with con HCl and then Con.H2SO4. Test sample Solubility was observed.

pH Value:

Potentiometrically pH value was determined by a glass electrode and a suitable pH meter. The pH of the BKM tablet was written in results column.

PHYSIO CHEMICAL STANDARDIZATION

Loss on Drying (Indian Pharmocoepia, 1996)

Loss on drying is the loss in percentage w/w resulting from water and volatile matter of any kind that can be driven off under a specified condition. A glass stopper, shallow weighing bottle was weighed accurately and the quantity of the sample as specified was transferred to the bottle was weighed accurately and the quantity of the sample as specified was transferred to the bottle covered and weighed. The sample was distributed evenly and the bottle was placed in the drying chamber. The sample was then dried for a specific period of time, and the bottle was removed from the chamber and allowed to cool at room temperature in a desiccators before weighing.

Total ash

Two grams of ground air dried powder of BKM was accurately weighed in a previously ignited and tared silica crucible. The drug was gradually ignited by raising the temperature to 450^{o}C until it was white. The sample was cooled in a desiccators and weighed. The percentage of total ash was calculated with reference to air-dried drug.

Acid insoluble ash

The ash was boiled with 25ml of 2M hydrochloric acid for 5 minutes, the insoluble matter was collected on an ash less filter paper, washed with hot water, ignited cooled in a desiccators, and weighed. The percentage of acid insoluble ash calculated with reference to the air-dried drug.

Water soluble extractive

Proceed as directed for the determination of Alocohol-soluble extractive , using chloroform water instead of ethanol.

Alcohol soluble extractive

Macerate 5g of the air dried drug, coarsely powdered, with 100ml of alcohol of the specified strength in a closed flask for 24 hrs, shaking frequently during six hours and allowing to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat

bottomed shallow dish, and dry at 105^{o},to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

Tablet disintegration test:

Each BKM tablet was placed in each of the six tubes of the basket present in the disintegration apparatus. The apparatus was operated by using water as the immersion fluid maintained at 35-39 °C. At the end of the 30 min, the basket is lifted from the fluid and the state of the tablet is observed. The disintegration time of BKM was recorded.(Loher Dr).

High Performance Thin Layer Chromatography

HPTLC is a major advancement of TLC principle requiring shorter time and better resolution.The basic difference between conventional TLC and HPTLC is only in particle and pore size of the sorbents.

The plates are similar to conventional TLC plates.Layers of HPTLC are available in the form of precoats.Silica gel of very fine particle size is widely used as sorbent in HPTLC.The use of smaller particle size helps in greater resolution and sensitivity.About 3-6cm solvent front migration is sufficient to effect proper separation.

Sample preparation:

About 1gm of sample was macerated with 0.1N Sulphuric acid and shaken vigorously for 15 minutes. Then it was filtered, to the filtrate add ammonia solution to pH 10, then the filtrate was extracted with chloroform. The chloroform layer was evaporated to dryness. The dried residue was made upto known volume and is used for TLC analysis.

Stationary phase	**:**	Silica Gel 60 F_{254}
Mobile phase	:	Toluene: Ethyl acetate: Diethylamine (70:20:10)
Procedure	:	Applied 10µ l,20 µl of test solution on a precoated silica gel 60 F_{254} HPTLC plate (E.Merck) of uniform thickness 0.2mm using Linomat5 sample applicator. Developed the plate in the solvent system to a distance of 8 cm.

Observed the plate under UV light at 254nm & 366nm using CAMAG REPROSTAR 3.

Wave length : 254nm & 366nm

Evalution : Test related to Alkaloids.

MICROBIAL CONTAMINATION

1. DETERMINATION TOTAL VIABLE AEROBIC MICROBIAL COUNT

Dissolve 10g or dilute 10ml of the preparation being examined, unless otherwise specified, in buffered sodium chloride-peptone solution pH 7.0 or any other suitable medium shown to have no antimicrobial activity under the conditions of test and adjust the volume to 100 ml the same medium. If necessary, adjust the pH to about 7.

Membrane filtration:

Use membrane filters 50 mm in diameter and having a nominal pore size not greater than 0.45 µm the effectiveness of which in retaining bacteria has been established for the type of preparation being examined. Sterilise and assemble the filtration apparatus described under tests for sterility, Appendix- III.

Transfer 10 ml or a quantity of each dilution containing 1 g of the preparation being examined to each of two membrane filters and filter immediately. If necessary, dilute the pretreated preparation so that a colony count of 10 to 100 may be expected. Wash each membrane by filtering through it three or more successive quantities, each of about 100 ml, of a suitable liquid such as buffered sodium chloride-peptone solution pH 7.0. For fatty substances add to the liquid polysorbate 20 or polysorbate 80. Transfer one of the membrane filters, intended for the enumeration of bacteria, to the surface of a plate of casein soyabean digest agar and the other, intended for the enumeration of fungi, to the surface of a plate of Sabouraud dextrose agar with antibiotics.

Incubate the plates for 5 days, unless a more reliable count is obtained in shorter time, at 30º to 35º in the test for bacteria and 20º to 25º in the test for fungi. Count the number of colonies that are formed. Calculate the number of micro-organisms per g or per ml of the preparation being examined, if necessary counting bacteria and fungi separately.

2.Plate Count: For Bacteria

Using Petri dishes 9 to 10 cm in diameter, add to each dish a mixture of 1 ml of the pretreated preparation and about 15 ml of liquefied casein soyabean digest agar at not more than 45º. Alternatively, spread the pretreated preparation on the surface of the solidified medium in a Petri dish of the same diameter. If necessary, dilute the pretreated preparation as described above so that a colony count of not more than 300 may be expected. Prepare at least two such Petri dishes using the same dilution and incubate at 30º to 35º for 5 days, unless a more reliable count is obtained in a shorter time. Count the number of colonies that are formed. Calculate the results using plates with the greatest number of colonies but taking 300 colonies per plate as the maximum consistent with good evaluation.

3. For Fungi

Proceed as described in the test for bacteria but use Sabouraud dextrose agar with antibiotics in place of casein soyabean digest agar and incubate the plates at 20º to 25º for 5 days, unless a more reliable count is obtained in a shorter time. Calculate the results using plates with not more than 100 colonies.

TESTS FOR SPECIFIED MICRO-ORGANISMS

Pretreatment of the sample being examined – Proceed as described under the test for total aerobic microbial count but using lactose broth or any other suitable medium shown to have no antimicrobial activity under the conditions of test in place of buffered sodium chloride-peptone solution pH 7.0.

1. Escherichia coli:

Place the prescribed quantity in a sterile screw-capped container, add 50 ml of nutrient broth, shake, allow it to stand for 1 hour (4 hours for gelatin) and shake again. Loosen the cap and incubate at 37º for 18 to 24 hours.

Primary test:

Add 1.0 ml of the enrichment culture to a tube containing 5 ml of Mac-Conkey broth. Incubate in a water-bath at 36º to 38º for 48 hours. If the contents of the tube show acid and gas carry out the secondary test.

Secondary test:

Add 0.1 ml of the contents of the tubes containing (a) 5 ml of Mac-Conkey broth, and (b) 5 ml of peptone water. Incubate in a water-bath at 43.5º to 44.5º for 24 hours and examine tube (a) for acid and gas and tube (b) for indole. To test for indole, add 0.5 ml of Kovac's reagent, shake well and allow it to stand for 1 minute; if a red colour is produced in the reagent layer indole is present. The presence of acid and gas and of indole in the secondary test indicates the presence of Escherichia coli. Carry out a control test by repeating the primary and secondary tests adding 1.0 ml of the enrichment culture and a volume of broth containing 10 to 50 Escherichia coli (NCTC 9002) organisms, prepared from a 24-hour culture in nutrient broth, to 5 ml of Mac-Conkey broth. The test is not valid unless the results indicate that the control contains Escherichia coli.

2. Salmonella:

Transfer a quantity of the pretreated preparation being examined containing 1 g or 1 ml of the product to 100 ml of nutrient broth in a sterile screwcapped jar, shake, allow to stand for 4 hours and shake again. Loosen the cap and incubate at 35º to 37º for 24 hours.

Test for Salmonella

Medium Description of colony

Bismuth Sulphite agar Black or green, Brilliant green agar Small, transparent and colorless, or opaque, pinkish or white (frequently surrounded by a pink or red zone) Deoxycholate- citrate agar Colorless and opaque, with or without blank centers Xylose -lysine -desoxy - cholate agar Red with or without black centers.

Primary test:

Add 1.0 ml of the enrichment culture to each of the two tubes containing (a) 10 ml of selenite F broth and (b) tetrathionate-bile-brilliant green broth and incubate at 36º to 38º for 48 hours. From each of these two cultures subculture on at least two of the following four agar media: bismuth sulphate agar, brilliant green agar, desoxycholatecitrate agar and xylose-lysine-desoxycholate agar. Incubate the plates at 36º to 38º for 18 to 24 hours. Upon examination, if none of the colonies conforms to the description given in Table 2, the sample meets the requirements of the test for the

absence of the genus Salmonella. If any colonies conforming to the description in Table 2 are produced, carry out the secondary test.

Secondary test:

Subculture any colonies showing the characteristics given in Table 2 in triple sugar-iron agar by first inoculating the surface of the slope and then making a stab culture with the same inoculating needle, and at the same time inoculate a tube of urea broth. Incubate at 36º to 38º for 18 to 24 hours. The formation of acid and gas in the stab culture (with or without concomitant blackening) and the absence of acidity from the surface growth in the triple sugar iron agar, together with the absence of a red colour in the urea broth, indicate the presence of salmonellae. If acid but no gas is produced in the sub culture, the identity of the organisms should be confirmed by agglutination tests. Carry out the control test by repeating the primary and secondary tests using 1.0 ml of the enrichment culture and a volume of broth containing 10 to 50 Salmonella abony (NCTC 6017) organisms, prepared from a 24-hour culture in nutrient broth, for the inoculation of the tubes (a) and (b). The test is not valid unless the results indicate that the control contains Salmonella.

3. Pseudomonas aeruginosa:

Pre-treat the preparation being examined as described above and inoculate 100 ml of fluid soyabean-casein digest medium with a quantity of the solution, suspension or emulsion thus obtained containing 1 g or 1 ml of the preparation being examined. Mix and incubate at 35º to 37º for 24 to 48 hours. Examine the medium for growth and if growth is present, streak a portion of the medium on the surface of cetrimide agar medium, each plated on Petri dishes. Cover and incubate at 35º to 37º for 18 to 24 hours. If, upon examination, none of the plates contains colonies having the characteristics listed in Table 3 for the media used, the sample meets the requirement for freedom from Pseudomonas aeruginosa. If any colonies conforming to thedescription in Table 3 are produced, carry out the oxidase and pigment tests. Streak represents suspect colonies from the agar surface of cetrimide agar on the surfaces of pseudomonas agar medium for detection of fluorescein and pseudomonas agar medium for detection of pyocyanin contained in Petri dishes.

Cover and invert the inoculated media and incubate at 33º to 37º for not less than 3 days. Examine the streaked surfaces under ultra-violet light. Examine the plates to determine whether colonies conforming to the description in Table 3 are present. If

growth of suspect colonies occurs, place 2 or 3 drops of a freshly prepared 1% w/v solution of N, N, N1, N1-tetramethyl-4-phenylenediamine dihydrochloride on filter paper and smear with the colony; if there is no development of a pink colour,changing to purple, the sample meets the requirements of the test for the absence of Pseudomonas aeruginosa.

4. Staphylococcus aureus:

Proceed as described under Pseudomonas aeruginosa. If, upon examination of the incubated plates, none of them contains colonies having the characteristics listed in Table 4 for the media used, the sample meets the requirements for the absence of Staphylococcus aureus. Carry out the control test by repeating the primary and secondary tests using 1.0 ml of the enrichment culture and a volume of broth containing 10 to 50 Salmonella abony (NCTC 6017) organisms, prepared from a 24-hour culture in nutrient broth, for the inoculation of the tubes (a) and (b). The test is not valid unless the results indicate that the control contains Salmonella.

Test for Staphylococcus aureus

Selective medium

Gram stain

Vogel	-	Johnson agar Black surrounded by yellow zones Positive cocci (in clusters)
Mannitol	-	salt agar Yellow colonies with yellow zones Positive cocci (in clusters) Baird -Parker agar Black, shiny, surrounded by clear zones of 1 to 5mm Positive cocci (in clusters)

If growth occurs, carry out the coagulase test. Transfer representative suspect colonies from the agar surface of any of the media listed in Table 4 to individual tubes, each containing 0.5 ml of mammalian, preferably rabbit or horse, plasma with or without additives. Incubate in water-bath at 37º examining the tubes at 3 hours and subsequently at suitable intervals up to 24 hours. If no coagulation in any degree is observed, the sample meets the requirements of the test for the absence of Staphylococcus aureus.

4.2.2. CHEMICAL ANALYSIS

PROCEDURE:

5gms of the drug was wighted accurately and placed in a 250ml clean beaker. Then 50ml of distilled water is added and dissolved well. Then it is boilded well for about 10 minutes. It is cooled and filtered in a 100ml volumetric flask and then it is make up to 100ml with distilled water. This fluid is taken for analysis.

QUALITATIVE ANALYSIS FOR BASIC RADICALS:

Test for Calcium:

2ml of the above prepared extract is taken in a clean test tube. To this add 2ml of 4% Ammonium oxalate solution. Formation of white precipitate indicates the presence of calcium.

Test for Iron (Ferric):

The extract is acidified with glacial acetic acid and potassium ferro cyanide. Formation of blue colour indicates the presence of ferric iron.

Test for Iron (Ferrous):

The extract is treated with concentrated Nitric acid and ammonium thio-cyanate solution. Formation of blood red colour indicates the presence of ferrous iron.

Test for Zinc:

The extract is treated with potassium ferro-cyanide. Formation of white precipitate indicates the presence of zinc.

QUALITATIVE ANALYSIS FOR ACIDIC RADICALS:

Test for Sulphate:

2ml of extract is added to 5% barium chloride solution. Formation of white precipitate indicates the presence of sulphate.

Test for Chloride:

The extract is treated with silver nitrate solution. Formation of white precipitate indicates the presence of chloride.

Test for Phosphate:

The extract is treated with ammonium molybdate and concentrated nitric acid. Formation of yellow precipitate indicates the presence of phosphate.

Test for Carbonate:

On treating the extract with concentrated hydrochloric acid giving brisk effervescence indicates the presence of carbonate.

Test for starch:

The extract is added with weak iodine solution. Formation of blue colour indicates the presence of starch.

Test for albumin:

The extract is treated with Esbach`s reagent. Formation of yellow precipitate indicates the presence of albumin.

Test for tannic acid:

The extract is treated with ferric chloride. Formation of bluish black precipitate indicates the presence of tannic acid.

Test for unsaturation:

The extract is treated with potassium permanganate solution. The dis-colourization of potassium permanganate indicates the presence of unsaturated compounds.

Test for the reducing sugar:

5ml of Benedict`s qualitative solution is taken in a test tube and allowed to boil for 2 minutes and added 8-10 drops of the extract and again boil it for 2 minutes. Any colour change indicates the presence of reducing sugar.

Test for amino acid:

One or two drops of the extract is placed on a filter paper and dried it well. After drying, 1% Ninhydrin is sprayed over the same and dried it well. Formation of violet colour indicates the presence of amino acid.

