PRE CLINICAL STUDY OF SIDDHA DRUG

MAVILINGU KASAYAM

FOR ITS

LITHOTRIPTIC, DIURETIC AND ANTI-SPASMODIC

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

THE GOVERNMENT SIDDHA MEDICAL COLLEGE

TIRUNELVELI-627002

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GOVT. SIDDHA MEDICAL COLLEGE,

PALAYAMKOTTAI-02

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"Pre clinical study of Siddha Drug Mavilingu Kasayam for it's Lithotriptic, Diuretic And Antispasmodic Activities"** is a bonafide and genuine research work carried out by me under the guidance of **Dr.A.Kingsly, M.D(s).,** Reader, Post Graduate Department of Gunapadam, Govt.Siddha Medical College, Palayamkottai,Tirunelveli-02uner and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

Date: **Signature of the Candidate**

Place: Palayamkottai **S.Mageshkannan**

GOVT. SIDDHA MEDICAL COLLEGE,

PALAYAMKOTTAI

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **"Pre clinical study of Siddha Drug Mavilingu Kasayam for it's Lithotriptic, Diuretic and Antispasmodic Activities"** is submitted to the Tamilnadu Dr.M.G.R. Medical University,Chennai-32, in partial fulfillment of the requirements for the award of degree of M.D (Siddha) is the bonafide and genuine research work done by **Dr.S.Mageshkannan** under my supervision and guidance and the dissertation has not formed the basis for the award of any Degree, Diploma, Associateship, Fellowship or other similar title.

Date: **Signature of the Guide**

Place: Palayamkottai. **Dr.A.KINGSLY. MD(S).**

Reader

GOVT. SIDDHA MEDICAL COLLEGE,

PALAYAMKOTTAI

BONAFIDE CERTIFICATE

This is to certify that the dissertation entitled **"Pre clinical study of Siddha Drug Mavilingu Kasayam for it's Lithotriptic, Diuretic and Antispasmodic Activities"** is a bonafide work done by **Dr.S.Magesh kannan**, a candidate of GSMC, Palayamkottai. In partial fulfillment of the University rules and regulations foraward of MD (Siddha)-Gunapadam under my guidance and supervision during the academic year of 2016

Name & Signature of the
Head of the Department

Name& Signature of the
principal

ACKNOWLEDGEMENT

I am extremely grateful to the lord almighty who empowered who empowered me with his blessings and grace to complete my dissertation work successfully.

First of all I thank the **'GOD ALMIGHTY** and **SIDDHARS**' for showering me with abundant blessing, strength and wisdom to achieve this task successfully.

I gratefully record my indebtedness to the respected **Vice Chancellor**, The Tamilnadu Dr.M.G.R.Medical University, Chennai and **Commissioner** of Indian Medicine and Homeopathy, Chennai.

I express my sincere thanks to our former Principal Prof. **Dr.S.Soundararajan, M.D. (S), B.L.,** Govt. Siddha Medical College, Palayamkottai for his kind permission to carry out my research work.

It's my unique pleasure to express my hearted thanks to my guide redear **Dr.A.Kingsly,M.D(S).,** Head of the department, P.G. Gunapadam Department, Govt siddha medical college, Palayamkottai for his excelled care, continuous support and optimisitic approach, which influenced me to accomplish this work successfully.I could never forger the help and priceless guidance throughout my life.

I express my sincere thanks to our former H.O.D **Dr.M.Ravichandran, M.D.(S), Ph.D** Department of P.G Gunapadam, Govt siddha medical college, Palayamkottai.

It gives me pride and pleasure to express my deep sense of gratitude to**, Dr.G.Essakkypandian,M.D(S).,** Lecturer, Government Siddha Medical college, Palayamkottai,for his constant support inspiring, invaluable guidance, and motivation that made me think and do this research work with confidence.

It is with immense pleasure that I place on record my deep sense of gratitude to Dr**.R.Antony Duraichi, M.D(S).,** Assistant Lecturer, Department of P.G Gunapadam, Government Siddha Medical College, Palayamkottai, for his untiring consultation, encouragement during my research work. I am indebted to him for all his valuable and generosity.

I have not been still finding a suitable word to express my ineffable sense of gratitude to **Dr.M.Kalaivanan M.Sc, Ph.D.,** Department of Pharmacology, Govt. Siddha Medical College, Palayamkottai, for his constant help and encouragement to complete the pharmacological work successfully.

I am grateful to **Mrs.N.Nagaprema, M.Sc., M. Phil.,** and Head of the Department of Bio-chemistry, Govt.Siddha Medical College, and Palayamkottai for her kind help and suggestions on biochemical aspects of this dissertation.

I am very much happy to thank **Mrs.S. sudha, M.Sc.,** Head of the Department of Herbal Botany and Herbal Pharmacognosy, Govt. Siddha Medical College, Palayamkottai for her kind help in botanical aspect of this study and valuable suggestions regarding drug identification.

I express my thanks to **Dr.R.Murugesan,M.Sc(Pharm), Ph.D.,** H.O.D, Department of pharmacology, Indian Institute of Technology (IIT),Chennai-36 for his valuable support in doing the Heavy metal analysis, scanning electron microscopic analysis of the trial drug.

I express my thanks to **Dr. N.Chidambaranathan,** H.O.D, Department of pharmacology, K.M.C.H College of pharmacy, coimbutore for the excellent help in pharmacology study.

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

I extended my gratitude to the animal Ethical Committee Members for their approval to do animal studies in pre clinical studies.

I am also my thankful to our Librarian **Mrs.Poongodi,** M.Lib.Sc, M.Phil, and staffs for their kind co-operation for my study.

I am also thankful to **Mrs.Suganthi**, DMLT, Pharmacist, and Post Graduate Department of Gunapadam for her kind co-operation to purification and preparation of the trail drug for my study and successful completion of dissertation.

I am also thankful to my college staffs for their kind co-operation for my study.

I should express my gratefulness to all my classmates and P.G Gunapadam students for landing their helping hands whenever needed during the course of study.

With immense pleasure I thank for the full support and co-operation given by my **Parents** and my sister S.Deepa, M.Sc., B.Ed., and my brother L.Magesh kumar M.pharm and my friends for the successful completion of this work.

Without the above, I might not be able to complete this dissertation as a successful one.

I owe everything to them. Besides this, several people have knowingly and unknowingly helped me in the successful completion of this project.

ABBREVIATIONS

ALT	Alanine amino transferase
MK	Mavilingu kasayam
MKC	Mavilingu kasaya chooranam
ANOVA	Analysis Of Variance
CCDs	Charge coupled devices
CPD	Calculi Producing Diet
FT-IR	Fourier Transform - Infra red Spectroscopy
SGOT	Serum Glutamate oxaloacetate transaminase
SGPT	Serum Glutamate pyruvate transaminase
IAEC	Institutional Animal Ethical Committee
ICP-OES	Inductively Coupled Plasma Optical Emission Spectroscopy
PCV	Packed cell volume
RBC	Red blood corpuscles
SEM	Scanning Electron Microscope
WBC	White blood corpuscles
CRB	Calcium Reserve Body
No.	Number
Mg	Milligram
Kg	Kilogram
LD_{50}	Lethal Dose $_{50}$
p.o	peros
ML	Milliliter

R&D	Research and Development
EDTA	Ethylene Diamine Tetra Acetic Acid
M	Male
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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GOVT. SIDDHA MEDICAL COLLEGE

PALAYAMKOTTAI

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CERTIFICATE OF APPROVAL

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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 **MAVILINGUKASAYAM** FOR ITS LITHNOTRIPTIC, DIURETIC, ANTI SPASMODIC 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/05/16.07.2015

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

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(Prof. Dr. M. Logamanian)

16/7/15

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This is to certificate that the project title PRECLINICAL STUDY OF SIDDHA DRUG MAVILINGOKASAYAM FOR ITS LITHNOTRIPITIC, DIURETIC, ANTI SPASMODIC ACTIVITIES.

has been approved by the IAEC/ KMCRET/MD(S)/14/2016-2017.