4.2.3. INSTRUMENTAL ANALYSIS

Fig.4 SCANNING ELECTRON MICROSCOPE (SEM)

The microstructure of the powders was examined using a Hitachi S 3000H scanning electron microscope

Introduction:

The scanning Electron Microscope is one of the most versatile instruments available for the examination and analysis of the micro structural characteristics of solid objects. The primary reason for the SEM's usefulness is the high resolution which can be obtained when bulk objects are examined; values of the order of 5nm (50degreeA) are usually quoted for commercial instruments. Advanced research instruments have been described which have achieved resolutions of about 2.5nm (25 degree A). Any solid material can be studied. Sample size is limited to specimens less than about 10μm in diameter

Principle:

The beam is then rastered over the specimen in synchronism with the beam of a cathode ray tube display screen. The eleastically scattered secondary electrons are emitted from the sample surface and collected by a scintillator, the signal from which is used to modulate the brightness of the cathode ray tube. In this way the secondary electron emission from the sample is used to form an image on the CRT display screen. (Goldstein, et. al., 1992)

SEM MECHANISM

Procedure:

An electron beam passing through an evacuated column is focused by electromagnetic lenses onto the specimen surface. Since an electron is a charged particle, it has a strong interaction with the specimen (due to coulomb interaction).So when an electron beam images on a specimen, it is scattered by atomic layers near the surface of the specimen. As a result, the direction of electron motion changes and its energy is partially lost. Once an incident electron (primary electron) enters a substance, its direction of motion is influenced by various obstructions (multiple scattering), and follows a complicated trajectory which is far from a straight line. Also, when electrons with the same energy are incident on the specimen surface, a portion of electrons is reflected in the opposite direction (back scattered) and the remainder is absorbed by the specimen (exciting X- rays or other quanta in the process). If the specimen issufficiently thin, the electron can pass all the way through the specimen (transmitted electrons, scattered or non-scattered).

The depth at which various signals are generated due to electron beam – specimen interaction indicates the diffusion area of the signals in the specimen in addition to the local chemistry of the specimen. Secondary electrons mainly indicate information about the surface of a specimen. Since secondary electrons do not diffuse much inside the specimen, they are most suitable for observing the fine-structures of the specimen surface. That is to say, sharp scanning images with high resolution can be expected from secondary electrons, because of the smaller influence on resolution by their diffusion.

As the incident electron energy increases, the probability of incident electrons

Colliding with elemental components of the specimen and releasing secondary electronsalso increases. In other words, as the incident energy increases, the emission of electrons from the specimen also increases. However, as the energy increases beyond a certain level, the incident electrons penetrate deeper into the specimen with the result that the specimen derived electrons use up most of their energy to reach the specimen surface. Consequently, the electron emission yield decreases. Therefore, the peak secondary electron emission yield occurs at a specific entry level of the incident electrons.

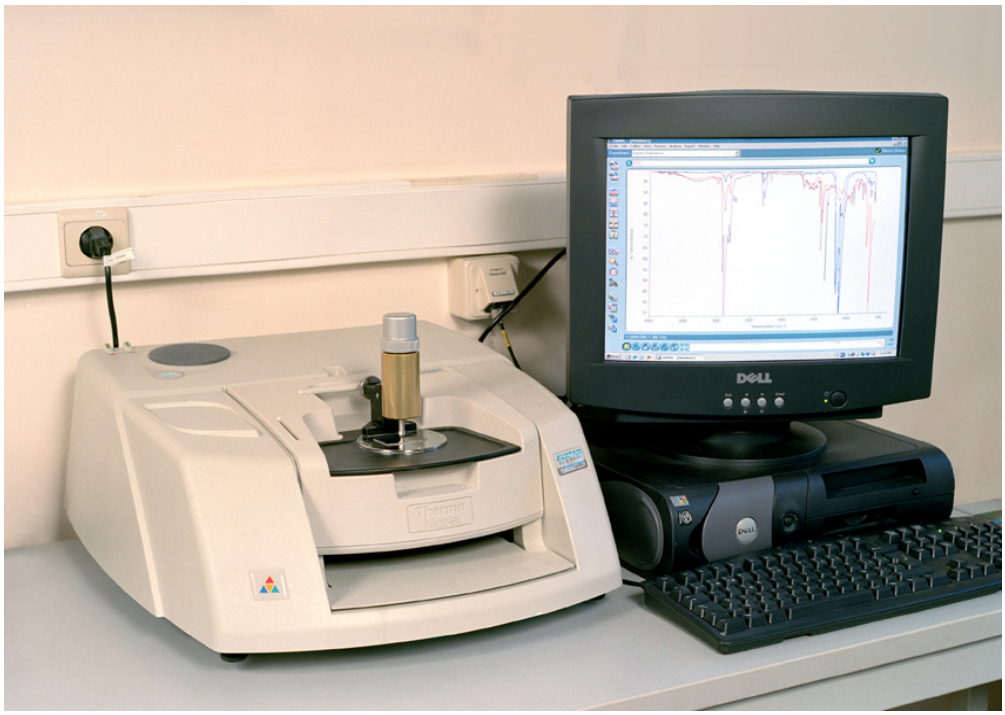
In order to verify the existence of a substance and recognize its shape, the image contrast must be well defined. In other words, even if a system boasts extremely high resolution, if image contrast is poor, it would be extremely difficult to determine the existence of a substance, let alone recognize its shape. Another important feature of the SEM is the three-dimensional appearance of the specimen image, which is a direct result of the large depth of field.

Applications:

The SEM is capable of examining objects at very low magnification. This feature is useful in viewing particle size and shape of any composition at various stages of preparation in *Siddha* system as well as other fields.The large depth of field available in the SEM makes it possible to observe 3-dimensional objects in stereo. Today, a majority of SEM facilities are equipped with X-ray analytical capabilities. Thus topographic crystallographic and compositional information can be obtained rapidly, efficiently and simultaneously from the same area.

The author was chosen this analysis for detecting Particle size of the classical *Siddha* herbo-mineral drug *Bosana kudori mathirai*. SEM results of *Bosana kudori mathirai* were represented in results section.

Fig :5 FOURIER TRANSFORM-INFRA RED SPECTROSCOPY (FT-IR)



Introduction:

Vibrational spectroscopy is an extremely useful tool in the elucidations of molecular structure .The spectral bands can be assigned to different vibrational modes of the molecule. The various functional groups present in the molecule can be assigned by a comparison of the spectra with characteristic functional group frequencies. As the positions of the bands are directly related to the strength of the chemical bond, a large number of investigations including intermolecular interactions, phase transitions and chemical kinetics can be carried out using this branch of spectroscopy. In IR spectroscopy, the resonance absorption is made possible by the change in dipole moment accompanying the vibrational transition. The Infrared spectrum originates from the vibrational motion of the molecule. The vibrational frequencies are a kind of fingerprint of the compounds. This property is used for characterization of organic, inorganic and biological compounds. The band intensities are proportional to the concentration of the compound and hence qualitative estimations are possible. The IR spectroscopy is carried out by using Fourier transform technique.

Principle:

Infra red spectroscopy involves study of the interaction of electromagnetic radiation with matter. Due to this interaction, electromagnetic radiation characteristic of the interacting system may be absorbed (or emitted). The experimental data consist of the nature (frequency of wave length) and the amount (intensity) of the characteristic radiation absorbed or emitted. These data are correlated with the molecular and electronic structure of the substance and with intra- and inter molecular interactions.

Source	:	Nernst Glower
Beam splitter	:	It is made up of a transparent material. Thin films of Silicondepositedon Potassium bromide (KBr) Bromide (KBr) Detectors: DeutratedTriGlycine Sulphate (DTGS).
Scan Range	:	MIR 450to 4000 cm-1
Resolution	:	4.0 cm-1
Sample required	:	50mg, solid or liquid
Sampling Techniques:		There are a variety of techniques for sample preparation physical form of the sample to be analyzed.
Solid	:	KBr or Nujol mull method.
Liquid	:	Csl / TIBr Cells
Gas	:	Gas cells

Measurements Techniques:

The procedure for recording the %T or %A is as follows:

- Air is first scanned for the reference and stored. The sample is then recorded andfinally the ratio of the sample and reference data is computed to give required %T or %A at various frequencies.
- Study of substances with strong absorbance bands and weak absorbance bands aswell as possible.
- Small amount of samples are sufficient
- High resolution is obtained.

Procedure:

Typically,1.5 mg of protein, dissolved in the buffer used for its purification, were centrifuged in a 30 K Centric on micro concentrator (Amicon) at 3000_g at 4oC until a volume of approximately 40 Al.

- Then, 300 Al of 20 mM buffer, prepared in H_20 or $2H_20$, pH or p2H 7.2, were added and the sample concentrated again. The p2H value corresponds to the pH
- meter reading + 0.4. The concentration and dilution procedure was repeated several times in order to completely replace the original buffer with the This buffer.
- The washings took 24 h, which is the time of contact of the protein with the 2H20
- medium prior FT-IR analysis. In the last washing, the protein was concentrated to fine a volume of approximately 40 Al and used for the infrared measurements.
- The concentrated protein sample was placed in CaF2 windows and a 6 Am tin spacer or a 25 Am Teflon spacer for the experiments in H20 or 2H20, respectively. FT-IR spectra were recorded by means of a Perkin-Elmer - Spectrum-1 FT-IR spectrometer using a deuteratedtriglycinesulfate detector.
- At least 24 h before, and during data acquisition, the spectrometer were continuously purged with dry air at a dew point of 40^oC. Spectra of buffers and samples were acquired at 2 cm_1 resolution under the same scanning and temperature conditions. In the thermal denaturation experiments, the temperature was raised in 5^oC steps from 20 to 95^oC.
- Before spectrum acquisition, samples were maintained at the desired temperature for the time necessary for the stabilization of temperature inside the cell (6 min).Spectra were collected and processed using the SPECTRUM software from Perkin-Elmer. Correct subtraction of H_20 was judged to yield an approximately flat baseline at 1900-1400 cm_ 1, and subtraction of $2H_20$ was adjusted to the removal of the$2H_20$ bending absorption close to 1220 cm_ 1

KBr Method

- The sample is grounded using an agate mortar and pestle to give a very fine powder.
- The finely powder sample is then mixed with about 100mg dried KBr salt.
- The mixture is then pressed under hydraulic press using a die to yield a transparent disc and measure about 13mm diameter and 0.3mm in thickness.

Nujol Mull Method:

- The sample is ground using an agate mortar and pestle to give a very fine powder.
- A small amount is then mixed with nujol oil to give a paste and this paste is thenapplied between two sodium chloride plates.
- The plates are then placed in the instrument sample holder ready for scanning.

Liquids:

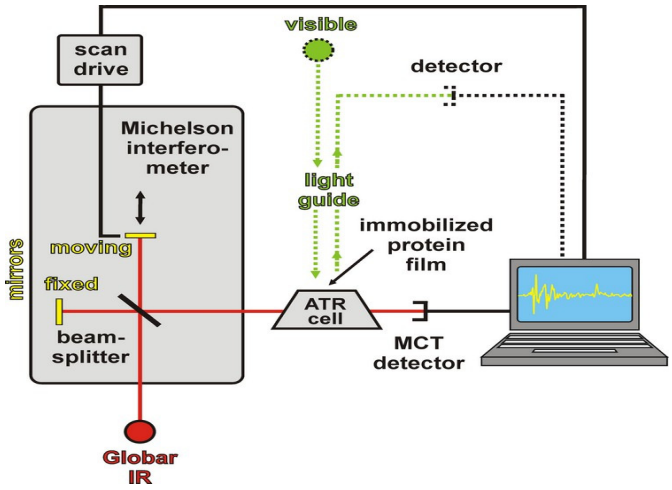
- Viscous liquids can be smeared in the cell and directly measured.
- For dilute solutions, liquid cells and variable path length cells are employed.

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.

Fig No. 6 Mechanism of FTIR analyzer

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 ions
- Detection limits vary greatly, but are sometimes $<10^{13}$ bonds/cm^3 or sometimes sub monolayer .Useful with solids, liquids, or gases.

Fig.No. 7 INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETRY(ICP-OES):

Introduction:

Inductively coupled plasma optical emission spectrometry (ICP-OES) is an analytical technique used for the detection of trace metals. It is a type of emission spectroscopy that uses the inductively coupled plasma to produce excited atoms and ions that emit electromagnetic radiation at wavelengths characteristic of a particular element. The intensity of this emission is indicative of the concentration of the element within the sample.

Mechanism:

The ICP-OES is composed of two parts: ICP and the optical spectrometer. The ICP torch consists of 3 concentric quartz glass tubes. The output or "work" coil of the radiofrequency (RF) generator surrounds part of this quartz torch. Argon gas is typically used to create the plasma.

When the torch is turned on, an intense electromagnetic field is created within the coil by the high power radio frequency signal flowing in the coil. This RF signal is created by the RF generator which is, effectively, a high power radio transmitter driving the "workcoil" the same way a typical radio transmitter drives a transmitting antenna. The argon gas flowing through the torch is ignited with a Tesla unit that

creates a brief discharge arc through the argon flow to initiate the ionization process. Once the plasma is "ignited", the Tesla unit is turned off.

The argon gas is ionized in the intense electromagnetic field and flows in a particular rotationally symmetrical pattern towards the magnetic field of the RF coil. Stable, high temperature plasma of about 7000 K is then generated as the result of the inelastic collisions created between the neutral argon atoms and the charged particles. A peristaltic pump delivers an aqueous or organic sample into a nebulizer where it is changed into mist and introduced directly inside the plasma flame. The sample immediately collides with the electrons and charged ions in the plasma and is itself broken down into charged ions. The various molecules break up into their respective atoms which then lose electrons and recombine repeatedly in the plasma, giving off radiation at the characteristic wavelengths of the elements involved.

Within the optical chamber(s), after the light is separated into its different wavelengths (colours), the light intensity is measured with a photomultiplier tube or tubes physically positioned to "view" the specific wavelength(s) for each element line involved, or, in more modern units, the separated colours fall upon an array of semiconductor photo detectors such as charge coupled devices (CCDs). In units using these detector arrays, the intensities of all wavelengths (within the system's range) can be measured simultaneously, allowing the instrument to analyse for every element to which the unit is sensitive all at once. Thus, samples can be analysed very quickly.

The intensity of each line is then compared to previously measured intensities ofknown concentrations of the elements and their concentrations are then computed by interpolation along the calibration lines. In addition, special software generally corrects for

interferences caused by the presence of different elements within a given sample matrix.

Applications :

ICP-OES is used in the determination of metals, arsenic present in Traditional medicines, and trace elements bound to proteins. ICP-OES is widely used in minerals processing to provide the data on grades of various streams, for the construction of mass balances.

The author used it for elemental identification and quantitative compositional information of the *Bosana kudori mathirai.*

4.3. TOXICOLOGICAL STUDIES:

4.3.1. ACUTE TOXICITY STUDY IN FEMALE WISTER RATS TO EVALUATE TOXICITY PROFILE OF *Bosanakudori Mathirai*

OBJECTIVES

The aim of this Study is to evaluate the toxicity of the test substance ***Bosanakudori Mathirai***, when administered orally to Female Wister Rats with different doses, so as to provide a rational base for the evaluation of the toxicological risk to man and indicate potential target organs.

Guidelines followed:

(a) OECD Guidelines No. 423,

Study Design and Controls:

1) Female Wister Rats in controlled age and body weight were selected.
2) ***Bosanakudori Mathirai*** was administered at **5 mg/kg, 50 mg/kg, 300 mg/kg, 1000 mg/kg, and mg/kg** body weight as (Water) as suspension along with blank.
3) The results were recorded on day 0, with single oral dosing period of 14 days.