Name of the Chairman / Member Secretary IAEC:

Name of the CPCSEA Nominee

Signature with Date

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Chairman / Member Secretary of IAEC

CPCSEA Nominee
(V. VINAYAK KULKARNI)

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

Certificate of Botanical Authenticity

Certified the following plant drugs used in Siddha formulation **"Mavilingu kasayam"** taken up for Post Graduation Dissertation Studies by **Dr. S. Mageshkannan (Reg. No:321312005),** PG Dept. of Gunapadam, are correctly identified and authenticated through Visual inspection / Organoleptic Characters / Experience, Education & Training Morphology / Microscopical and Taxonomical methods.

Drug : MAVILINGU KASAYAM

INGREDIENTS:

S.No	Name	Botanical Name	Parts Used
1	Chirukanpeelai	Aerva lanata	Root
2	Nerunjil	Tribulus terrestris	Root
3	Mavilingam	Crataeva magna	Root
4	Peramutti	Pavonia odarata	Root

Station : Palayamkottai

Date : 08.07.15.

08/07/15

Authorized Signature

Dr. (Mrs.) S. Sulfin Nihar, M.D., (S
Reg. No: 515
Asst. Lecturer
Govt. Siddha Medical College
Palayamkottai - 627 002

The Tamil Nadu Dr.M.G.R. Medical University

#69, Anna salai, Guindy, Chennai-600 032.

This certificate is awarded to

Dr./~~Mr.~~/~~Ms.~~ S.MAGESH KANNAN

for participating as ~~Resource Person~~ / Delegate ✓ in the Fifteenth 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 23.06.2014 to 27.06.2014.

Dr. N. KABILAN M.D. (Siddha) Reader, Dept. of Siddha	**Dr. JHANSI CHARLES,** M.D. Registrar	**Prof. Dr. D. SHANTHARAM,** M.D., D.Diab., Vice-Chancellor

GOVERNMENT SIDDHA MEDICAL COLLEGE
PALAYAMKOTTAI

CME

Conducted by

POST GRADUATE DEPARTMENT OF POTHU MARUTHUVAM

Certificate

This is to certify that Dr. MAGESH KANNAN. S

of GUNAPADAM *department has participated in the*

Continue Medical Education Programme on ***Renal Diseases***

held at ***Government Siddha Medical College Palayamkottai*** *On 08 - 06 - 2016 Wednesday*

Prof. Dr. A. MANOHARAN M.D (S)
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(A UNIVERSITY U/S 3 OF THE U.G.C. ACT, 1956)

Centre For Advanced Research In Indian System Of Medicine (CARISM)

Certificate of Participation

This is to certify that Dr. **S.MAGESHKANNAN** of **Government Siddha Medical College, Palayamkottai** participated in Ministry of AYUSH supported training programme on **"Characterization Techniques in the Standardization of Ayurvedha & Siddha Herbo-Metallic Preparations"** held during 28 to 30 march 2016.

SHANMUGHA ARTS, SCIENCE, TECHNOLOGY & RESEARCH ACADEMY

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Convener
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Registrar
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1. INTRODUCTION

Siddha system is one of the oldest systems of medicine in India. Siddha system is based on truth and philosophy. This medicine system has the unique feature like removal the root cause of the disease and perfect remedy for body, mind and soul. The Siddha system has explained that five basic elements namely, 1. Prithivi 2. Appu 3. Theyu 4.Vayoo 5. Agayam

According to the Siddhars concept

அண்டத்தில் உள்ளதே பிண்டம்

பிண்டத்தில் உள்ளதே அண்டம்

-சட்டை முனி ஞானம்.

The Siddha system based on namely *vatham, pitham and kabam.* Siddha is the first system to emphasize on food habits. If human beings have any alteration in food habits, it will affect the vital elements of their body. According to Thiruvalluvar in his Thirukkural,

மாறுபாடில்லாத உண்டி மறுத்துண்ணி

ஊறு பாடில்லை உயிர்க்கு.

Yugi one of the ancient Siddhar had classified the diseases into 4448 types. He had classified urinary disorders into

1. Neerinai Arukkal Noigal
2. Neerinai Perukkal Noigal

Kalladaippu disease comes under Neerinai Arukkal Noigal.

In our Siddha system Urolithiasis may be compared to Kalladaippu. Kalladaippu is the most common diseases of present society due to modern life style and abnormal diet habits clinical features of Kalladaippu are lower abdominal pain, dysuria, oliguria, burning micturition, nausea, vomiting haematuria and fullness of abdomen.

The efficacy of invasive therapies such as extra corporal shock-wave lithotripsy and ureteroscopy has been proven by several studies. However these techniques are not risk free and they are problematic and quite expensive and complication.

In our Siddha system the number of drugs has been prepared from the medicinal plants which are source of drugs for Kalladaippu.

According to Agasthiyar, in his *vathiya kaviyam 1500* there was a preparation of *MAVILINGU KASAYAM* which is indicated for Kalladaippu and to evaluate its Lithotriptic, Diuretic, Anti-Spasmodic (smooth muscle relaxants) activities.

We must praise safe guard and follow the Siddha systems of medicine and also furnish it to take up with the up to date advancement.

2. AIM AND OBJECTIVES

AIM:

The main aim of this dissertation work is to do a scientific validation of **MAVILINGU KASAYAM** and its efficacy in treating Kalladaippu disease.

Urolithiasis constitutes one of the commonest diseases in our country and pain due to urolithiasis is known as worse than that of labor pain. In India, 5% of people are seeing affected by kidney stones and 8-10% people who have life time risk of passing the kidney stone. The recurrence rate for urinary calculi is very high, approximately 50% among the Indian population.

OBJECTIVES:

The main objective of the present study is to safety and efficacy of the mavilingu kasayam in the treatment of kalladaippu, the following methodology was adopted to evaluate the drug and its standardization studies.

- Collection of literature evidence regarding the trial medicine.
- Identification of the drugs in the mavilingu kasayam.
- Preparation of the trial medicine as per the text.
- Physic-chemical analysis of the test drug
- Evaluation of the toxicity of the test drug.
- Evaluation of pharmacological activity of the test drug.

3. REVIEW OF LITERATURE

3.1 AERVA LANATA

3.1.1GUNAPADAM ASPECT

சிறுபீளை – chirukanpeelai

Aerva lanata

OTHER NAME:

Chirukan peelai, karpaethi, pasanapaethi kanpeelai.