EXPERIMENTAL PROCEDURE

1. ANIMALS

Supply

A total of 15 Female Wister Rats with an approximate age of 6 weeks and purchased from M/s.Venkateshwara Enterprises Pvt. Ltd, Bangalore. On their arrival a sample of animals was chosen at random and weighed to ensure compliance with the age requested. The mean weights of Female Wister Rats were 100-150 g respectively. The animals were housed in metabolic cages (55 x 32.7 x 19 cm), with sawdust litter, in such a way that each cage contained a maximum of 3 animals of the same sex.

All animals underwent a period of 20 days of observation and acclimatization between the date of arrival and the start of treatment. During the course of this period,

the animals were inspected by a veterinary surgeon to ensure that they fulfilled the health requirements necessary for initiation of the Study.

Housing

The Female Wister Rats were housed in metabolic cages (55 x 32.7 x 19 cm), placed on racks. From the week before initiation of the treatment, each cage contained a maximum of 6 mice of the same sex and treatment group.

Each cage was identified by a card, color coded according to the dose level. This card stated the cage number, number and sex of the animals it contained, Study number, test substance code, administration route, dose level and Study Director's name, date of the arrival of the animals and initiation of treatment.

The temperature and relative humidity were continuously monitored. Lighting was controlled to supply 12 hours of light (7:00 to 19:00 hours) and 12 hours of dark for each 24-hour period.

The cages corresponding to each experimental group were distributed on racks in such a manner that external factors, such as environmental conditions, were balanced as far as possible.

2. DIET

All the rats had free access to a pelleted rat diet. The diet was analyzed by the manufacturer to check its composition and to detect possible contaminants.

Water

The water was offered ad libitum in bottles.

3. ADMINISTRATION ROUTE AND PROCEDURE

The test substance was administered orally. The Female Wister Rats belonging to the control group were treated with the vehicle (Water) at the same administration volume as the rest of the treatment groups.

Numbering and Identification

The animals were marked on body with picric acid solution prepared in water. The marking within the cage was as below.

Group No	Animal Marking
1	Head
2	Body
3	Tail

The group no., cage no., sex of the animal and animal no. were identified as indicated below using cage label and body marking on the animals

Cage No	Group No	Animal Marking	Sex
1	I	H,B,T	Female
2	II	H,B,T	Female
3	III	H,B,T	Female
4	IV	H,B,T	Female
5	V	H,B,T	Female

Doses

The doses for the study were selected based on literature search and range finding study. Following the period of fasting, the animals were weighed and then drug was administered orally as single dose using a needle fitted onto a disposable syringe of approximate size at the following different doses.

GROUP	DOSE
GROUP	DOSE
Group-I	5 mg/kg
Group-II	50 mg/kg
Group-III	300 mg/kg
Group-IV	1000 mg/kg
Group-V	2000 mg/kg

The test item was administered as single dose. After single dose administration period, all animals were observed for day 14.

Dose Preparation

Bosanakudori Mathirai was added in distilled water and completely dissolved to form oral for administration. The dose was prepared of a required concentration before dosing by dissolving, in distilled water. It was mixed well. The preparation for different doses was vary in concentrations to allow a constant dosage volume.

Administration

The test item was administered orally to each Female Wister rats as single dose using a needle fitted onto a disposable syringe of appropriate size at the following different doses. The concentration was adjusted according to its body weight. The volume was not exceeding 10 ml/kg bodyweight. Variability in test volume was minimized by adjusting the concentration to ensure a constant volume at all dose levels.

Observation period

All animals were observed for any abnormal clinical signs and behavioral changes. The appearance, change and disappearance of these clinical signs, if any, were recorded for approximately 1.0, 3.0 and 4.0 hours post-dose on day of dosing and once daily thereafter for14 days. Animals in pain or showing severe signs of distress were humanely killed. The cageside observation was included changes in skin, fur, eyes and mucous membranes, occurrence of secretions and excretions. Autonomic activity like lacrimation, piloerection, pupil size and unusual respiratory pattern, changes in gait, posture, response to handling, presence of clonic or tonic movements, stereotypes like excessive grooming and repetitive circling or bizarre behavior like self-mutilation, walking backwards etc were observed. At the 14th day, sensory reactivity to stimuli of different types (e.g. auditory, visual and proprioceptive stimuli) was conducted. Auditory stimuli responses were measured by clicker sound from approximately 30 cm to the rats; visual stimuli response were measured with the help of shining pen light in the eye of rats and placing a blunt object near to the eye of rats. Response to proprioceptive stimuli was measured by placing anterior/dorsal surface of animals paw to the table edge. The responses of reactions for these three

exercises were normal in animals belonging to both the controls as well as drug treatment dose groups.

Mortality and Morbidity

All animals were observed daily once for mortality and morbidity at approximately 1.0, 3.0 and 4.0 hours post dose on day of dosing and twice daily (morning and afternoon) thereafter for 14 days.

4.3.2. SUB-ACUTE TOXICITY STUDY IN WISTER RATS TO EVALUATE TOXICITY PROFILE OF *BOSANAKUDORI MATHIRAI*

Objective

The objective of this '**Sub-Acute Toxicity Study of *Bosanakudori Mathirai* ON Wister Rats**' was to assess the toxicological profile of the test item when treated as a single dose. Animals should be observed for 28 days after the drug administration. This study provides information on the possible health hazards likely to arise from exposure over a relatively limited period of time.

Test Guideline Followed

OECD 407 Method - Sub-Acute Toxic Class Method (Repeated Dose 28-Day Oral Toxicity Study in Rodents)

Test Item Detail

Bosanakudori Mathirai

Test System Detail

The study was conducted on 5 male 5 female Wister rats. These animals were selected because of the recommended rodent species for oral studies as per followed guideline and availability of Animals 8-12 weeks old male and female rats were selected after physical and behavioral examination. The body weight range was fallen within ± 20% of the mean body weight at the time of Randomization and grouping. The rats were housed in standard laboratory condition in Polypropylene cages, provided with food and water *adlibitum* in the Animal at M/s. Sree Venkateshwara Enterprises Pvt. Ltd, Bangalore. The experimental protocol was approved by Institutional Animal Ethical Committee as per the guidance of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, government of India.

Acclimatization

The animals were selected after veterinary examination by the veterinarian. All the selected animals were kept under acclimatization for a week.

Randomization & grouping

One day before the initiation of treatment (days 0- last day of acclimatization), the selected animals were randomly grouped into three different groups containing minimum 6 male animals per group.

Numbering and Identification

The animals were marked on body with picric acid solution prepared in water. The marking within the cage was as below.

Group No	Animal Marking
1. CONTROL	H,B,T,HB,NM (MALE) H,B,T,HB,NM (FEMALE)
2. LOW DOSE OF *Bosanakudori Mathirai* 300mg/kg	H,B,T,HB,NM (MALE) H,B,T,HB,NM (FEMALE)
3. MIDDLEDOSE OF *Bosanakudori Mathirai* 600mg/kg	H,B,T,HB,NM (MALE) H,B,T,HB,NM (FEMALE)
4. HIGH DOSE OF *Bosanakudori Mathirai* 900mg/kg	H,B,T,HB,NM (MALE) H,B,T,HB,NM (FEMALE)

The group no., cage no., sex of the animal and animal no. were identified as indicated below using cage label and body marking on the animals:

Case No	Group No	Animal Marking	Sex
1	**1. CONTROL**	**H,B,T,HB,NM** **H,B,T,HB, NM**	**Male** **Female**
2	**2. LOW DOSE OF *Bosanakudori Mathirai* 300mg/kg**	**H,B,T,HB,NM** **H,B,T,HB, NM**	**Male** **Female**
3	**3. MIDDLEDOSE OF *Bosanakudori Mathirai* 600mg/kg**	**H,B,T,HB,NM** **H,B,T,HB, NM**	**Male** **Female**
4	**4. HIGH DOSE OF *Bosanakudori Mathirai* 900mg/kg**	**H,B,T,HB,NM** **H,B,T,HB ,NM**	**Male** **Female**

Husbandry

Housing

The Wister rats were housed in standard polypropylene cages with stainless steel top grill. Paddy husk was used as bedding. The paddy husk was changed at least twice in a week. From the week before initiation of the treatment, each cage contained a maximum of 6 mice of the same sex and treatment group.

Environmental conditions

The animals were kept in a clean environment with 12 hour light and 12 hour dark cycles. The air was conditioned at $22\pm3^{0}C$ and the relative humidity was maintained between 30-70% with 100% exhaust facility. The cages corresponding to each experimental group were distributed on racks in such a manner that external factors, such as environmental conditions, were balanced as far as possible.

Feed & feeding schedule

'Sai Durga Animal Feed, Bangalore. Feed was provided *adlibitum throughout* the study period, except over night fasting (18-20 hours) prior to dose administration. After the substance has been administered, food was with held for a further 3-4 hours.

Water

The water was offered *adlibitum* in bottles. There was periodically analyzed to detect the presence of possible contaminants

Doses

The doses for the study were selected based on literature search and range finding study. Following the period of fasting, the animals were weighed and then extract was administered orally as single dose using a needle fitted on to a disposable syringe of approximate size at the following different doses.

TEST GROUP	DOSE TO ANIMALS (mg/kg body-weight/day)	NUMBER OFANIMALS
Group-1	**1. CONTROL**	10 (5MALE and5 FEMALE)
Group-II	**2. LOW DOSE OF *Bosanakudori Mathirai* 300mg/kg**	10(5MALE and 5 FEMALE)
Group-III	**3. MIDDLEDOSE OF *Bosanakudori Mathirai* 600mg/kg**	10(5MALE and 5 FEMALE)
Group-IV	**4. HIGH DOSE OF *Bosanakudori Mathirai* 900mg/kg**	10(5MALE and 5 FEMALE)

The test item was administered as single dose. After single dose administration period, all animals were observed for 28 days.

Dose Preparation

Bosanakudori Mathirai was added in distilled water and completely dissolved to for oral for administration. The dose was prepared of a required concentration before dosing by dissolving ***Bosanakudori Mathirai*** in distilled water. It was mixed well. The preparation for different doses was vary in concentrations to allow a constant dosage volume.

Administration

The test item was administered orally to each rat as single dose using a needle fitted on to a disposable syringe of appropriate size at the following different doses. The concentration was adjusted according to its body weight. The volume was not exceeding 10 ml/kg body weight. Variability in test volume was minimized by adjusting the concentration to ensure a constant volume at all dose levels.

OBSERVATIONS

These observations were also performed on week-ends. The observations included but were not limited to changes in skin and fur, in the eyes and mucous membranes, in the respiratory, circulatory, central nervous and autonomous systems, somatomotor activity and behavior.

Clinical signs of toxicity

All the rats were observed at least twice daily with the purpose of recording any symptoms of ill- health or behavioral changes. Clinical signs of toxicity daily for 28 days.

Food intake

Prior to the beginning of treatment, and daily, the food intake of each cage was recorded for period of 28 days and the mean weekly intake per rats was calculated.

Water intake

Water intake was checked by visual observation during the Study. In addition, the water consumption in each cage was measured daily for a period of 28 days.

Bodyweight:

The body weight of each rat was recorded one week before the start of treatment, and during the course of the treatment on the day of initial, 3rd, 7th, 10th, 14th, 17th, 20th, 24th and 28th days (day of sacrifice). The mean weights for the different groups and sexes were calculated from the individual weights.

Blood Collection

Blood was collected through retro-orbital sinus from all the animals of different groups on 28th day. The blood was collected in tubes containing Heparin/EDTA as an anticoagulant. Animals were fasted over night prior to the blood collection.

LABORATORY STUDIES

During the 4th week of treatment, samples of blood were withdrawn from the orbital sinus of 6 males from each group, under light ether anesthesia after fasting for 16 hours. The blood samples are used to evaluate Hematological parameters like

RBC, WBC, and PLATELETS etc….. The collected blood samples also centrifuged 10000 rpm in 10 minutes to separate the serum. The separated serum used to evaluate biochemical parameters like SGOT, SGPT, ALP and BILIRUBIN ect……...

Hematology

The following hematological parameters were analysed using Autoanalyser

Hb	:	Haemoglobin (g %)
PCV	:	Packed Cell Volume
WBC	:	White Blood Corpuscles (x103/cmm)
RBC	:	Red Blood Corpuscles (x106/cmm)
		Blood Platelet count (x103/cmm)

Differential WBC count:

N	:	Neutrophils (%)
L	:	Lymphocytes (%)
M	:	Monocytes (%)
E	:	Eosinophils (%)
RDW	:	Red Cell Distribution Width.
MPV	:	Mean Platelet Volume

Clinical Biochemistry:

The following clinical Bio parameters were analysed using Auto analyser

Total serum protein (g/dl)

ALT/SGPT	:	Alanine amino transferase (U/L)
AST/SGOT	:	Aspartate amino transferase (U/L)
ALP	:	Alkaline serum phosphatase (U/L)
CHL	:	Cholesterol (mg/dL)
HDL	:	High density lipoprotein
TG	:	Triglyceride

TERMINAL STUDIES

Sacrifice and macroscopic examination

On completion of the 4 weeks of treatment, 18 Wister rats were sacrificed by ether inhalation. A full autopsy was performed on all animals which included examination of the external surface of the body, all orifices, cranial, thoracic and

abdominal cavities and their contents both *in situ* and after evisceration. As the number of animals exceeded the number that could be sacrificed in one day, the autopsies were carried out over three consecutive days at the end of the treatment period.

Organ weights:

After the macroscopic examination the following organs were weighed after separating the superficial fat: Brain, Heart, Spleen Kidneys, Testes, Liver, Lungs, pancreas and stomach.

ESTIMATION OF HEMATOLOGICAL PARAMETERS:

Collection of blood for hematological studies

After the treatment period the animals were anaesthetized by ketamine hydrochloride and the blood was collected from Retro-orbital sinus by using capillary into a centrifugation tube which contains EDTA for haematological parameters The haematological parameters like RBC, WBC and Hb percentage, Differential cell count, MCV, MCHC, Hematocrit, MCH, platelet count were estimated by the following procedures.

ENUMERATION OF RED BLOOD CELLS

Reagents : RBC diluting fluid

Procedure:

Using a red blood cell pipette of haemocytometer, well mixed blood was drawn up to 0.5 mark and RBC diluting fluid was taken up to mark II. The fluid blood mixture was shaken and transferred onto the counting chamber. The cells were allowed to settle to the bottom of the chamber for 2 min. See the fluid does not get dried. Using 45X or high power objective the RBC's were counted uniformly in the larger corner squares.

The cells were expressed as number of cells $x10^{12}/l$

ENUMERATION OF WBC

Reagents:

Turk's fluid: Turk's fluid was prepared by mixing 2ml of acetic acid with 100 ml of distilled water. To this 10 drop of aqueous methylene blue 3 % w/v) was added. This solution haemolysis the red cells due to acidity so that counting of white cells becomes easy.

Procedure:

Using a white blood cell pipette of haemocytometer, well mixed blood was drawn up to 0.5 mark and WBC diluting fluid was taken up to mark II. The fluid blood mixture was shaken and transferred onto the counting chamber. The cells were allowed to settle to the bottom of the chamber for 2 min. See the fluid does not get dried.

Using 10X or low power objective the WBC's were counted uniformly in the larger corner squares.

The cells were expressed as number of cells/10mm.

DIFFERENTIAL LEUCOCYTE COUNT

Reagent:

Leishmann's stain: 150mg of powdered leishmann's stain was dissolved in 133ml of acetone free methanol.

Procedure:

A blood film stained with leishmann's stain was examined under oil immersion and the different types of WBCs were identified. The percentage distribution of these cells was then determined. Smears were made from anticoagulant blood specimens and stained with leishmann's stain. The slides were preserved for counting the number of lymphocytes and neutrophils, per 100 cells were noted.

From the different Leukocyte count and WBC count, absolute lymphocyte and neutrophil count were calculated.

$$\text{Absolute neutrophil count} = \frac{\text{Number of neutrophils}}{100} \times \text{TWBC}$$

$$\text{Absolute lymphocyte count} = \frac{\text{Number of lymphocytes}}{100} \times \text{TWBC}$$

J. C. Dacie and S. M. Lewis, Practical haematology, London: Churchill Livingstone, 1984, pp. 5.

Measurement of biochemical parameters estimation

Haemoglobin (Hb), was estimated using whole blood. Remaining parameters were measured in serum. All of the above biochemical parameters were estimated using semi-autoanalyzer (Photometer 5010 $_{V5+}$, Germany) with enzymatic kits procured from Piramal Healthcare limited, Lab Diagnostic Division, Mumbai, India.

Determination of aspartate aminotransferase (AST)

Aspartate aminotransferase, also known as Glutamate Oxaloacetate Transaminase (GOT) catalyses the transamination of L-aspartate and α keto glutarate to form oxaloacetate and L- glutamate. Oxaloacetate formed is coupled with 2,4-

Dinitrophenyl hydrazine to form hydrazone, a brown coloured complex in alkaline medium which can be measured colorimetrically.

Reagents

Buffered aspartate (pH 7.4); 2,4- DNPH reagent; 4N sodium hydroxide; working pyruvate standard; solution I (prepared by diluting 1 ml of reagent 3 to 10 ml with purified water).

Procedure

Rietman and Frankle method was adopted for the estimation of SGOT. (Reitmann S, Frankel S, 1957. A colorimetric method for the determination of serum oxaloacetic and glutamic pyruvate transminases. American Journal of Clinical Pathology.28: 56-63.The reaction systems used for this study included blank, standard, test (for each serum sample) and control (for each serum sample). 0.25 ml of buffered aspartate was added into all the test tubes. Then 0.05 ml of serum was added to the test group tubes and 0.05 ml of working pyruvate standard into the standard tubes. After proper mixing, all the tubes were kept for incubation at 37^{o}C for 60 min, after which 0.25 ml each of 2,4- DNPH reagent was added into all the tubes. Then, 0.05 ml of distilled water and 0.05 ml of each serum sample was added to the blank and the serum control tubes respectively. The mixture was allowed to stand at room temperature for 20 min. After incubation, 2.5 ml of solution I was added to all test tubes. Mixed properly and optical density was measured in a spectrophotometer at 505 nm within 15 min.

The enzyme activity was calculated as:-

AST (GOT) activity in IU/L) = [(Absorbance of test **-** Absorbance of control)/ (Absorbance of standard - Absorbance of blank)] x concentration of the standard

Determination of alanine aminotransferase (ALT)

Alanine aminotransferase, also known as Glutathione Peroxidase (GPT) catalyses the transamination of L-alanine and α keto glutarate to form pyruvate and L-Glutamate. Pyruvate so formed is coupled with 2,4 – Dinitrophenyl hydrazine to form a corresponding hydrazone, a brown coloured complex in alkaline medium which can be measured colorimetrically.

Reagents

Buffered alanine (pH 7.4), 2,4–DNPH, 4N sodium hydroxide, working pyruvate standard, solution I (prepared by diluting 1 ml of reagent 3 to 10 ml with purified water).

Procedure

Rietman and Frankle method was dopted for the estimation of SGPT. The reaction systems used for this study included blank, standard, test (for each serum sample) and control (for each serum sample). 0.25 ml of buffered alanine was added into all the test tubes. This was followed by the addition of 0.05 ml of serum into the test group tubes and 0.05 ml of working pyruvate standard into the standard tubes. After proper mixing, all the tubes were kept for incubation at 37^{o}C for 60 minutes, after which 0.25 ml each of 2,4- DNPH reagent was added into all the tubes. Then, 0.05 ml of distilled water and 0.05 ml of each serum sample was added to the blank and the serum control tubes respectively. The mixture was allowed to stand at room temperature for 20 min. After incubation, 2.5 ml of solution **I** was added to all test tubes. Mixed properly and optical density was read against purified water in a spectrophotometer at 505 nm within 15 min.

The enzyme activity was calculated as:- ALT (GPT) activity in IU/L) = [(Absorbance of test **-** Absorbance of control)/ (Absorbance of standard - Absorbance of blank)] x concentration of the standard.

Determination of alkaline phosphatase (ALP)

Alkaline phoshatase from serum converts phenyl phosphate to inorganic phosphate and phenol at pH 10.0. Phenol so formed reacts in alkaline medium with 4-aminoantipyrine in presence of the oxidising agent potassium ferricyanide and forms an orange-red coloured complex, which can be measured spectrometrically. The color intensity is proportional to the enzyme activity.

Reagents:

Buffered substrate

Chromogen Reagent

Phenol Standard, 10 mg%

Procedure:

ALP was determined using the method of Kind (Kind PRM, King EJ, 1972. *In-vitro* determination of serum alkaline phosphatase. Journal of Clinical Pathology 7: 321-22\). The working solution was prepared by reconstituting one vial of buffered substrate with 2.2 ml of water. 0.5 ml of working buffered substrate and 1.5 ml of purified water was dispensed to blank, standard, control and test. Mixed well and incubated at 37^0C for 3 min. 0.05 ml each of serum and phenol standard were added to test and standard test tubes respectively. Mixed well and incubated for 15 min at 37^0C. Thereafter, 1 ml of chromogen reagent was added to all the test tubes. Then, added 0.05 ml of serum to control. Mixed well after addition of each reagent and the O.D of blank, standard, control and test were read against purified water at 510 nm.
Serum alkaline phosphatase activity in KA units was calculated as follows
[(O.D. Test-O.D. Control) / (O.D. Standard- O.D. Blank)] x 10

Determination of bilirubin

In toxic liver, bilirubin levels are elevated. Hyperbilirubinemia can result from impaired hepatic uptake of unconjugated bilirubin, such a situation can occur in generalized liver cell injury, certain drugs (e.g Rifampin and probenecid) interfere with the rat uptake of bilirubin by the liver cell and may produce a mild unconjugated hyperbilirubinemia. Bilirubin level rises in diseases of hepatocytes, obstruction to bilirubin excretion into duodenum, in haemolysis and defects of hepatic uptake and conjugation of Bilirubin pigment such as Gilbert's disease.

Elevation of total serum bilirubin may occur due to:

1) Excessive haemolysis or destruction of the red blood cells.Eg:Haemolytic disease of the new born.
2) Liver diseases.Eg.Hepatitis and cirrhosis.
3) Obstruction of the biliary tract.Eg.Gall stones.

The method is based on the reaction of Sulfonilic acid with sodium nitrite to form azobilirubin which has maximum absorbance at 546nm in the aqueous solution. The intensity of the color Produced is directly proportional to the amount of direct or total bilurubin concentration present in the sample.

Reagents

1. Diazo A-(Reagent-R1) :Ready to use
2. Diazo B-(Reagent-R2):Ready to use
3. Bilirubin Activater :Ready to use

Procedure

Kind & King's method was followed for the estimation of Bilirubin. Five hundred µl of working reagent was added to 50 µl of rat serum & incubated for 5 min at 37°C. Absorbance was measured AT 546 NM in semi auto analyzer against the standard.

The Bilirubin content was calculated using the following equation:

Total bilirubin (mg/dt) = Abs of the sample blank x 15.

Estimation of Urea

Urea is the nitrogen-containing end product of protein catabolism. States associated with elevated levels of urea in blood are referred to as hyper uremia or azotemia.

Method

Estimation of urea was done by Urease-GLDH: enzymatic UV test.

Principle

$$\text{Urea} + 2H_2O \xrightarrow{\text{Urease}} 2NH_4 + 2HCO_3$$

$$\text{2- Oxoglutarate} + NH_4^+ + \text{NADH} \xrightarrow{\text{GLDH}} \text{L- Glutamate} + NAD^+ + H_2O$$

Reagents

R 1	TRIS pH 7.8	120 mmol/l
	2-Oxoglutarate	7 mmol/l
	ADP	0.6 mmol/l
	Urease	≥ 6 KU/l
	GLDH	≥ 1 KU/l
R 2	NADH	0.25 mmol
R 3	Standard	40 mg/dl

Procedure

- Take 1000 µl of reagent-1 and 250 µl of reagent-2 in 5 ml test tube.
- To this, add 10 µl of serum.
- Mix well and immediately read the test sample at 340 nm Hg 334 nm Hg 365 nm optical path 1 cm against reagent blank (2-point kinetic).
- And note down the value.
- **Normal range:** 10 – 50 mg/dl.

ESTIMATION OF URIC ACID

Uric acid and its salts are end products of the purine metabolism. In gout the most common complication of hyperuricemia, ie. Increased serum levels of uric acid lead to formation of monosodium urate crystal around the joints.

Method

Enzymatic photometric test using TOOS (N ethyl- N (hydroxyl -3-sulfopropyl)-m- toluidin)

Principle

$$\text{Uric acid} + H_2O + O_2 \xrightarrow{\text{uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

$$\text{TOOS} + 4\text{ aminoantipyrine} + 2H_2O_2 \xrightarrow{\text{POD}} \text{Indamine} + 3H_2O$$

Reagents

R1	Phosphate buffer pH 7.0	100mmol/l
	TOOS	1mmol/l
	Ascorbate oxidase	≥1 KU/l
R2	Phosphate buffer pH 7.0	100mmol/l
	4- amino antipyrine	0.3mmol/l
	K_4 (Fe(CN)$_6$)	10µmol/l
	Peroxidase	≥1KU/l
	Uricase	≥50U/l

Procedure

- Take 800µl of reagents -1 in a2ml centrifuge tube.
- To this add 20µl of serum.
- Mix well and incubate at 30°c for 5 minutes.
- Then add 200µl of reagent2
- Mix well incubate for 5min at 37°c
- Measure the not down the values.

Normal range: 1.9-8.2mg/dl

ESTIMATION OF CREATININE:

Estimation of Creatinine by Jaffe Method (modified)

Principle:

Creatinine forms a coloured complex with picrate in alkaline medium.

The rate of formation of the complex is measured.

Reagents:

Reagent 1 Standard Creatinine (2mg/100ml)

Reagent 2 Picric acid solution

Reagent 3 Sodium hydroxide solution

Procedure:

Take 500 µl of reagent -2 and 500 µl of reagent -3 in a 5ml test tube. To this add 100 µl of serum. Mix well and immediately read the test sample at Hg 492 nm 1cm light path and note down the values.

Normal range is 0.6 -1.1 mg/dl.

4.4. PHARMACOLOGICAL STUDIES

4.4.1. EVALUATION OF ANTIULCER ACTIVITY OF *Bosanakudori Mathirai* ON PYLORIC LIGATION INDUCED ULCER IN RAT

1. PYLORIC LIGATION MODEL

The ulcer protective effect of ***Bosanakudori Mathirai*** was studied as per the method of Shay et al., (1945).The ulceration is caused by accumulation of acidic gastric juice in the stomach and by this method several parameters can be estimated.

Animals:

AlbinoWister Rats weighing 150-200gm, procured from SreeVenkateshwara Enterprises Pvt Ltd, Bangalore, were used for the study.

Housing of the Animals:

Animals were kept for one week to acclimatize to laboratory conditions before starting the experiment; they were given free access to water and standard rat feed but 12 hrs prior to an experiment, the rats were deprived of food but not water.

Method:

Albino Wister rats of either sex weighing between 150 to 200gms were divided into Six groups of 6 animals each.

Group I	:	Control (saline 5 ml/kg)
Group II	:	Only pylorus ligation
Group III	:	pylorus ligation + Ranitidine 30 mg/kg body weight, oral.[2]
Group IV	:	pylorus ligation + ***Bosanakudori Mathirai*** 14.364 mg/kg (po)
Group V	:	pylorus ligation + ***Bosanakudori Mathirai*** 71.82 mg/kg (po)
Group VI	:	pylorus ligation + ***Bosanakudori Mathirai*** 143.64 mg/kg (po)

In this method Albino Wister Rats were fasted in metabolic cages for 24 h. Care was being taken to avoid Coprophagy. Control vehicle, three doses of ***Bosanakudori Mathirai*** and standard drug (Ranitidine 30 mg/kg) were administered by different doses orally for five days. At the end of the fifth day the animals were kept fasted for 14 hrs with water ad libitum, animals were treated with ***Bosanakudori Mathirai*** 30 minutes before ligation and the abdomen was opened the pylorus was ligated under light ether anesthesia, care beingtaken not cause bleeding or to occlude

blood vessels. The abdomen was then sutured. After 6h of pyloric ligation, the animals were sacrificed with excess of anesthetic ether, and the stomach was dissected out. Gastric juice was collected and its volume, pH, free acidity and total acidity was measured. Ulcer index was determined. The excised stomach was used to evaluate gastric wall mucus, antioxidant enzymes ,lipidperoxidation, Myeloperoxidation, prostaglandin estimation and Histopathological evaluation.

Ulcer index:

Procedure

The glandular portion of the stomach was opened along the greater curvature and fixed on a cork plate and the number and severity of ulcers was registered with a stereo-microscope using the following scores.

Mean ulcer score for each animal was expressed as Ulcer Index.

Calculation:

Ulcer index was calculated as;

Ulcer index (UI)=[10*ulcerated area (mm2)/ total stomach area (mm2)]

The percentage protection was calculated using the formula:

$$\text{Percentage of ulcer protection} = \frac{Ut}{Uc} \times 100$$

Where Ut = Ulcer index of treated group and

Uc = Ulcer index of the control

% of ulcer protection was calculated by

% of ulcer protection = Mc – Mt/Mc x100

Ulcer scores

Sl. No.	Stomach colours	Ulcer score
1	Normal colour	0
2	Red colour	0.5
3	Red spots	1
4	Hemorrhagic streaks	1.5
5	3 > 5 ulcers	2
6	< 5 ulcers	3

REAGENTS FOR BIOCHEMICAL ESTIMATIONS OF FREE AND TOTAL ACIDITY

1) REAGENTS FOR ESTIMATION OF FREE AND TOTAL ACIDITY:

- Freshly prepared 0.01N oxalic acid solution (BDH) was used to standardize sodium hydroxide.
- Freshly prepared 0.01N sodium hydroxide
- Topfer's reagent. It is dimethylaminoazobenzene 0.5% in absolute ethanol available 100ml package.
- Freshly prepared 1% Phenolphthalein (BDH) solution prepared in 50% absolute ethanol.

METHODS FOR BIOCHEMICAL ESTIMATION OF FREE AND TOTAL ACIDITY IN GASTRIC JUICE:

COLLECTION OF GASTRIC JUICE:

Gastric juice was collected from pylorus ligated rats. The gastric thus collected was centrifuged and the volume of gastric juice as well as pH of gastric juice was noted. The gastric was subjected to determination of free and total acidity as follows:

DETERMINATION OF FREE AND TOTAL ACIDITY:

One ml of gastric juice was pipetted into a 100ml conical flask, added 2 or 3 drops of Topfer's reagent and titrated with 0.01N Sodium hydroxide until all traces of red color disappears and the color of the solution was yellowish orange. The volume of alkali added was noted. This volume corresponds to free acidity. Then 2 or3 drops of phenolphthalein solution was added and titration was continued until a definite red tinge appears. Again the total volume of alkali added was noted. The volume corresponds to total acidity.