CHIRUPEELAI OTHER NAME:

சிறுபீளை பேர்தனையே செப்பக்கேளு
செயமான பாஷாணம் பேதியாகுஞ்
சிறுபீளை சங்கி சிலா பேதாசும்
பேதனாசைவோ பலபேதத் துன்மியாகும்
நறுபூளை நாகத்தினி சத்துருவே யாகும்
நலமாயுப்புச் சத்தை நாசமாக்கிக்
சறுபூளைத் திரிதோஷ யுரியுமாகுஞ்
செப்பியதோர் பேரெல்லாம் சிறுபீளைக்கே.

Bogar nigandu-1700

Pasana paethi, Sangisilapaethasum, Palapaetha thunmi, Nagaththin sathuru, Uppuchaththai nasamakki Thiridhosauri.

VERNACULAR NAME:

English	- Comman way side weed
Telugu	- Pindi-kanda
Sanskrit	- Pashanabhed
Malayalam	- Cherupila

PARTS USED:

Whole plant

ORGANOLEPTIC CHARACTERS:

Taste	- bitter
Potency	- heat
Biotransformation	- pungent

PROPERTIES:

Cuvai	- kaippu
Gunam	- ilaku kurmai
Virium	- veppam
Pirivu	- karppu

- The Siddha pharmacopeia of India part 1

ACTION:

Diuretic

Lithotriptic

- Gunapadam mooligai vagupu

Lithotriptic
Diuretic
Astringents
Bitter
Emollient
Vermifuge
Suppurative

- Indian medicinal plants vol – 2

Anthelmintic
Demulcent

-medicinal plants in India vol-1

Demulcent
Diuretic

- Materia medica of India and their therapeutics

Anthelminitic
Diuretic

-Glossary of Indian medicinal plants

Diuretic
Demulcent

-

- Wealth of India vol II-A and Glossary of Indian medicinal plants.

GENERAL CHARACTERISTICS :

பாண்டு பெரும்பாடு பகர் மூத்திரக்கிரிச்சம்
பூண்ட திரிதோடமிவை போகுங்காண்-தாண்டிப்
பரிய வேளைத் துரத்தும் பாரவயிக் கண்ணாய
சிறிய பீளைக்குச் சிதைத்து

நீரடைப்பு கல்லடைப்பு நீங்கா குடல் சூலை
பேரிட இரத்த கணம் போக்கும் காண்- வாரி
பூண்முலையே கேளாய் பொருந்தும் சிறுபீளை
யாமிது கற்பேதி அறி.

Kalladaippu (kidney stone), pandu (anemia), (menorrhogia), neererichal (burning micturition), muppini, neeradaippu, kudar soolai, kuruthi soodu.

சிறுபீளை குணத்தை கேட்பாய்
உறுசீதம் லகுவேஸ் நிக்தம் உயர் பிரமேகஞ் சூலை
செறி மூத்ரக்ருச்ர மூலம் திரிதோஷ பீலிகாரோகம்
பெரு குன்ம மிதயரோகம் பேர்திடும் வஸ்தி செய்யும்.

Piramagam
Soolai
Moothirakirucharam
Moolam
Thiridhosam
Kunmam
Iruthayarogam

இரு விதப் பீளை தம்மா விலங்கு நீர்க் கல்லடைப்பு
பெருகிய பாண்டு வீக்கம் பெரிய பைசாசதோஷம்
மருவிய வாதரத்தம் மண்டிரு மலையின் பிண்ணாக்கோ
டொருவிடா திடித்து காற்றினுறு நஸ்யம் விடத்தை தீர்க்கும்

Neeradippu
Kalladaippu
Pandu

Veekam
Paisasa dhosam
Vadha raktham
Sarpa vizham

-pathartha guna mangari

MEDICINAL USES:

- The flowers are used to cure the kidney stone and gonorrhea.
- The roots and flowers are used for cure the headache.
- The whole plant was used in lithiasis, cough, indigestion, sorethoroat, wounds, and diabetes.
- The plant was used to treat lithiasis boils, cephalgia, and cough.

- Indian medicinal plants vol-1

- Plant is used as antidote against poisoning of arsenic.

-Materia medica of Indian and their therapeutics

- Roots are credited with tonic properties and given to pregnant women.

-The wealth of India volume II A

- The plant decoction is used in catarrh of bladder.
- The root paste is applied on the forehead in headache.

-Hand book of medicinal plant

- The decoction of the plant can dissolve kidney stones in a few days, which is scientifically proven. It is also a good remedy for bladder stones. Aerva Lanata flowers are also used for this purpose.

- It is used as a treatment for gonorrhea.

- The roots of Aerva Lanata are used for the treatment of snake bite.

- The decoction of root and flowers gives relief from headache.

- The decoction of the root is also used as a tonic during pregnancy.

- Roots of Aerva Lanata are effective as demulcents.

- Juice obtained from the leaves of Aerva Lanata is used as a remedy for painful urination and kidney stones. Advised to be taken in small quantities.

- The decoction of the leaves of Aerva Lanata is used to treat sore throat by gargling and also used against guinea-worm.

- When infants are affected with malaria, decoction of leaves is used to bath. The smoke of the burning plant is also used for this purpose.

- Ash obtained by burning the leaves of Aerva Lanata, after removing impurities when rubbed on the lower back gives relief from back pain.

- Fresh leaves of Aerva Lanata are taken and crushed to apply on injuries or wounds, as it cures inflammation and reduces pain.

- Leaves of Aerva Lanata are powdered and used as a medicine for Diabetics. The powder can be stored and taken two times a day. It helps in controlling the sugar level in blood.

- ❖ Leaf-sap is used for eye problems.Infusion of aerva Lanata is used to treat Diarrhea, Inflammation and also can be used during Childbirth.

OTHER PREPARATION WHICH CONTAINS CHIRUPEELAI:

1. CHIRUPEELAI VER KUDINEER

Indication -kalladaippu

-Uyir kakkum Siddha maruthuvam

2. KALLADAIPPU KUDINEER

Dose : ulakkalau
Indication : kalladiappu

-Yugi muni vaithiya kaviyam

3. RAJA SINTHAMANI ENNAI

Dose : ¼-1/2 palam 2 times
Indication : Megavayu

-kannusamy parambarai vaithiyam

4. KALLADAIPPU MARUNTHI

Indication : Kalladaippu
Duration : 3 days

-Sarabaenthirar vaithiya muraiga
Pitharogachigichai

5. CHIRUPEELAI SAMOOLA KUDINEER

Indication : kalladaippu

-Gunapadam – mooligai

6. THIRACHATHI CHOORANAM

Dose : Thirikadi 2 times

Indication : neerkaduppu, neererichal, neerkattu

Duration : 20 days

- Kannusamy paramparai vaithiyam

7. KALLUDAI KUDORI MATHIRAI

Dose : ulunthu size 2 times a day

Indication : kalladaippu

- Siddha vaithiya thirattu.

3.1.2 BOTANICAL ASPECT:

Botanical Name - Aerva lanata

TAXONOMICAL CLASSIFICATION

Division	- Spermatophyta
Subdivision	- Angiosperma
Class	- Dicotyledonae
Subclass	- Monochlamydeae
Series	- Curvembryeae
Family	- Amarantaceae
Genus	- Aerva
Species	- Lanata

HABITAT:

Tropical, cosmopolitan in distribution found all over tropical India as a common weed in fields and waste places. It also found Africa, Indomalaysia, and Arabia.

HABIT:

Herbs (or) under shrubs
Erect (or) prostrate
Tomentose herb
Branches woolly.

LEAVES:

Simple, alternate, elliptic, obovate, pubescent above white woolly beneath-upto 1" Long.