Acidity was calculated by using the formula:

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH} \times 100}{} \text{ mcq/lit/100gm}$$

1. Poonam D, PushpeshM, RakeshM, VinayC, GautamP. Desmodiumgangeticum: A potent antiulcer agent. I j exp bio2005;43:517-521.
2. Anoop A, Jegadeesan M. Biochemical studies on the anti-ulcerogenic potential of Hemidesmusindicus R.Br. var. indicus. J Ethnopharmacology 2003; 84: 149-156.
3. Amresh G, Hussainzeashan, Ram Jigupta, Ravikant, Chandanavenkateswararao, Parasnathsingh. Gastroprotective effects of ethanolic extract from cissampelospareira in experimental animals. J Nat med 2007; 61:323-328.
4. Maity S, Vedasiromoni JR, Ganguly DK.Anti-ulcer effect of the hot water extract of black tea *(Camelliasinensis).*J Ethnopharmacol1995; 46:167-174.
5. HawkPB, Oser BL, Summerson HW. Practical physiological chemistry,Churchill, London.1974.

4.4.2. IN-VITRO ANTISPASMODIC ACTIVITY OF *Bosanakudori Mathirai* ON EXCISED RAT ILEUM

ISOLATION OF RAT ILEUM:-

Rats were anesthetized and sacrificed by cervical displacement followed by exsanguinations. The ileum was dissected out, immersed in Tyrode'ssolutionand cleaned off the mesentery. Respective segments of 2-3cm long were mounted in a 25ml tissue organ bath, filled with a mixture of 95% O2 and 5% CO2 and maintained at 37 o C. The composition of Tyrode's solution (in mM for l lit) was 9 mg KCl, 0.1 mg NaCl, 0.1mg NaHCO3, 0.42mg NaH2PO4, 0.6 mg Glucose and pH value was 7.4.

ANTI-SPASMODIC ACTIVITY ASSAY PROCEDURE:-

1. Firstly concentration dependent responses of acetylcholine were recorded (with dose of 0.1ml, 0.2ml, 0.4ml, 0.8ml, 1.6ml, 3.2ml) using Sherrington's recording drum with a frontal writing lever. Contact time of 60 sec, and base line of 30sec time cycle were opted for proper recording of the responses in presence of plane Tyrode's solution as stock-I solution.

2. Then same concentration dependent responses of acetylcholine (Ach) using same procedure for a mixture of Tyrode's solution+ Lantana camara extract (with a concentration of 1mg/ml) as a stock-II solution were recorded.

3. Lastly the same concentration dependent responses of Ach for a mixture of Tyrode's solution+ Atropine (as a standard antispasmodic agent) as a stock-III solution were recorded.

4.4.3. ANTI DIARRHOEAL ACTIVITY:

EFFECT OF *BOSANAKUDORI MATHIRAI* ON SMALL INTESTINAL TRANSIT IN RATS

The experiment was performed according to the method of Venkatesan et al. (2005). Rats were fasted 48 h, and water was given *ad libitum*. The water was withdrawn 1 h before starting the experiment. Male Wister rats were given Normal saline (orally), atropine sulfate (intraperitoneally, i.p) and test compounds (orally). Thirty minutes later, they were orally fed with 1 ml of 3% deactivated charcoal (in 0.5% CMC). Thirty minutes after the deactivated charcoal feeding, the rats were sacrificed with intraperitoneal injection of pentobarbital sodium (50 mg/kg) and the gastrointestinal tract was removed. Total length of the small intestine (pylorus to caecum), and the distance of the deactivated charcoal movement were measured. The small intestine transit was calculated and expressed as percentage of the deactivated charcoal movement.

Animal Grouping

Group I	-	Control (Distilled Water)
Group II	-	Castor oil +Charcoal+ Atropine
Group III	-	Castor oil + Charcoal+ ***Bosanakudori Mathirai*** (14.364 mg/kg (po)
Group IV	-	Castor oil + Charcoal+ ***Bosanakudori Mathirai*** (71.82 mg/kg (po)
Group V	-	Castor oil + Charcoal+ ***Bosanakudori Mathirai***] (143.64 mg/kg (po)

DOSAGE SCHEDULE:

The required dose for mice/rat will be calculated by using the standard dose calculation procedure from recommended clinical dose.

CONVERSION FORMULA:

Human dose is 798mg

Total clinical dose (a) x conversion factor (b) 0.018 = (c) per 200 gm of rat

798 mg x 2(a) x 0.018 (b) = 14.364mg/kg (c) /200gms of rat

14.364x1000/200 = 71.82mg/kg

Experimental Doses Calculated as per the standard procedures are

S.No	Groups	Dose /kg, weight	Dose /200 gms. weight	Volume of administration
1	Vehicle Control	--	--	0.5 ml
2	Therapeutic Dose	71.82mg	14.364mg	0.5 ml
3	Average Dose	359.1mg	71.82mg	0.5 ml
4	High Dose	718.2mg	143.64mg	0.5 ml

4.5. MICROBIOLOGICAL ANALYSIS

4.5.1. ANTI - MICROBIAL ACTIVITIES BY WELL DIFFUSION METHOD

Aim:

The antimicrobial activity of *venthamaraiyathi chooranam* was adapted through Well diffusion method(Agar diffusion testing).

PRINCIPLE

The antimicrobials present in the plant extract are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters.

Components of Muller Hinton agar medium:

Beef extract - 2gm/lit

Acid Hydrolysate of Casein - 17.5 gm/lit

Starch - 1.5 gm/lit

Agar – 17gm/lit

Distilled water - 1000 ml

PH - 7.3± 0.1 at 25^0C

PROCEDURE (Murray *et al.,* 1995)

Petriplates containing 20ml Muller Hinton medium were seeded with 24hr culture of bacterial strains. Wells were cut and 20 µl of the plant extracts (aqueous) were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993). Streptomycin was used as a positive control. standard for an In-vitro antimicrobial activity of *venthamaraiyathi chooranam* was screened against bacteria strains such as *Klebsiella pneumonia, Streptococcus mutans, Staphylococcus aureus, Escherichia coli, Pseudomonas.aerugina, Enterococcus faecalis.*

Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken HR Manual of Clinical Microbiology, 6th Ed. ASM Press, Washington DC, 1995; 15-18.

5. RESULTS AND DISCUSSION

STANDARDISATION OF BOSANA KUDORI MATHIRAI

The test drug ***Bosana Kudori Mathirai*** had been subjected to various studies to establish the works of Siddhar`s to be true. Literary collections, physico-chemical and elemental analysis, pharmacological study, toxicological study and antimicrobial study are done to prove the activity of ***Bosana Kudori Mathirai*** as an antiulcer, antispasmodic and antidiarrhoeal activity.

Table – 2 Physico Chemical Standardisation.

SL. NO.	PARA METER	RESULTS
1.	Organo leptic characters	
	a. Color	Brown
	b. Odour	Pleasant odour
	c. Sense of touch	Hard
	d. Appearance	Solid
	e. Taste	Salt in taste
2.	Physico chemical standard	
	a. Loss of drying	11.1%
	b. PH	7.0
3.	Microbial Limit Testing	
	a. Total viable aerobic count	1.4×10^4 col/g
	b. Total entero bactriacea	Nil
4.	Total fungel count	
	Test for speuitic pathojen	2.2×10^2 col/g
	Salmondla SP	Nil
	Staphylococcus aurea	Nil
	E-coli	Nil
	Pseudomonas aeruginosa	Nil

Interpretation:

The physical parameters like colour, odour touch, appearance revealed that Bosana Kudori Mathirai is a Brown. Pleasant odour, salt in taste having the PH 7.0 slightly alkaline Ph. The drug act as antacid.

Determination of loss of dying normal:

The loss of drying test is designed to measure the amount of volatile matters in a sample when the sample is dried under specified conditions moisture is one of the major factors. Responsible for the deterioration of the drugs and formulations low moisture content is always desirable for higher stability of days.

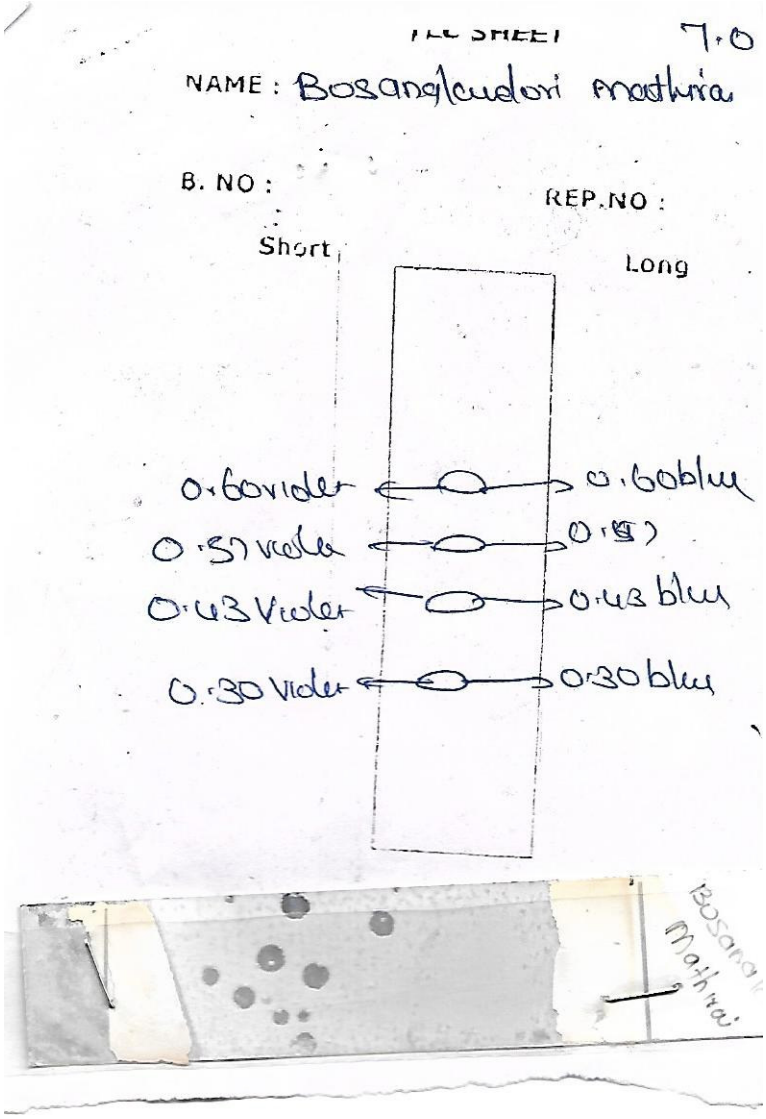
The percentage of loss on drying was within acceptable range to thus implying that the formulation can be stored for a long period and would not easily be attacked by microbes.

Microbial Limit Tests

The total bacterial count and the total fungal count of the drug were found to be within the WHO prescribed limits which indicate that the drug is free from microbial condamination. The other pathogens like Escherichia coli, Salmonella sps, Pseudomonoas aeruginosa and Staphylococcus aureus were found to be completely absent in the drugs.

Fig No.8 Thin Layer Chromotography :

Under UV 254nm and 366nm



Interpretation :

Under UV 254nm and 366nm test related to alkoloid it shows major spots shorts at Rf 0.6 (violet), 0.57 (violet), 0.43 (D.violet), 0.30 (Violet) and Long at 0.60 (Blue), 0.43 (Blue), 0.30 (Blue) 7 major compounds are found.

CHEMICAL ANALYSIS:

Table – 3 Results of Preliminary test for basic and acidic radicals

S.NO	EXPERIMENT	INFERENCE
1.	Test for Calcium	**Present**
2.	Test for Sulphate	Absent
3.	Test for Chloride	**Present**
4.	Test for Carbonate	Absent
5.	Test for Starch	**Present**
6.	Test for Ferric Iron	Absent
7.	Test for Ferrous Iron	**Present**
8.	Test for Phosphate	**Present.**
9.	Test for Albumin	Absent
10.	Test for Tannic Acid	Absent
11.	Test for Unsaturated Compounds	**Present**
12.	Test for Reducing Sugar	Absent
13.	Test for Amino Acid	**present**
14.	Test for Zinc	Absent

Result:

The biochemical analysis of Bosana Kudori Mathirai contains the following chemical constituents, Calcium, Chloride, Starch,ferrous iron, Phosphate, Unsaturated compounds, Amino acid.

1. Calcium :

Calcium is absorbed more readily from the upper part of the small intestine calcium ions are necessary for the maintenance and regulation of acid base balance and water balance in the body calcium ions are necessary for muscle contraction.

2. Chloride :

Chloride regulate the acid base balance of the body fluids, by maintaining the osmotic pressure of the body fluids. In severe diarvtnoea vomiting, large amount of

water and electrolytes are lost from body. The dehydraction has to be treated by administering water and these electrolytes.

3. Amino Acid :

- Amono acids are involved in protein synthesis.
- Amino acid nourishes smooth muscles of GIT
- The body can also use aminoacid for energy when lack of carbohydrates and fats.

4. Ferrous Iron :

Iron is easily soluble and readily absorbed from intestine and involved

5. Starch:

Starch functions much like dietary fibre. They provide nutrition for the beneficial bacteria in the colon, keeping them thriving and health. Dietary fibre in starch reduces effects of haemorrhoids, diverticulosis & controls blood pressure.

6. Phosphate:

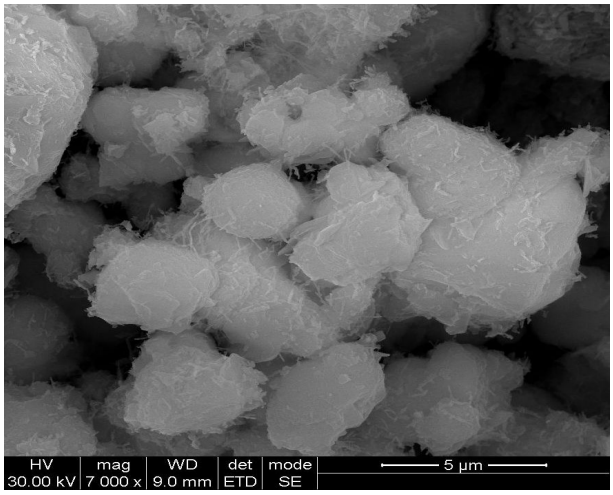
Parathyroid hormone(PTH), and calcitriol also regulate phosphate in the body. PTH helps lower blood phosphate levels. It does this by reducing the reabsorption of phosphates dissolved in urine in the kidneys, causing more excretion of phosphates. Calcitriol raises the level of phosphate in the blood by promoting its absorption by the intestine.

7. Unsaturated compound:

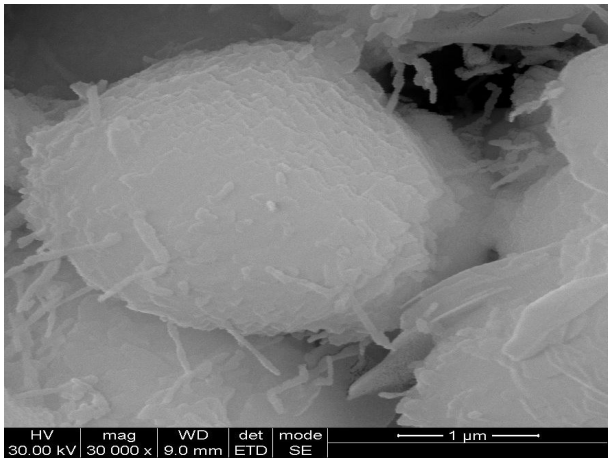
Monounsaturated and polyunsaturated fats can replace saturated fat in the diet, trans unsaturated fats should not. Replacing saturated fats with unsaturated fats helps to lower levels of total cholesterol and LDL cholesterol in the blood.

INSTRUMENTAL ANALYSIS:

Scanning Electron Microscope (SEM)



SEM -4000 Magnification



SEM -8000 Magnification

Figure - 9 Showing SEM Results of Trial Drug *(bosana kudori mathirai)*

Interpretation :

The morphology of the Bosana Kudori Mathirai samples can be determined by Environmental SEM (FEI Quanta). A representative portion of each sample must be sprinkled onto a double side carbon tape and mounted on aluminium stubs, in order to get a higher quality secondary electron image for SEM examination.The SEM photographs revealed that particles were spherical in shapes and sizes were in the range from 1µm to 5µm.Although the particle sizes of different batches showed similarity, it seems that these particles were aggregates of much smaller particles.

When dispersed in an aqueous medium, these preparations form a negatively charged hydrophobic particle suspension. This hydrophobicity gave these particles a tendency to aggregate together to form larger particles. ***bosana kudori mathirai*** exhibited larger sizes and agglomeration of the particles. Therefore, the comparatively larger size may be due to the agglomeration of the particles by repeated cycles of calcinations involved in preparation.SEM analysis of the ***bosana kudori mathirai*** shows most of the particles present in the sample are nano size, average particle size is **20-30 nm**. So, very minimal quantity of the medicine is enough to treat the disease. *Siddhars* were the great scientist in ancient times. They used nano technology for the preparation of *parpam*, *chenduram* to treat chronic diseases. Nano particles have beneficial properties that can be used to improve drug delivery system. Target cells take up these nano particles quickly .Because of their smaller size,lesser particles enhance the bio absorption and bio availability resulting efficacy of the drug will be increased. Larger particles could not enter in to the target cell because of their size, resulting in excretion from the body. If a drug is cleared too quickly from the body, this could force a patient to use high dose, poor bio distribution is a problem that can affect normal tissue through wide spread distribution but the particles from drug delivery systems lower the volume of distribution and reduce the effect on non target tissue. Adjuvant and detoxification (Purification) is also important factors for drug transport.

FOURIER TRANSFORM-INFRAREDSPECTROSCOPY(FTIR)

Fourier Transform Infra-Red Spectroscopy (FTIR) analysis results in absorption spectra that provide information about the functional group and molecular structure of a material IR relates with the sample and the bonds among atoms in the molecule stretch and bend, absorbing infrared energy and creating the infrared spectrum. It is of two kinds of bending and stretching.

FT-IR is a very useful tool in the recognition of the functional groups of bio molecules, thus aiding in their structural elucidation, so confirming the presence of active molecules responsible for the therapeutic activity of Siddha drugs. The results of Table no: 14 and Fig no: 9shows the presence of functional group and inorganic compounds of ***bosana kudori mathirai***

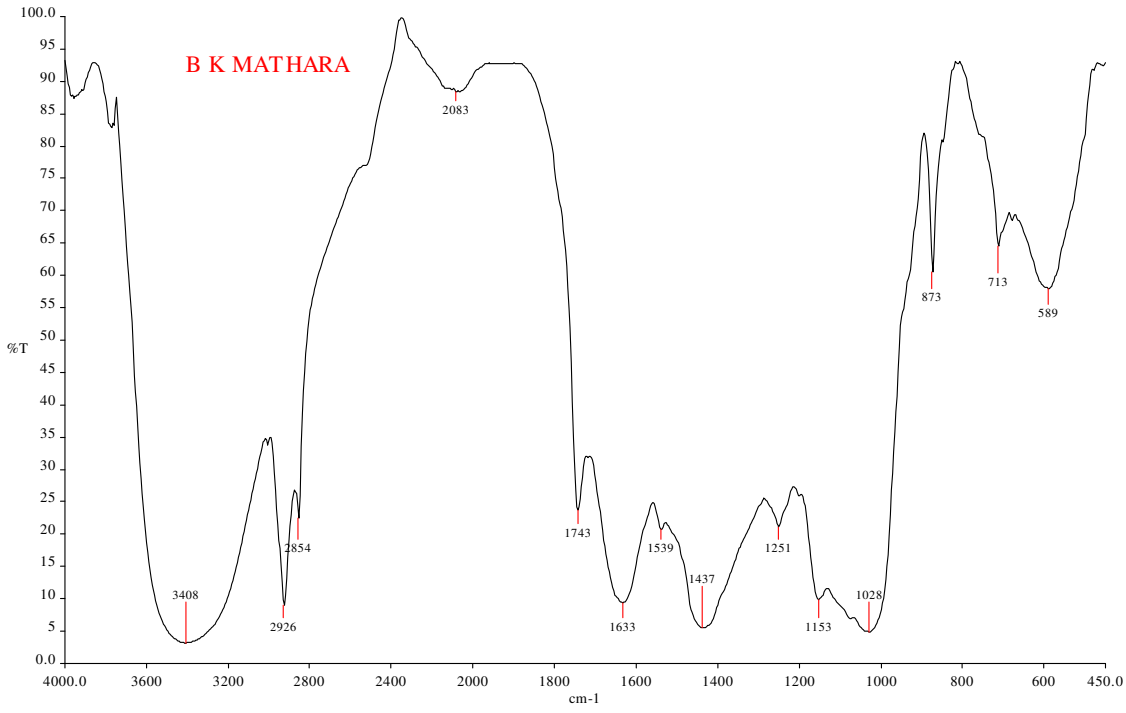


Figure -10 Showing FTIR Image of *bosana kudori mathirai*

Table – 4 Interpretation of FTIR Spectrum

CHARACTERISTIC ABSORPTION IN Wave number (cm-1)	Vibrational modes of *bosana kudori mathirai* in IR region	FUNCTIONAL GROUPS
3408	N–H Stretch	Primary secondary amines, amides
2926	O-H Stretch	Carboxylic acid
2854	O-H Stretch	Carboxylic acid
2083	C=O Stretch	Alkynes
1743	C=O Stretch	Carbonils
1633	N-H Bend	Primary amines
1539	N-O Stretch	Nitro Compounds
1437	C-C Stretch	Aromatics
1251	C-O Stretch	Alcohols, carboxylic acids
1153	C-H Wag	Alkyl halides
1028	C-N Stretch	Aliphatic amines
873	N-H Wag	Primary secondary amines
713	=C-H Bend	Alkynes
589	C-Br Stretch	Alkyl halides

Interpretation:

- In FTIR the wavenumbers between 4000^{cm-1} – 400^{cm-1} is known as functional group area. $<400^{cm-1}$ wavenumbers is known as fingerprint area.The Infrared bands for inorganic materials appear in the lower wavenumbers than those observed for organic materials. These warmth stable organic functional groups might have been derived from the lemon juice used in the drug preparation. There is a possibility for the formation of organo-metallic complex with these

functional groups. Stretching and bending modes shows the vibrational frequencies in the IR region.

- It confirms that ***bosana kudori mathirai*** constitutes Alkyl Halides, Alkyne, Carboxylic acids Aromatics, Alcohol, phenols, primary amines, Nitro compounds, Aliphatic amines as functional groups.

Table - 5 ICP-OES of BOSANA KUDORI MATHIRAI

BASANA KUDORI MATHIRAI

(wt:0.14715g)

Al 396.152	BDL
As 188.979	BDL
Ca 315.807	01.160 mg/L
Cd 228.802	BDL
Cu 327.393	BDL
Hg 253.652	BDL
K 766.491	13.114 mg/L
Mg 285.213	01.324 mg/L
Na 589.592	25.310 mg/L
Ni 231.604	BDL
Pb 220.353	BDL
P 213.617	124.341 mg/L
Zn 206.200	01.288 mg/L

BDL: Below Detectable Limit(Normal-1ppm)

1% = 10000ppm,

1ppm = 1/1000000 or 0.0001%

Toxic metals and the permissible limits

Heavy metals	WHO & FDA limits
Arsenic (As)	10ppm
Mercury (Hg)	1ppm
Lead (Pb)	10ppm
Cadmium (Cd)	0.3ppm

Interpretation:

The result indicate that the formulation is extremely safe as it contains heavy metals within specified limits.

- ICP-OES reveals high concentration of K in ***bosana kudori mathirai*** 13.821mg/L.
- Concentration of Na is 25.310 mg/L, phosphorous is 124.341 mg/L and Zn 01.288 mg/L
- It also has physiologically important minerals like Na, K,P.

The main ingredient of the drug is Calcium, but the final product shows below detection limit of the minerals. Below detection limit(BDL) of heavy metals As(arsenic), Hg(Mercury), Cd (Cadmium), Pb(Lead), Cu (Copper) and trace elements like Ni(Nikkal), Al(Aluminum) is seen.This reveals the safety of the drug.It is evident that the effectiveness of *Siddha* medicine has been proved by the modern scientific way.

TOXICITY STUDY RESULT

ACUTE TOXICITY OF *BOSANAKUDORI MATHIRAI*

RESULT Table –6 Physical and behavioral examinations.

Group no.	Dose(mg/kg	Observation sign	No. of animal affected.
Group-I	5mg/kg	Normal	0 of 3
Group- II	50mg/kg	Normal	0 of 3
Group-III	300mg/kg	Normal	0 of 3
Group-IV	1000mg/kg	Normal	0 of 3
Group-V	2000mg/kg	Normal	0 of 3

Table-7 Home cage activity

Functional and Behavioural observation	Observation	5mg/kg Group (G-I)	50mg/kg (G-II)	300mg/kg (G-III)	1000mg/kg (G-IV)	2000mg/kg (G-V)
		Female n=3	Female n=3	Female n=3	Female n=3	Female n=3
Body position	Normal	3	3	3	3	3
Respiration	Normal	3	3	3	3	3
Clonic involuntary Movement	Normal	3	3	3	3	3
Tonic involuntary Movement	Normal	3	3	3	3	3
Palpebral closure	Normal	3	3	3	3	3
Approach response	Normal	3	3	3	3	3
Touch response	Normal	3	3	3	3	3
Pinna reflex	Normal	3	3	3	3	3
Pinna reflex	Normal	3	3	3	3	3
Tail pinch response	Normal	3	3	3	3	3

Table-8 Hand held observation

Functional and Behavioral observation	Observation	Control	5 mg/ kg (G-I)	50 mg/kg (G-II)	300mg/kg (G-III)	1000mg/kg (G-IV)	2000mg/kg (G-V)
		Female n=3	Female n=3	Female n=3	Female n=3	Female n=3	Female n=3
Reactivity	Normal	3	3	3	3	3	3
Handling	Normal	3	3	3	3	3	3
Palpebral closure	Normal	3	3	3	3	3	3
Lacrimation	Normal	3	3	3	3	3	3
Salivation	Normal	3	3	3	3	3	3
Piloerection	Normal	3	3	3	3	3	3
Pupillary reflex	Normal	3	3	3	3	3	3
Abdominal tone	Normal	3	3	3	3	3	3
Limb tone	Normal	3	3	3	3	3	3

Table-9 Mortality

Group No	Dose no(mg/kg)	Mortality
Group-I	5(mg/kg)	0 of 3
Group-II	50(mg/kg)	0 of 3
Group-III	300(mg/kg)	0 of 3
Group-IV	1000(mg/kg)	0 of 3
Group-V	2000(mg/kg)	0 of 3

Result:

From acute toxicity study it was observed that the administration of ***Bosanakudori Mathirai*** at a dose of 2000mg/kg, to a rats. From acute toxicity study it was observed that the administration of ***Bosanakudori Mathirai*** at a dose of 2000 mg/kg to the rats do not produce drug-related toxicity and mortality. So No-Observed-Adverse-Effect- Level (NOAEL) of ***Bosanakudori Mathirai*** is 2000 mg/kg.

DISCUSSION

Bosanakudori Mathirai was administered single time at the dose of 5mg/kg, 50mg/kg , 300mg/kg, 1000mg/kg and 2000mg/kg to rats and observed for consecutive 14 days after administration. Doses were selected based on the pilot study and literature review. All animals were observed daily once for any abnormal clinical signs. Weekly body weight and food consumption were recorded. No mortality was observed during the entire period of the study. Data obtained in this study indicated no significance physical and behavioural signs of any toxicity due to administration of ***Bosanakudori Mathirai*** at the doses of 5mg/kg, 50mg/kg , 300mg/kg, 1000mg/kg and 2000mg/kg to rats.

At the 14th day, all animals were observed for functional and behavioral examination. In functional and behavioral examination, home cage activity, hand held activity were observed. Home cage activities like Body position, Respiration, Clonic involuntary movement, Tonic involuntary movement, Palpebral closure, Approach response, Touch response, Pinna reflex, Sound responses, Tail pinch response were observed. Handheld activities like Reactivity, Handling, Palpebral closure, Lacrimation, Salivation, Piloercetion, Papillary reflex, abdominaltone, Limb tone were observed. Functional and behavioral examination was normal in all treated groups.Food consumption of all treated animals was found normal as compared to normalgroup.

Body weight at weekly interval was measured to find out the effect of ***Bosanakudori Mathirai*** on the growth rate. Body weight change in drug treated animals was found normal.

SUMMARY & CONCLUSION:

Summary:

The present study was conducted to know single dose toxicity of ***Bosanakudori Mathirai*** on female Wister rats. The study was conducted using 15 female Wister rats. The female animals were selected for study of 8- 12 weeks old with weight range of within ± 20 % of mean body weight at the time of randomisation. The groups were numbered as group I, II, III, IV and V and dose with 5mg/kg, 50mg/kg, 300mg/kg, 1000mg/kg and 2000mg/kg of ***Bosanakudori Mathirai***. The drug was administered by oral route single time and observed for 14 days. Daily

the animals were observed for clinical signs and mortality. Body weight of all animals was recorded once in a week.

There were no physical and behavioral changes observed in albino mice of 5mg/kg, 50mg/kg , 300mg/kg, 1000mg/kg and 2000mg/kg to rats during 14 days.
Body weight of all animals did not reveal any significant change as compared to vehicle control group.

Food consumption of all group animals was normal.

Mortality was not observed in any treatment groups.

Interpretation:

The study shows that ***Bosanakudori Mathirai*** did not produce any toxic effect at dose of 5mg/kg, 50mg/kg , 300mg/kg, 1000mg/kg and 2000mg/kg to rats. So No-Observed-Adverse-Effect-Level (NOAEL) of ***Bosanakudori Mathirai*** is 2000 mg/kg.