INFLORECENCE:

Spike, axillary less than 0.5 long.

FLOWERS:

Bisexual or polygamous, minute in small dense greenish white heads or spikes. Bracteate and 2 small bracteoles present.

PERIANTH:

5 petals, calycine in nature, membranous, the lobes are equal.

ANDROECIUM:

5 stamens, filaments subulate, connate with interposed linear staminodes in a hypogenous cup, anthers 2 celled.

GYNOEXIUM:

2 carpels, syncarpous. The ovary unilocular, superior, ovule, pendulous from a long basal funicle style – simple, stigma – 2.

FRUIT

A membranous utricle

SEED:

Inverse testa coriaceous, embryo annular surrounds the floury albumen, cotyledons linear, radical superior.

- Flora of presidency of madras vol II JS Gamble.

DESCRIPTION:

MACROSCOPIC

Root - Tap-root, laterally branched, cylindrical, up to 0.8 cm. in thickness and about 25 cm. long pieces, externally light brown and rough but cut surface white and smooth; fracture fibrous and hard.

Stem - Nearly cylindrical, branching alternate, external surface shows slight ridges and furrows, hairy and light brown in colour; cut surface white; fracture granular.

Leaf - Simple, opposite, alternate, shortly petiolate, lamina 2.0 to 2.5 cm. long and 1.0 to 1.6 cm.broad, elliptic-orbicular or ovate, acute, reticulate veined, margin entire, densely pubescent on both surfaces.

Flower - Minute cluster as axillary spike; greenish-white; perianth 5, bracteolate; actinomorphic,bisexual; stamen 5, opposite to perianth, anthers 2 lobed; stigma bifid, superior ovary, unilocular with campylotropous ovule.49

Fruit - A greenish, roundish, compressed membranous, utricle or circumscissile capsule with a coriaceous upper part or lid and containing a single seed.

Seed - Seed minute, 0.5 to 0.7 cm. in dia., black, polished, lenticular; taste pungent.

MICROSCOPIC

Root - Shows 5 to 7 layers of cork cells, upper 2 or 3 layers filled with brownish content;

secondary cortex a wide zone consisting of circular to oval, elongated, thin walled parenchymatous cells, most of the cells containing rosette crystals of calcium oxalate; endodermis not distinct;pericycle present in the form of interrupted ring of pericyclic fibres; anomalous secondary growth present;

secondary xylem and phloem tissues in form of 3 or 4 alternating rings; medullary bundles present; phloem consisting of sieve tubes, companion cells and phloem parenchyma; xylem consists of vessels, tracheids, fibres and xylem parenchyma; vessels circular to oval having simple pits; pith cells circular in shape containing rosette crystals of calcium oxalate.

Stem - Shows slightly wavy outline, corresponding to ridges and furrows; epidermis single layered covered with thick cuticle; trichomes multicellular, end cells pointed or vesicular, warty and thick walled; cortex 6 or 7 layers with 3 or 4 layers below ridges being collenchymatous and 3 or 4 layers below furrows chlorenchymatous; rest of the cells oval to elongated, elliptical, thin walled and parenchymatous, with a few cells containing rosette crystals of calcium oxalate; endodermis single layered; pericycle present in the form of a ring, single or groups of 2 to 4 fibres; anomalous secondary growth present; vascular bundles arranged in 2 or 3 rings showing included phloem alternating with parenchymatous tissue; phloem consists of sieve tubes, companion cells and phloem parenchyma; xylem composed of vessels, tracheids, wood fibres and xylem parenchyma;vessels round to oval having simple pits; pith wide consisting of circular to polygonal havingintercellular spaces, rosette crystals of calcium oxalate present in this region.

Leaf

Petiole - Shows single layered epidermis covered with cuticle; trichomes multicellular present on both surfaces; cortex consisting of 2 or 3 layers, upper collenchymatous and lower parenchymatous; vascular bundle collateral and 3 in number; rosette crystals of calcium oxalate present in cortical cells.

Midrib - Epidermis, cuticle and trichomes, similar to those in petiole; cortex 5 to 7 layers, upper 3 collenchymatous and lower 3 or 4 circular, thin walled and parenchymatous; vascular bundles 3 in number, 2 accessory and one middle; xylem towards the upper and phloem towards lower epidermis; rosette crystals of calcium oxalate present in cortical region.

Lamina - Epidermis, cuticle and trichomes similar as in petiole and midrib; palisade 1 or 2 layers;spongy parenchyma 3 to 5 layers composed of thin walled parenchymatous cells with intercellular spaces, a few rosette crystals of calcium oxalate present in spongy parenchyma; anomocytic stomata present on both surfaces; palisade ratio 2 or 3; stomatal index on upper surface 12 to 15 and on lower surface 16 to 18; vein -islet number 4 or 5 per square mm

- The Siddha parmacopia ofIndia part I

CHEMICAL CONSTITUENTS:

Plants contains four flavonoid,glycosides (I, II, III and IV) tannin which comprises β sitosteryl palmitate, α amyrin, β sitosterol and its glucoside betulin, alkaloid canthin -6-one and β –carboline.

- Medicinal plants of india volume -2 tamilnadu

CONSTITUENTS:

β sito sterol, β sito sterol palmitate, campesterol, stigma sterol, stigma sterol acetate, daucosterol, ergosterol, a-amyrln, β amyrln, lupeol,betulin,olean-12-en-2foic acid, 16-dioxymethyl ester, hentriacontane, chrysin, 3 glo flavones, 4 methoxy, 3 glo 3 methoxy flavones, kaemferol- 3 galactoside, kaemferol -3-rhammno galacto side aeruine,methylaeruine, aervoside, aervolomine, free sugars-fructose, galactose, rhamnose and 3G sucrose.

- The Siddha pharmacopeia of india part I

USES:

- Aerva Lanata is antimicrobial. It helps is fighting against pathogens. It protects both the skin and the body from pathogens.

- Aerva Lanata is diuretic which helps in promoting the production of urine, effective in urethral problems, lithiasis and gonorrhea.

- Aerva Lanata acts as a demulcent which helps in getting relief from pain and inflammation.

- Aerva Lanata is anthelmintic which helps in destroying parasitic worms and reducing sores and injuries on the skin.

- Aerva Lanata acts as an astringent thereby helping in reducing bleeding in piles. It is also used as a treatment for diarrhea and hemorrhages.

- Aerva Lanata stem acts as an antioxidant which helps in balancing the free radicals.

- Aerva Lanata us lithontriptic and antilithic which gives the plant the power to destroy stones in kidneys and bladder.

- Aerva Lanata behaves as a medicinal tonic.

- Aerva Lanata is also known for increasing memory power.

- Aerva Lanata is also used to treat headache, abdomen and digestion problems. It is effective for neck and back pain, fever, urine problems and also regulates body metabolism.

- Aerva Lanata is considered to be effective for hepatitis and inflammation of the liver. It is a good remedy for strangury, i.e condition of painful or frequent urination.

- Aerva Lanata is also used for treatment of many health problems like Anemia, Alzheimer, Cholesterol, lung problems, bone problems and also blood circulation.

LATERAL RESEARCH OF AERVALANATA LINN:

The aqueous extract of aervalanata was evaluated for antiurolithiatic activity in male albino wistarrats. The aqueous extracts of aevalanata were safe orally and exhibited. No gross behavioral changes in the rats in hyper calculi animals, the oxalate, and calcium and phosphate excretion grossly increased.