Table – 10 EFFECT OF SUB-ACUTE DOSES (28 DAYS) OF *Bosanakudori Mathirai* ON BODY WEIGHT IN gms (PHYSICAL PARAMETER)

GROUP	CONTROL	P K M.L.D	P K M.M.D	P K M.H.D
1st day	117.8±1.315	112.5±2.63	111.5±3.969	110.3±2.175
7th day	123.75±1.65202	118.5±2.72336	118.5±1.55456	119.25±1.43614
14th day	130.75±0.853913	127±3.58236	132±1.41421	126.75±1.88746
21st day	138.75±1.93111	141.25±1.49304	135±1.29099	139.5±1.32288
28th day	143.8±1.25	141.3±1.493	142.8±0.8539	140.5±0.866

Values arae expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by dunnett's (n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

Table – 11 EFFECT OF SUB-ACUTE DOSES (28 DAYS) OF *Bosanakudori Mathirai* ON FOOD INTAKE IN gms

GROUP	CONTROL	P K M.L.D	P K M.M.D	P K M.H.D
1st day	39.5±2.217	32.75±2.81	33±1.958	30.75±1.797
7th day	44.5±3.617	44.75±3.038	38.75±2.016	38.75±1.493
14th day	44±2.858	45.5±2.533	54.75±10.25	45±2.415
21st day	49.25±2.097	53.5±2.754	56.25±4.09	57.25±5.121
28th day	50.75±2.175	46.25±2.839	47±3.391	54.5±2.102

Values arae expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by dunnett's (n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

Table – 12 EFFECT OF SUB-ACUTE DOSES (28 DAYS) OF *Bosanakudori Mathirai* ON WATER INTAKE IN ml

GROUP	CONTROL	P K M.L.D	P K M.M.D	P K M.H.D
1st day	40±6.88	41.5±7.984	34±4.062	83±7.234
7th day	30.75±4.95606	48±5.09902	40.75±4.23035	49±8.07259
14th day	88±6.364	65.25±6.713	87.5±4.173	81±7.382
21st day	89±4.60073	96.25±7.37535	80.25±2.32289	92±2.97209
28th day	95±3.851	88±2.16	94.5±3.403	85±7.969

Values arae expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by dunnett's (n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

Table – 13 EFFECT OF SUB-ACUTE DOSES (28 DAYS) OF *Bosanakudori Mathirai* ON ORGAN WEIGHT IN gms

GROUP		CONTROL	P K M.L.D	P K M.M.D	P K M.H.D
BRAIN		1.005±0.5249	1.002±0.5081	0.9933±0.5223	1.07±0.5356
HEART		0.398±0.1998	0.3847±0.1924	0.3807±0.1904	0.4167±0.2109
LIVER		4.403±2.204	4.368±2.187	4.279±2.141	3.137±1.578
LUNGS		0.8183±0.4539	0.9497±0.4782	1.295±0.1549	1.566±0.7866
TESTIS		1.529±0.7658	1.618±0.8092	2.067±1.035	1.545±0.7727
UTRES		0.4967±0.2487	0.512±0.258	0.4667±0.2421	0.4633±0.2317
KIDNEY	L	0.526±0.0394	0.6487±0.07753	0.527±0.09359	0.4957±0.08158
	R	0.5077±0.05045	0.5p03±0.08391	0.5953±0.0452	0.543±0.1544

Values are expressed as the mean ± S.D. Statistical significance (p) calculated by one way ANOVA followed by dunnett's. ns- not significant $^{**}P< 0.05$ calculated by comparing treated group with control group

Table – 14 EFFECT OF SUB-ACUTE DOSES (28 DAYS) OF *Bosanakudori Mathirai* ON HAEMATOLOGICAL PARAMETERS

GROUP	CONTROL	P K M.L.D	P K M.M.D	P K M.H.D
RBC (X10^6µL)	4.097±0.7541	5.83±0.7032	6.413±0.6639	7.333±0.8819
WBC(X10^3µL)	11.93±1.337	10.83±1.539	11.77±1.507	9.933±1.844
HB (g/dl)	9.23333±1.02686	11.7333±2.88694	10.5667±1.08372	10.3±2.76466
POLYMORPHS %	8.33333±0.666667	10.3333±1.45297	8.66667±2.02759	8±1
LMPHOCYTES%	91.67±2.333	88.67±0.6667	84±3.786	83±4.041
MONOCYTES %	7.333±0.8819	7±0.5774	5.667±0.3333	6.333±0.8819

Values are expressed as the mean ± S.D. Statistical significance (p) calculated by one way ANOVA followed by dunnett's. ns- not significant $^{**}P< 0.05$ calculated by comparing treated group with control group

Table – 15 EFFECT OF SUB-ACUTE DOSES (28 DAYS) OF *Bosanakudori Mathirai* ON BIOCHEMICAL PARAMETERS

GROUP	CONTROL	P K M.L.D	P K M.M.D	P K M.H.D
SGOT (U/L)	92.6±10.42	107.5±15.87	88.8±5.325	75.03±2.709
SGPT(U/L)	36.44±4.64251	93.6667±19.7425	53.5±4.05956	37.0333±3.96414
ALP (U/L)	129.667±2.18581	137±6.08276	122.333±2.96273	151.4±27.1048

Values are expressed as the mean ± S.D. Statistical significance (p) calculated by one way ANOVA followed by dunnett's. ns- not significant $^{**}P< 0.05$ calculated by comparing treated group with control group

GROUP	CONTROL	P K M.L.D	P K M.M.D	P K M.H.D
TOTAL BILURUBIN (g/ml)	1.323±0.04096	1.44±0.09165	1.777±0.1938	1.517±0.09528
UREA (mg/dl)	14.5333±1.64756	20.2333±1.21701	17.9333±4.11677	18.8±1.61658
URIC ACID (mg/dl)	3.2±0.305505	2.35±1.11168	2.16667±0.176383	2.43333±0.34801
CREATININE (mg/dl)	0.34±0.043589	0.406667±0.0233333	0.503333±0.0433333	0.603333±0.0995546

Values are expressed as the mean ± S.D. Statistical significance (p) calculated by one way ANOVA followed by dunnett's. ns- not significant $^{**}P< 0.05$ calculated by comparing treated group with control group

Table – 16 EFFECT OF SUB-ACUTE DOSES (28 DAYS) OF *Bosanakudori Mathirai* ON ELECTROLYTES

GROUP	CONTROL	P K M.L.D	P K M.M.D	P K M.H.D
SODIUM (mg/ml)	3.075±1.048	6.625±0.278	4.6±1.562	4.035±1.416
CALCIUM (mg/ml)	1.083±0.3642	4.3±0.8	2.655±0.8905	4.025±1.404
PHOSPHORUS (U/L)	0.3125±0.1075	1.12±0.7862	0.6367±0.0393	0.6067±0.07839

Values are expressed as the mean ± S.D. Statistical significance (p) calculated by one way ANOVA followed by dunnett's. ns- not significant $^{**}P< 0.05$ calculated by comparing treated group with control group

RESULTS:

CLINICAL SIGNS:

All animals in this study were free of toxic clinical signs throughout the dosing period of 28 days.

Mortality:

All animals in control and in all the treated dose groups survived throughout the dosing period of 28 days.

Body weight:

Results of body weight determination of animals Table-1 from control and different dose groups exhibited comparable body weight gain throughout the dosing period of 28 days.

Food consumption:

During dosing and the post-dosing recovery period, the quantity of food consumed by animals from different dose groups was found to be comparable with that by control animals.

Organ Weight:

Group Mean Relative Organ Weights (% of body weight) are recorded in Table No.4 Comparison of organ weights of treated animals with respective control animals on day 29 was found to be comparable similarly.

Hematological investigations:

The results of hematological investigations (Table 4) conducted on day 29 revealed following significant changes in the values of different parameters investigated when compared with those of respective controls; however, the increase or decrease in the values obtained was within normal biological and laboratory limits or the effect was not dose dependent.

Biochemical Investigations:

Results of Biochemical investigations conducted on days 29 and recorded in Table 2 revealed the following significant changes in the values of hepatic serum

enzymes studied. When compared with those of respective control. However, the increase or decrease in the values obtained was within normal biological and laboratory limits.

DISCUSSION:

1. All the animals from control and all the treated dose groups up to 900 mg/kg survived throughout the dosing period of 28 days.
2. No signs of toxicity were observed in animals from different dose groups during the dosing period of 28 days.
3. Animals from all the treated dose groups exhibited comparable body weight gain with that of controls throughout the dosing period of 28 days.
4. Food consumption of control and treated animals was found to be comparable throughout the dosing period of 28 days
5. Haematological analysis conducted at the end of the dosing period on day 29, revealed no abnormalities attributable to the treatment.
6. Biochemical analysis conducted at the end of the dosing period on day 29 no abnormalities attributable to the treatment.
7. Organ weight data of animals sacrificed at the end of the dosing period was found to be comparable with that of respective controls.

Interpretation :

In conclusion ***Bosanakudori Mathirai*** can be considered safe, as it did not cause either any lethality or adverse changes with general behavior of rats and also there were no observable detrimental effects (300 to 900 mg/kg body weight) over a period of 28 days. Our results have demonstrated that the ***Bosanakudori Mathirai*** is relatively safe when administered orally in rats.

PHARMACOLOGICAL STUDIES

ANTI ULCER ACTIVITY

Table – 17 EFFECT OF *Bosanakudori Mathirai* ON BODY WEIGHT ANALYSIS IN PYLORUS LIGATED GASTRIC ULCER IN RATS

Group	Control	Only pylorus	Pylorus+ Ranitidine 30 mg/kg	Pylorus + PMK.L.D	pylorus+ PMK.M.D	pylorus+ PMK.H.D
Body Weight	133.75±0.853913	133.5±1.70783	131.5±1.70783	140.5±1.70783	138.5±3.5	129±1.29099
Final Body Weight	123.75±0.853913	131.5±1.70783	135±1.29099	142±1.41421	139.75±1.65202	133±1.73205

Values are expressed as the mean ± S.D; Statistical significance (p)calculated by one way ANOVA followed by dunnett's $^{c}P< 0.001$, $^{b}P< 0.01$, $^{a}P < 0.05$ calculated by comparing treated group with CONTROL group

Table – 18 EFFECT OF *Bosanakudori Mathirai* ON BODY FREE AND TOTAL ACIDITY IN PYLORUS LIGATED GASTRIC ULCER IN RATS

Group	Control	Only pylorus	Pylorus+ Ranitidine 30 mg/kg	Pylorus + PMK.L.D	pylorus+ PMK.M.D	pylorus+ PMK.H.D
FREE ACIDITY	0±0	3.7±0.3317	4.5±0.1225	7.6±0.3367	6.2±0.07071	3.35±0.3227
TOTAL ACIDITY	0±0	15.23±0.5105	6.125±0.1887	8.9±0.4166	8.075±0.1109	9.285±0.6378

Values are expressed as the mean ± S.D; Statistical significance (p)calculated by one way ANOVA followed by dunnett's $^{c}P< 0.001$, $^{b}P< 0.01$, $^{a}P < 0.05$ calculated by comparing treated group with CONTROL group.

Table – 19 EFFECT OF *Bosanakudori Mathirai* ON GASTRIC PH AND GASTRIC VOLUME IN PYLORUS LIGATED GASTRIC ULCER IN RATS

Group	Control	Only pylorus	Pylorus+ Ranitidine 30 mg/kg	Pylorus + PMK.L.D	pylorus+ PMK.M.D	pylorus+ PMK.H.D
GASTRIC PH	0±0	2.85±0.13229***	5.825±0.1315***	3.525±0.36372***	2.95±0.25***	2.6±0.2582***
GASTRIC VOLUME	0±0	16.5±0.78528***	8.4±0.19579***	12.175±0.32243***	7.475±0.28687***	8.5325±0.133***

Values are expressed as the mean ± S.D; Statistical significance (p)calculated by one way ANOVA followed by dunnett's $^{c}P< 0.001$, $^{b}P< 0.01$, $^{a}P < 0.05$ calculated by comparing treated group with CONTROL group

Table – 20 EFFECT OF ON ULCER SCORE AND ULCER INDEX IN PYLORUS LIGATED GASTRIC ULCER IN RATS

Group	Ulcer Score	% Inhibition	Ulcer Index	% Inhibition
Control	0±0	---	0±0	---
Only Pylorus	8.425±0.2085	0.0	9.225±0.2175	0.0
Pylorus+ Ranitidine 30 Mg/Kg	4.565±0.2144	45.85 %	7.448±0.336	19.30 %
Pylorus + P K M. L.D	5.978±0.4089	30.98 %	3.925±0.2742	47.31 %
Pylorus+ P K M. M.D	4.748±0.3127	19.93 %	5.16±0.2434	31.63 %
Pylorus+ P K M. H.D	5.835±0.2277	22.15 %	5.275±0.06946	2.13 %

Values are expressed as the mean ± S.D; Statistical significance (p)calculated by one way ANOVA followed by dunnett's $^{c}P< 0.001$, $^{b}P< 0.01$, $^{a}P < 0.05$ calculated by comparing treated group with CONTROL group

Interpretation

Effect of BKM against pylorus ligation induced gastric ulcer shows that the standard drug ranitidine 10mg /100g has significantly reduced ulcer score (16.0%) Free HCL in units (10), Total HCL in units (22). In pylorus induced ulcer model the trial drug BKM have significantly reduced the ulcer score 11.3% free HCL (2.5) Total HCL (11) in units. The antiulcer activity is evident from its significant reduction in degree of ulceration, free HCL and total HCL respectively. Hence this study represents BKM has good antiulcer activity.

ANTI SPASMODIC ACTIVITY

Table – 21 Dose Response Relationship Observations of Acetylcholine

Sl.No	Concentration/dose	Acetylcholine
		Response (cm)
1	0.1 ml	3.4 cm
2	0.2 ml	3.6 cm
3	0.4 ml	4.2 cm
4	0.8 ml	5.1 cm
5	1.6 ml	5.4 cm

Table – 22 DoseResponse Relationship Observations of Atropine

Sl.No	Concentration/dose	atropine
		Response (cm)
1	0.1 ml	-
2	0.2 ml	-
3	0.4 ml	-
4	0.8 ml	-
5	1.6 ml	-

Table – 23 Dose Response Relationship Observations of Acetylcholine and *Bosanakudori Mathirai*

Sl.No	Concentration/dose	Acetylcholine + ***Bosanakudori Mathirai***
		Response (cm)
1	0.1 ml +0.1 ml	2.2 cm
2	0.2 ml +0.2 ml	2.8 cm
3	0.4 ml +0.4 ml	3.6 cm
4	0.8 ml +0.8 ml	4.1 cm
5	1.6 ml + 1.6 ml	4.6 cm

Table – 24 Comparative Dose Response of Ach and Ach followed by *Bosanakudori Mathirai*

Si No	Treatment	Dose(ml)	response	% of response
1	Acetylcholine	0.1 ml	3.4 cm	--
2		0.2 ml	3.6 cm	--
3		0.4 ml	4.2 cm	--
4		0.8 ml	5.1 cm	--
5		1.6 ml	5.4 cm	--
6	Acetylcholine + ***Bosanakudori Mathirai***	0.1 ml +0.1 ml	2.2 cm	35.29 %
7		0.2 ml +0.2 ml	2.8 cm	22.22 %
8		0.4 ml +0.4 ml	3.6 cm	14.28 %
9		0.8 ml +0.8 ml	4.1 cm	19.60 %
10		1.6 ml + 1.6 ml	4.6 cm	14.81 %

RESULTS:-

Effect of Acetylcholine on excised rat ileum reflected an increase in spasmodic activity (response) with an increase in dose

DISCUSSION

From the present study results it was observed that acetylcholine (Ach) alone causes contraction of excised rat ileum but when acetylcholine was given in presence of ***Bosanakudori Mathirai*** there was a marked decrease in contraction of ileum was observed.

This revealed that ***Bosanakudori Mathirai*** possess a high degree of spasmolytic (anti-spasmodic) activity by blocking cholinergic receptors.

INTERPRETATION

From all observations and results obtained for the present study it was concluded that ***Bosanakudori Mathirai***.,(Ghaneri) exhibits promising anti-spasmodic activity. Also when compared with a standard anti-spasmodic agent (atropine), it was found that ***Bosanakudori Mathirai*** has comparatively less potent spasmolytic activity than atropine.