International journal of pharmaceutical science review and research.
www.globlare search online.net
int.J.pharm.sci rev.res.17 (2) 2012

3.2TRIBULUS TERRESTRIS

3.2.1 GUNAPADAM ASPECT

நெருஞ்சில் – Nerunjil

Tribulus terrestris

OTHER NAME:

Thirikandam, Thirikandagam, Thirithandam, Nerunjiputhum, Asuvasatiram, Suvathattam, Gokandam, Kamarasi, Suvathukandam, Kittiram, Gondam, Sutham.

VERNA CULARNAME:

Assamese	– Gokshura, Gukhurkata.
Bengali	– Gokkshura, Gokhuri.
Gujarati	– Be tha gokharu mithogokharu.
Kashmiri	– Michirkana, Pakhada.
Marathi	– Gokharu, Sarate.
Oriya	– Gukhura, Gokhyura.
Panjabi	– Bhakhra, Gokhru.

-The Siddha Pharmacopocia of India Part 1

VERBACULAR NAME:

English	– Small Caltrops Land Caltrops Puncture Vine.
Telugu	– Palleru.
Sanskrit	- Gokshura, Svadamstra, Trikanta.
Hindi	– Gokhru.
Malayalam	– Nerunji.

Arab	– Khara-Khusk.
Bengali	– Gokhuri.
Kannada	– Negil-mullu, Neggilu, Sannana ggilu.
English	– Small Caltrops.
Gwalior Hindi	– chota gokhru.

PART USED:

Whole plant.

fruit and root

ORGANOLEPTIC CHARACTERS:

Taste	– bitter, sweet
Potency	– cool
Biotransformation	– sweet.

PROPERTIES:

Cuvai	– Inippu, Tuvarppu.
Gunam	– Noymai, Tinmai.
Virium	– Tadpam.
Pirivu	– Inippu.

- The Siddha Pharmacopoeia of India Part 1

THERAPATIC ACTION:

PLANT AND DRIED SPINY FRUIT:

Cooling, Diuretic, Tonic, Demulcent, Aphrodislac, Astringent.

STEM:

Astringent.

INFLORESCENCE:

The flowers are 4- 10mm wide,with 5 lemon yellow paetals, 5 sepals, and 10 stamens. In Sothern California it blooms from April through October.Where it is highly invasive in waste places and disturbed sites.

FRUIT:

A week after each flower blooms it is followed by a fruit that easily falls apart into 5 nutletes (or) burns.

The nutlets are hard and bear 2-4 sharp spines 10 mm Long and 4-6 mm broad point-to-point. These nutlets strikingly resemble goats (or) bulls' heads.

GENERAL CHARACTERS:

நல்ல நெருஞ்சிலது நாளுங்கிரிச்சரத்தை
வல்ல சுரமனலை மாற்றுங்காண்-மெல்லியலே
மாநிலத்தின் கல்லடைப்பும் வாங்காத நீர்கட்டும்
கூனுறுமெய் வாதமும் போக்கும்.

மேகவெட்டை நீர்சுறுக்கு வீறுதிரி தோடம்புண்
வேகாதசுர தாகவெப்பம் விட்டொழியும்-போகந்
தரஞ்சின மதலை மொழித் தையலே நல்ல
நெருஞ்சிலதனை நினை.

- Gunapadam mooligai,

Sottuneer, Suravaethumbal, Kalladaippu, Neeradaippu, Mudavayu, Vellai, Siruneererichal, Mukutram, Neervetkai, Vepum.

MEDICINAL USES:

It is believed to be helpful in treating some cardiovascular conditions including angina, high blood pressure, high cholesterol, anemia and poor circulation. Digestive disorders that may benefit from the use of puncture vine include constipation and flatulence. It is used in Chinese medicine as a liver tonic, and for food poisoning and overeating. It is has many uses in the Ayurvedic tradition, including pacifying vata, as a diuretic and to treat disorders of the genitourinary system. In other medical traditions, this herb is also used as a diuretic to treat hypertension and for angina pectoris. It is believed by some to help prevent both oxalate and struvite kidney stones. It is believed to have antiseptic and diuretic properties that can reduce the incidence of urinary tract infections. It is also used as a treatment internally and externally for immune disorders, such as allergic skin conditions, eczema and psoriasis.

OTHER PREPARATION WHICH CONTAINS NERUNJIL

1. THIRIKANDATHI CHOORANAM

Adjuvent (anupanam) – Honey (or) Ghoat milk.

Duration – 7 days.

Indication – kidney stones.

-Anubaa vithiya Deva ragasiyam.

2. NERUNJIL LEGIYAM.

Dose – 24 gm.

Indication – Siruneer erichal, Neerdaippu, Sathaiadaippu.

-Gunapadam mooligai vaguppu.

3. NEER – MALA KATTUKU. NERUNJIL CHOORANAM.

Dose – 5-10 kundri or 6.50 mg-1.3 gm.

Daily - 2-3 times

Adjuvent (Anupanam) – Elaneer (or) Venneer.

Indication - Piramegam, Neeradaippu, Saihaiadaippu, Kalladaippu Kaikalerivu, Elumburiki, Kunmanoi.

-Agathiyar Pallu 200

4. KUMKUMA POO LEGIYAM.

Dose – 5 gm. 2 times.

Indication –Meganeer, Vellai, Vettai Neeradaippu, Neerkaduppu, Sathaiadaippu, Kalladaippu, Madhumoothiram, Nerilizhu, Rakthapiramagam, Perumpadu, Kustam, kiranthi, Soolai, Kirukirupu, Kaikal eruvu, Pithavettai, Megam, Asthisuram, Araiyappu, maladu.

-Pirana Rakshamirtha Sindhu. Part 2.

6. NILAPANAI CHOORANAM.

Dose – Thirikadi.

Anupanam – Milk.

Indication – Neerkaduppu, Vellai, Thathupalaveenam.

7. NEERMULI KUDINEER

Dose - 1/2 alakku 2 times a day

Indication - neerkattu, sobai

8. MANDOORATHI AADI KUDINEER

Dose - ¼ alakku
2-3 times a day

Indication - pandu, sobai, kamalai magotharam

- Siddha vaithiya thirattu.

3.2.2 BOTANICAL ASPECT

Botanical Name – Tribulus terrestris.

TAXONOMICAL CLASSIFICATION

Kingdom	– Plantae(Plants).
Subkingdom	– Tracheoblosta. (Vascular plants).
Division	– Spermatophyta (Seed plants).
Subdivision	– Angiospermae.
Class	– Dicotyledonae.
Subclass	– Polypetalae.
Series	– Disciflorae.
Order	– Geraniales.
Family	– Zygophyllaceae.
Genus	– Tribulus.
Species	– Terrestris.

- Benthem and Hooker.

FAMILY

Genera 22, Species 160, Xero and halophytes of tropical and subtropical regions.

GENUS

A Genus of ascending (or) Prostrate herbs, Commonly known as Caltrops, distributed in the tropics and warm temperature regions of the world.

HABITAT

It is native to warm temperature and tropical regions of the old world in Southern Europe, Southern Asia, throughout Africa and Australia. It can thrive even in desert climates and poor soil.

LEAVES AND STEM

Stems branch from the crown and are densely hairy Leaves are opposite and pinnately compound. Densely hairy Leaflets are opposite and up to 1/8 inch (0.0032m) Long.

MORPHOLOGICAL CHARACTERS

A Variable prostrate annual upto 90 cm in Length, commonly found throughout India. The herb is a common weed, springing up everywhere soon after the first showers. The herb flowers and fruits almost throughout the year. Leaves paripinnate, Leaflets 5-8 pairs, subequal, oblong to linear oblong, flowers leaf opposed, solitary, pale yellow to yellow, flowering starts with in 20-35 days fruits globose spinous (or) tuberculate consisting if 5-12 woody cocci each with 2 pairs of hard, sharp, divaricate spines, one pair longer than the other, seeds. Several in each coccus with transverse partitions between them. Fruits matures in 14 days after the formation of seeds, roots slender, cylindrical, fibrous 10-15cm long, light brown and faintly aromatic.

-The wealth of India. Vol X

ACTION AND USES

Plant and dried spiny fruit are esteemed as cooling demulcent, diuretic, tonic and aphrodisiac. The diuretic properties if the plant, no doubt are due to the Large quantities of the nitrates present as well as the essential oil which occurs in the

seeds.The fruit and root are sweetish, cooling, tonic, fattening, aphrodisiac, alternative, Improve appetite, useful in strangury, urinary discharges, vesicular calculi, pruritis ani, alternative burning sensation, reduce inflammation, remove tridosha, cough, asthma, pain, cure skin, and heart disease, piles, leprosy.

The seeds are cooling, fattening, diuretic, aphrodisiac, removes inflammation urinary troubles, stones in bladder.

-Nadkarni's Indian Materia Medica.Vol-I

DESCRIPTION

A.MACRPSCOPIC

Drug consists of root, 7 to 8 cm Long and 0.3 to 7 cm in diameter, slender, cylindrical, fibrous, frequently branched bearing a number of small rootlets, tough, woody and yellow to light brown in colour, surface becomes rough due to presence of small nodules, fracture fibrous, odour, aromatic, taste sweetish and astrigyent.

MICROSCOPIC

Transverse section of primary root show a Layer of epidermis followed by 4 (or)5 Layers of thin walled parenchymatous cortex, endodermis distinct, pericycle enclosing diarch stele, in mature root, cork 4 to 6 Layered cork cambium single Layered followed by 6-14 Layers of thin walled paranchymatus cells with groups of fibres, distributed throughout, some secondary cortex cells show secondary wall formation and reticulate thickening, secondary phloem divided into two zones, outer zone characterized by presence of neumrous phlowm fibers with a few sieve tubes slightly collapsed, inner zone frequently paranchymatous devoid of fibres, often showing sieve tubes and cambium cells, phloem rays distinct a few cells get converted into fibres in outer region cambium 3 to 5 Layered wood composed of vessels, tracheids parenchyma and fibres and traversed by medulary rays, vessels scattered arranged in singles or doubles towards inner side in groups of three (or) four on outer side having bordered pits, tracheids Long narrows with simple pits xylem

parenchyma rectangular (or) slightly enlongated with simple pits and reticulate thickening a few xylem fibres, medullary rays, hetrogenous 1 to 4 cells wide starch grains and rosette crystals of calcium oxalate present in secondary cortex phloem and medullary rays cells, a few prismatic crystals also present in xylem ray cells.

-The Siddha Pharmacopia of India Part I

CONSTITUENTS

Diogenin, Hecogenin, Gitogenin, Tiyogenin, Neotigogenin, Stiymasterol, βsitoserol and Campesterol.

-The Siddha Pharmacopia of India Part I

The fruit are regared as cooling, diuretic, tonic and aphrodistic, and are used in painful micturition, calculus affection, urinary disorders and impotence.

In Southern India, the fruit is highly valved as a diuretic, In many cases where this has been tried the result was quite perceptible in the increase of urinary secretion.

-Indian Medicinal plants Part 1

The fruits are credited with diuretic and tonic properties and are used for the treatment calculus affections and painful micturition.

-The Wealth of India. Vol-X.

ACTION AND USES IN AYURVEDHA AND SIDDHA

Mathura rasam, Seetha veeryam, Mootralam, Vrishyam, Dipanam, Balakaram, Pustikaram, Prameham arsas, Kricharam, Swasakasam, hridrogam.

ACTION AND USES IN UNANI:

Murakabul khuva, Diuretic, Aphrodisiac, Increase semen, removes stone, caused nugl in madda, in colic due to heat.

USES

Plant and dried spiny fruit are used in decoction (or) infusion in cases of spermatorrhoea, phosphaturia, diseases of the genitourinary system such as dysuria, gonorrhoea, gleet, chronic cystistis, calculus affections, urinary disorder incontinence of urine, gout, and impotence also in uterine disorder, after parturition and to ensure fecundity.

- Indian Meteria Medica Vol I.

The decoction (or) powder of seeds is given in calculi and sexual weakness.

-Medicinal plants and folklores

- Anti-ageing, Anti-bacterial, Anti-fungal,Anti-inflammatory
- Anti-urolithiatic its juice/powder helps in urinary/kidney stone preventing and stone dissolving activities.
- Anti-malarial
- It improves libido function to normal rate and erectile function.
- It helps to increase the number and motility of spermatozoa.
- It helps in alleviating some symptoms associated with male menopause
- It increase energy levels and provide health hormone function
- It enhances muscle movement during exercise
- It improves muscle growth and body strength
- It increases in strength of contraction of the heart muscle

- It helps to reduce sodium and fluid retention
- It is very useful and effective in treating Urinary Tract Infection and Urination problems
- It helps to control high blood pressure
- It helps to reduce in cholesterol
- It reduces body heat
- It relieves eye problems

  Sterols in Tribulus help to protect prostrate from swelling and protect prostrate from cancer

LATERAL RESEARCH OF TRIBULUS TERRESTRIS LINN

- Evaluation of the aphrodisiac activity of Tribulus terrestris linn in sexually sluggish male albino rats. J.Pharmacol Pharmacother 2012;3:43-7.

A dose dependent Improvement in sexual behaviour was observed with the Lyophihized aqueous extract of the dried fruits of tribulus terrestris (LAET) treatment.The enhancement of sexual behaviour was more prominent on chronic administration of LAET. Chronic administration of LAET produce significant increase in serum testosterone levels with no significant effect on the sperm count finding of the present study validate the traditional use of T.Terrestris as a sexual enhancer in management of sexual dysfunction in males.