ANTI DIARHOEAL ACTIVITY

Table – 25 EFFECT OF *BOSANAKUDORI MATHIRAI* ON CASTOR OIL - INDUCED SMALL INTESTINAL TRANSIT IN RATS

GROUP	Total Length of Intestine	Distance Travelled By Marker CHARCOAL	**% Intestinal Transit**
Control saline (2ml/kg i.p)	90.4±1.3267	0±0	-----
Castor oil (1ml p.o) +charcoal meal (1ml p.o)	78.4±3.9192	65±2.68328	28.09 %
Castor oil (1ml p.o) +Atropine (3mg/kg i.p)	85.4±2.2271	46.6±10.1025	40.56 %
Castor oil (1ml p.o) + PKM- L.D14.364mg	78.2±6.6363	55±1.84391	35.59 %
Castor oil (1ml p.o) + PPKM - M.D71.82mg	79.2±3.7336	49.8±7.90822	36.31 %
Castor oil (1ml p.o) + PKM - H.D 143.64 mg/kg	81.8±4.1761	41.6±7.11056	47.47 %

Values arae expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by dunnett's (n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

Interpretation

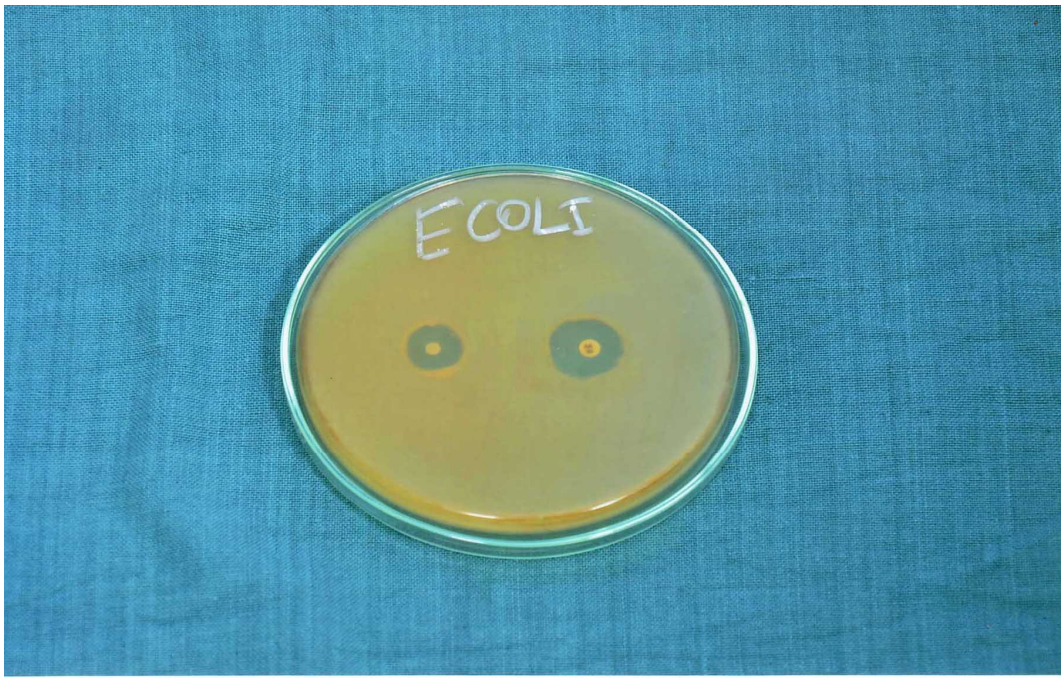
The test drug "*Bosana Kudori Mathirai"* has got significant action.

MICROBIOLOGICAL ANALYSIS

Table : 26 ANTI - MICROBIAL ACTIVITIES BY WELL DIFFUSION METHOD

s.no	Organism (Culture)	Susceptibility	Zone inhibition	
			Streptomycin zone size	Medicine size
1.	E.coli	Sensitive	20mm	18mm
2.	Klebsiella pnemoniae	Sensitive	24mm	11mm
3.	Staphylococcus aureus	Resistant	24mm	–
4.	Streptococcus mutant	Sensitive	18mm	22mm
5.	Enterococcus faecalis	Moderate resistant	24mm	25mm
6.	Pseudomonas aeruginosa	Resistant	14mm	–

ANTI-MICROBIAL ACTIVITY RESULT



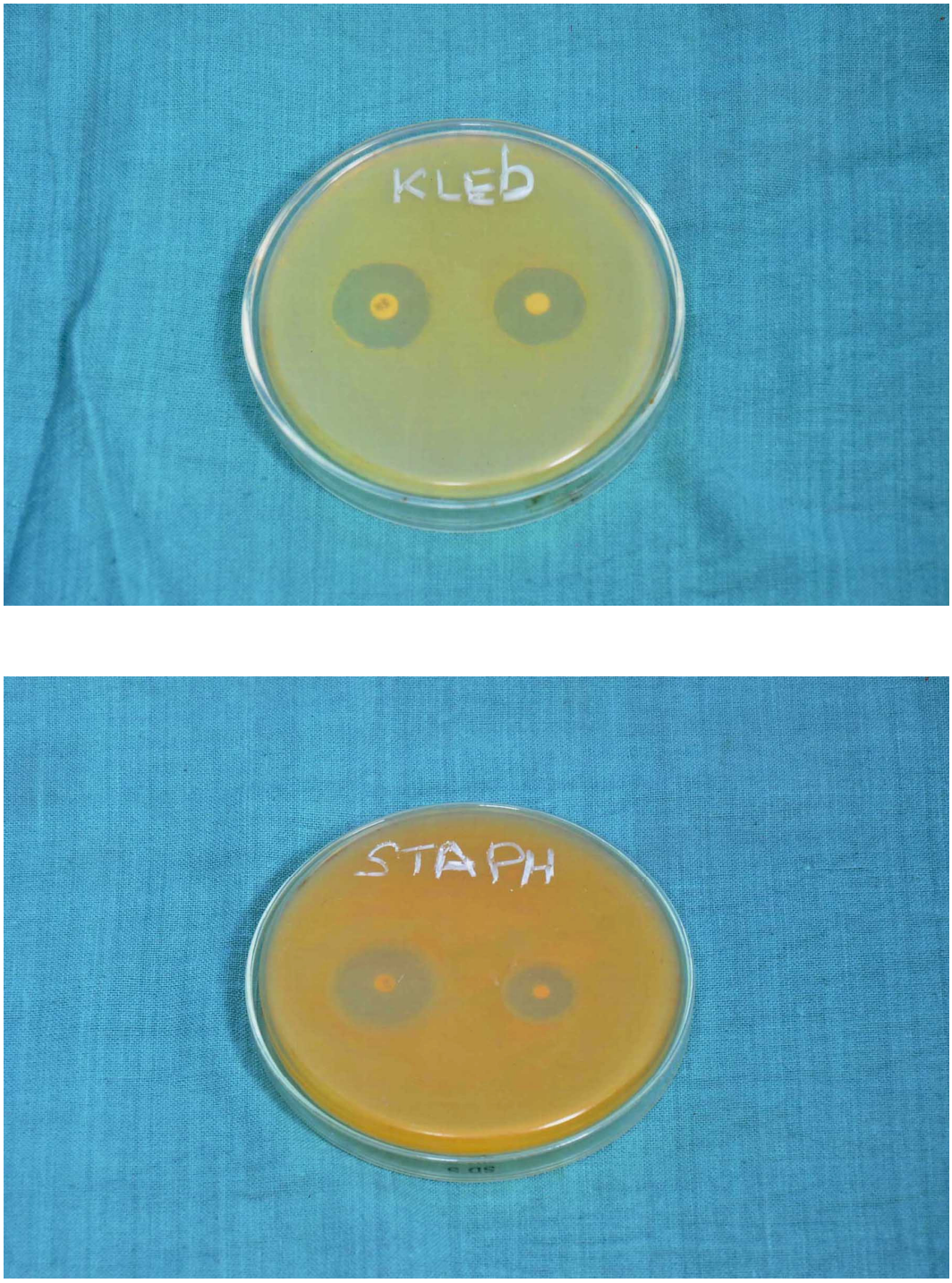


FIGURE - 11: ANTI-MICROBIAL ACTIVITY RESULT

INTERPRETATION:

It was observed that anti microbial studies of ***Bosana Kudori Mathirai*** showed that it is sensitive against *Escherichia coli,* Klebsiella pnemoniae and sensitive against Streptococcus mutant when compared to the standard drug (Streptomycin) which was evident from the zone inhibition.The herbel drug BKM showed inhibition of the growth of the micro organism at 100mg/ml concentration for the organism.Our result confirmed the traditional use of BKM has Anti microbial activity.

6. SUMMARY

Siddha system of medicine believes that herbo mineral formulation to be more effective than herbal formulation for chronic disease.

Herbomineral formulation are gaining popularity world wide due to its nano medicine form increased bioavailability minimal side effect, longer shelf life, period, and need less dosage.

From the ancient siddha literature (Anuboga vaidhiya bramha ragasiyam) in siddha literature book. Bosana Kudori Mathirai was selected for the preclinical study to establish the Anti-ulcer, Anti-spasmodic and Anti-diarrhoeal activity.

The test drug was prepared properly by the given procedure all the ingredients were identified and authenticated by the experts.

Collection of relevant literature evidences used in the preparation of Bosana Kudori Mathirai for the ingredients which claims supports area Anti-ulcer, Anti spasmodic and anti-diarrhoeal activity.

The preparation of trial drug was standardized by physicochemical and chemical analysis.

The physicochemical analysis the drug shows is a brown and pleasant odour and salt taste, Ph is 7.0

pH is alkaline, so it can be used as an antacid. So it increases the gastric secretion and it cures indigestion.

As per the siddha literature diseases are caused due to changes in mukkutram in peptic ulcer disease vatham is affected to treat vatha the drug must be in suitable taste that lower the effect of vatha.

So according to Kannaswamyiam

“வாத மேலிட்டால் மதுரம் புளியுப்பு”

Hence the drug BKM is have salt in taste, it normalise the vadha and it is given as a anti ulcer drug.

The biochemical analysis the drug shows, calcium, chloride, ferrous ion Tannic (acid) , Starch, Phosphate and unsaturated compounds, amino acid play an important role in acid base balance and determining the pH and acid level in the stomach.

FTIR-analysis related the presence amines, Amides, Alkanes, Aromatics, Aliphatic amines, Carboxylic acid, Carbonyles, Nitro compounds, Alchols, alkyne halides.

ICP- OES result shows the trial drug has below detection level of arsenic, mercury, cadmium, nickel and copper. It is evident that the drug BKM is devoid of heavy and toxic metals. This reveals the safety of the drug.

ICPOES analysis the showed calcium, potassium, magnesium, sodium, phosphorus and zinc.

Phosphate acts as a buffer and it is important for the maintenance of pH in blood and in the cells.

The acute and subacutae toxicological studies proved that the drug is non toxic and safe.

Acute and subacute toxicity results shows that the drug BKM does not produce any toxic signs in wister albino rats. It can be concluded that the dose level of 1 gm mentioned in Siddha literature is the safety dose for human consumption

Pharmacological analysis shows that the drug has got significant anti-ulcer, anti-spasmodic anti diarrhoeal activities.

It can be concluded that the drug may reduce the total Hcl, free Hcl revels and degree of ulceration and it has soud anti ulcer activities.

Anti microbial activity against E-coli, Streptococus mutant, klebsila, has indicated good microbial activity.

7. CONCLUSION

The trial drug of Bosana Kudori Mathirai was selected for the elaborate study of its efficacy on ***Gunmam*** from the literature review physicochemical, biochemical, pharmacological, microbiological, instrumental analysis, it has been good, anti ulcer, anti spasmodic and anti diarrhoeal activity and hence be effective for ***Gunmam.***

8. FUTURE SCOPE

The active principle which is responsible for the activity has to be find out through modern scientific analysis having made up of Tablet form. Bosana Kudori Mathirai is extra ordinary promise for the preventaion and treatment of Gunmam Thus the ancient wisdom siddhars will remains as one important source of future medicine and therapeutics.

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Department: OF GUNAPADAM

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For its ANTI ULCER, ANTI SPASMODIC, ANTI DIARRHOEAL ACTIVITIES

has been approved by the screening committee.

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3	Sirappu Maruthuvam	Dr.S.Kaniraja MD(S).,	
4	Kuzhanthai Maruthuvam	Dr.D.K.Soundararajan MD(S).,	
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F.No.GSMC/5676/P&D/Res/IEC/2014 Date: 16.07.2015

CERTIFICATE OF APPROVAL

Address of Ethical Committee	Government Siddha Medical College, Palayamkottai, Tirunelveli, Tamil Nadu, India. Pincode: 627002.
Principal Investigator	Dr.B.N.Rajeswari MD(s)- II year, Department of Gunapadam , Reg. No.: 321312007.
Guide	Dr. M.RAVICHANDRAN MD(s), ph.D H.O.D., Department of Gunapadam Govt. Siddha Medical College and Hospital, Palayamkottai, Tirunelveli District.
Dissertation Topic	PRE CLINICAL EVALUATION OF SIDDHA HERBAL FORMULATION **"BOSANA KUDORI MATHIRAI"** ANTI ULCER, ANTI SPASMODIC&ANTI DIARRHOEAL ACTIVITIES
Documents Filed	1) Protocol
Clinical / Non Clinical Trial Protocol	Non Clinical Trial Protocol
Informed Consent Document	NA
Any other Documents	NA
Date of IEC Approval & its Number	GSMC-II-IEC/2015-Br.-II/07/16.07.2015

We approve the trial to be conducted in its presented form.

The Institutional Ethical Committee expects to be informed about the process report to be submitted to the IEC atleast annually of the study, any changes in the protocol and submission of final report.

16/7/2015

Chairman
(Prof. Dr. M. Logamanian)

Member Secretary
(Prof. Dr. S. Soundararajan)

AUTHENTICATION CERTIFICATE

Date: 08.07.2015

Certified that the following mineral drug, submitted for identification by Dr.B.N.Rajeshwari, PG Department of Gunapadam, Govt.Siddha Medical College, palayamkottai are identified as

1. Inthuppu (sodium chloride)

Dr.A.KINGSLY,M.D(S).,
Lecturer,
Department of PG Gunapadam
Govt.Siddha medical college,
Palayamkottai.

CERTIFICATE OF BOTANICAL AUTHENTICITY

Certified the following plant drugs used in Siddha formulation ***"Bosana Kudori Mathirai"*** taken up for Post Graduation Dissertation Studies by **Dr. B. N. Rajeswari** PG Dept. of Gunapadam, are correctly identified and authenticated through Visual inspection / Organoleptic Characters / Experience, Education & Training Morphology / Microscopical and Taxonomical methods. The identified raw drugs is preserved to air tight container for further reference.

Drug : BOSANA KUDORI MATHIRAI

INGREDIENTS:

S.No.	Name	Botanical Name
1.	Chukku	Zingiber officinale (Dryginger)
2.	Perungayam	Ferula Asafoetida
3.	Inji	Zingiber officinale (Freshginger)
4.	Inthuppu	Sodium chloride impura
5.	Seeragam	Cuminum cyminum
6.	Milagu	Piper nigrum
7.	Elumichai	Citrus Limon

Station : Palayamkottai

Date : 08.07.15

08/07/15

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for participating as ~~Resource~~ ~~Person~~ / Delegate in the Sixteenth Workshop on

"Research Methodology & Biostatistics"

for AYUSH Post Graduates & Researchers

Organised by the Department of Siddha

The Tamil Nadu Dr. M.G.R. Medical University from 04.08.2014 to 08.08.2014

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23rd January 2016

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organised by S.A. Raja Pharmacy College and sponsored by Association of Pharmaceutical Teachers of India, Tamil Nadu Branch.

Dr.S.A. Jacob Raja	**Dr.N.Balakrishnan**	**Dr.R.Xavier Arulappa**	**Dr.K. L.Senthil Kumar**
Chief Patron	Convener & Principal	Organizing Secretary	President, APTI (TN Branch)

KMCH COLLEGE OF PHARMACY – COIMBATORE

IAEC - CERTIFICATE

This is to certificate that the project title PRECLINICAL STUDY OF HERBOMINERAL FORMULATION OF BOSANAKUDORI MATHIRAI FOR ITS ANTI ULCER, ANTISPASMODIC & ANTI DIARRHOEAL ACTIVITIES.

has been approved by the IAEC/ KMCRET / MD(S) /12 / 2016 - 2017.

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