Activity of certain fraction of Tribulus terrestris fruits against experimentally induced Urolithiasis in rats on subsequent fractionation of ethanol extract maximum activity was localised in the 10% aqueous methanol fraction.It provide significant protection against deposition of calculogenic material around the glass bead Implantation in albino rat

3.3 CRATAEVA MAGNA

3.3.1GUNAPADAM ASPECT

மாவிலங்கம் – MAVILINGAM

Crataeva Magna

OTHER NAME:

Mavilingu, kumaragam, varani

MAVILINGAM OTHER NAME:

மாவிலங்கத்தின் பேர் தனையே வகுக்க கேளு
வானோ வகு திச்சாகங்
கோவிலங்கைக் குமாரகன சுவேத புஷ்பி
குணமான சாருகா விகமு மாகும்
யேவிலங்கை யெழும்பிலதிச் சுடருமாகும்
யேத்தமாம் வன்னிதான் தீபனியாகும்
பாவிலங்கை மாந்தத்தை யகத்தியாகும்
அழகான மாவிலங்கை யாண்மையாமே

Kumaragan, Suveatha Buspi, Sarugavigam, Elumbilathi Sudar, Vanni, Deepani, Manththai Agachi

- Bogar nigandu 1200

VERNACULAR NAME:

English - Three leaved caper
Telugu - Urumatti

Malayalam	- Nirvala
Hindi	- Banan
Sanskrit	- Pashungandha, Asmarighna
Hindi	- Tapla Bilas
Kanada	- Narumbela

- GUNAPADAM MOOLIGAI

VERNACULAR NAME:

English	-Three leaved caper holy garlicpear.
Tamil	- Mavilinga Maram, Maralingam, Marilinga
Kumarakam, Varani.	
Telugu	- Uskia, Urumatti.
Malayalam	- Nirvala, Vilva-Patrani
Hindi	- Tapla, Bilas, Barna
Bengali	- Tikoshak, Textasak, Barun.
Kanada	- Nirvala, Narumbeli, Bilapatri
Punjabi	- Barna
Marathi	- Ramala, Kumlakoomla, Varuna,Haravarna
Burma	- Ka-Dat
Gujarti	- Vaivarna vayavarna
Cing	- Bilapatra
Sanskrit	- Koomvaurna, ashmarighana, tapla, belpatra

PART USED:

Leaves, bark, root

ORGANOLEPTIC CHARACTERS:

Taste	- Bitter
Potency	- Heat
Biotransformation	- Pungent

PROPERTIES:

Cuvai - Kaippu
Gunam - Ilaku varadci
Virium - Veppam
Pirivu - Karppu

- The Siddha pharmacopeia of India part I

ACTION:

Stomachic
Febrifuge
Tonic
Rubefacient
Laxative
Lithotriptic

-Gunapam mooligaivagupu

Lithotriptic
Laxative
Stomachic
Increase the biliary
Secretion.

- Indian materia medica

Anti periodic
Tonic
Demulcent

- The wealth of India.

Lithotriptic
Liver stimulant
Laxative
Promotes appetite

- Medicinal plants of india vol-II

Lithotriptic

Diuretic

Astringent

Carminative

Anthelminitic

Stomachic

Laxative

Stimulant

Expectorant

Demulcent

Anti periodic &Tonic

-Indian medicinal plants vol-II (orient Longman)

GENERAL CHARACTERISTICS:

சுரங் கடியின் தோடந் தொலையாத வாதம்

உரம் பெறு விடங் களொழியும் – சுரமுங்

கருமா வடுவயிலுங் கண்டஞ்சுங் கண்ணாய்

ஒரு மாவிலங்குக் குரை.

It cures fever, unknown bite, snake bite, thiratha vali noi

- GUNAPADAM MOOLIGAI VAGPPU

THERAPEUTIC USES:

Leaves decoction cures, fever, indigestion. The decoction of bark is internally administrated to cure diseases like renal calculi, dysuria, helminthiasis, inflammations and abscesses. The decoction exhibits actions like carminative, laxative, thermogenic, diuretic, lithontriptic, expectorant and demulcent. The leaf and stem bark have been evaluated for their antioxidant activity and inhibition of key enzymes relevant to

hyperglycemia. The leaves of Crataeva magna are used in traditional medicine for the treatment of various kinds of wounds.

OTHER PREPARATION WHICH CONTAINS MAVILNGU:

VARUNATHI KIRUTHAM

Indication : Kidney stones, dysuria

-Agnivasarin agasamgithai part 3

MAVILINGU KUDINEER

Indication : kidney stone.

-Valam tharum thavarangal part 5

MAVILINGU CHOORANAM

Dose : 1 gm per day

Indication : kidney stone, fever, ulcer

- Kosaii anubava vaithiya bramma ragasiyam

AYAKANTHATHI KULIGAI

Dosage : 1 kuligai (pakkalavu)

Indication : jaundice

- Agathiyar 2000 part 3

VATHA SURA KUDINEER

Indication - Vatha suram, nadukku vatham.

- Siddha vaithiya thirattu.

3.3.2 BOTANICAL ASPECT

Botanical Name – Crataeva magna

TAXONOMICAL CLASSIFICATION

KINGDOM : Plant kingdom
CLASS : Dicotyledons
SUBCLASS : Polypetalae
SERIES : Thalamiflorae
ORDER : Parietales
FAMILY : Capparaceae
GENUS : Crataeva
SPECIES : Magna

HABITAT:

Tropical, cosmopolitan, commonly found in Tamilnadu, central India, Bengal, Assam, Malabar and tropical Africa

HABIT:

A moderate sized deciduous tree with much branches and grey bark.

ROOT:

Long tap-root.

STEM:

Wood, branched with long horizontal wrinkles, shiny, yellowish white, moderately hard, smooth and close grained.

LEAVES:

Long petals, trifoliate, exstipulate, leaflets ovate (or) ovate lanceolate pale beneath upper surface is thin and dark green.

INFLORESCENCE:

Corymb.

FLOWERS:

White in colour, pedicellate, ebracteate, bisexual, actinomorphic, hypogynous, greenish yellow (or) white with red stamens.

CALYX:

Four sepals, deciduous.

COROLLA:

Four petals, polypetalous, ovate, long clawed petals.

ANDROECIUM:

Stamens numerous, free, adnate to the base of GYNOPHORE.

GYNOECIUM:

Bicarpellary, syncarpous, sessile stigma, ovary superior, 1-celled, raised on long gynophores, placentation parietal many ovules.

FRUIT:

Berry, many seeded.

SEED:

Woody, non-endospermic.

PHYTO CHEMISTRY:

Bark contains - Saponin tannin.

- The wealth of India

Root contains - Triterpene alcohol.

THERPEUTIC USES:

- Bark of the crataeva tree taken with buttermilk cures stone in the bladder, megam veneral affections.

  -Tamil-English dictionary of medicine volume V

- Bark of the crataeva given in calculi and other urinary tract infections.

- Mavilingam bark, leaf and root used in urinary calculi ulcer, skin eruptions, vatha diseases, utricaria, snake bite, fever.

- Varuna bark used in urinary calculi headache, carcinoma, cardiac diseases, gout, disorders of blood, rheumatic arthritis, worm infestations, gastritis, abdominal disorders.

  - Medicinal plants of India volume II

- Bark is used in urinary disorders and calculous affections, dropsy combained with tribulus terrestris.

- The compound powder is given in calculus, ascites, and chronic enlargements of glands as liver, spleen and in affections of the bladder and uterus.

- Fresh leaves and roots are mixed with coconut juice and ghee is given in rheumatism.

- Paste of the leaves is applied to soles of the feet to rheumatism.

- Paste of the leaves is applied to soles of the feet to relieve swelling and burning sensation.

  - Flora of British India part I..

- Crataeva used as lithotriptic action in renal calculi. Bark is especially useful in urinary complaints such as kidney and bladder stones

  - Materia medica of india and their therapeutics

- Compound decoction contains its root bark and leaves and small calotropis bark, ginger, carbonate of potash, honey and water is very useful in urinary tract infections and calculi.

- 2 ounces of bark decoction three times daily as a good antiperiodic and tonic.

- Bark is also used in snake bite

-.Indian material medica

DESCRIPTION

MACROSCOPIC

Thickness of bark varies, usually 1 to 1.5 cm. according to the age and portion of the plant
from where the bark is removed; outer surface, greyish to greyish-brown with ash-grey patches; at places, surface rough due to a number of lenticels, shallow fissures and a few vertical or
longitudinal ridges; inner surface smooth and creamy white in colour; fracture tough and short;
odour indistinct; taste slightly bitter

MICROSCOPIC:

Transverse section of mature stem bark shows an outer cork composed of thin walled rectangular and tangentially elongated cells, phellogen single layered with thin walled tangentially elongated cells followed by a wide secondary cortex, consisting of thin walled, polygonal to tangentially elongated cells with a number of starch grains, starch grains mostly simple, occasionally compound with 2 or 3 components also present large number of stone cells in groups of two or more found scattered in secondary cortex single stone cells not very common stone cells vary in size and shape being circular to rectangular or elongated with pits and striation on their walls, stone cells distributed somewhat in concentric bands in phloem comparatively a wide zone, consisting of sieve tubes, companion cells, parenchyma and groups of stone cells, alternating with medullary rays sieve elements found compressed forming certatenchyma in outer phloem region, where as in inner region of phloem, intact, medullary rays mostly multiseriate composed of thin-walled, radially elongated cells, tangentially elongated towards outer periphery, a number of starch grains similar to secondary cortex also present in phloem and ray cells, few rhomboidal crystals of calcium oxalate also found in the region.

- The Siddha pharmacopia of India part I.

CONSTITUENTS:

Cadabacaine, cadabacine diacetate (-) catechin, (-) epicatechin-5-glucoside, (-) epiafzelechin, isothicyanate glusoside glucocapparin, taraxasterol, lupeol, 3-epilupeol, lupeolacetate, diogenim, friedelin, betulinic acid, ceryl alcohol and spinasterol acetate.

LATERAL RESEARCH OF CRATAEVA MAGNA:

Free radical scavenging activity, wound healing activity and estimation of phenolic flavonoid and proanthocyanide contents of the plane crateva magna.

- Asian journal of pharmaceutical and clinical research vol 5 supp 3 2012.

Effect of crataeva magna in experimental urolithiasis oral administration of crataeva magna bark decoction on calcium oxalate lithiasis has been studied in rats the increased deposition of stone forming constituents in kidney of calculogenic rats was lowered with decoction administration. The increased urinary excretion along with lowered magnesium excretion found in stone forming rats was partially reversed by decoction treatment.

Journal of ethno pharmacology vol-28 Issue march 1990

3.4 PAVONIA ODARATA

3.4.1 GUNAPADAM ASPECT

பேராமுட்டி – PERAMUTTI

Pavoniaodarata

OTHER NAME:

Tamil – Peraamutti, Kastoori venda

English – Fragrant Sticky Mallow

Ayurvedic – Vaalaka, Baalaka, Baala, Barhishtha, Hrivera, Ambu, Jala, Nira, Paya, Toya, Udichya, Vaari, Muurdhaja, Sugandhbaalaa(also equated with Valeriana Jatamansi). In the South, Celus vettiveroides is equated with Baalaka.

Other Name:

பேராமுட்டி பேர்தனையே புகலக்கேளு
பேரான மகாபலா வருஷபுஷ்பி
தாராமுட்டித் தருவாத்தி யாஷகாசு
மிறுத்தா வாச தேவிக்க பிதாபி தாஷ்பி
பேராமுட்டி யிறுது பிலா யிருவயோ வல்லி
யெளிதான அஸ்த சுயிலுத்து நோனா சனி
யாரமுட்டி யங்கத்தின் தாபம் போக்கி
யாண்மையாம் பேராமுட்டி நாமமாமே.

-Bogar Nigandu -1200

Mahapala, Varusapuspi, Tharuvathi, yasakasu, Miruthavancha devi, Kapitha, Pithasta, Iruthupala, Iruvayovalli, Asthakayurthunonasani, Angaththin thabampokki.

VERRNACULAR NAME:

Hindi - bala,sugandha-bala, sugandhabala

Kannada - bala raakshasi, bala rakkasi, bala-rakkasi-gida, balaraakshi gida, balarakkasi, balarakkasi gida, balarakkasigida, balarakshasi, kareebaalada beru, madivaala, mudivaala, mudivala, peramuti beru

Malayalam - iruveli, kuruntotti

Marathi - kaalaavaala, kala-vala, kalavala, randodaki, sugandhabala, sughandabala

Sanskrit - ambu, ambunamaka, bala, balaka, barhishtha, harivera, hribera, hrivela, hrivera, kachamoda, keshanama, keshanamaka, keshya, kuntala, kuntaloshira, lalanapriya, toya, udichya, vajra, vala, valaka, valakah, valakam, varapinga, vari, varida,varinamaka

Tamil - anantai, anantavariti, anantavariti, anantavariticceti, antai, antaiyitan, arttavacceti, arttavam, ataiyalavan, avibattam, avipatam, avipattam, cakapita, carapantini, centotti, cukantapala, cunkattintakampokki, curiyamantiram, curiyarkkam, cutcayekavalli, cuvacakam, cuvaccalam, cuvacitam,cuvacitamutti, cuveccam, erunti, irutupala, karapattini, kayotalankam, kontulankam, kuruvicci, kuruvinci, makapala3, makapalam, makapela, malaittarikam, miruturoki, nattukkantam, pala, palututainetti, paramutti, paramutty, paratakam, pataippariyan, pattakam, pera muttiver, peraamutti,peramootie vayr, peramutiver, peramutti, peramutti, perunkuruntotti, pitaputpi, pitaputpi, rutupala, suvesagam, taramutti, tavarattiyali, totikam,totikamutticceti, totipela, totippari, totippariccceti, tuvaratini, vantirecceti, vantiyam, vantiyam, varantiyam, vataiyavalan, vattiracuram, vilivilanki, yokavalli

Telugu - chirubenda, chitlebunda, chitti benda, chittibenda, cittibenda, erra-kuti, errakooti, errakuti, ettakuti, ,muthupalaagamu, muttaavapulagam, muttavapulagamu, mutthava pulagam chettu, muttupalagamu, pudubodapu, theegabenda, thigebenda, tiga benda, tigebenda

Tibetan - ba-la-ka

PART USED:

Whole plant

.

ORGANOLEPTIC CHARACTERS:

Taste - bitter
Potency - hot
Biotransformation -sweet

ACTION:

Cooling
Carminative
Diuretic
Diaphoratic

PLANT:

Anti- inflammatory
Spasmolytic

USES:

Rheumatic affections

ROOT:

Stomachic

Astringent

Demulcent

USES:

Dysentery

Haemorrhages

Ulcers

Bleeding disorders

GENERAL CHARACTERS:

வாதசுரம் தாகம் மதலை கணமாந்தம்

சீதசுரம் பித்தாமனச் செப்பணங்கும் – ஓதுநம்பாற்

சேராமுட்டி கேகுஞ் செய்ய மடமயிலே

பேராமுட்டி உரைத்து பேசு.

-Gunapadam mooligai

Valisuram, Neervaetkai, Mantham, Kanam, Iyya suram, Thekkutram.

MEDICINAL USES:

2 teaspoons of fresh juice of the leaves of Pavonia Odarata along with black pepper for treating dysentery of babies.

These leaves also have emollient properties and the fresh juice extracted from the leaves of Sugandha Bala herb was used for soothing and softening the skin.

OTHER PREPARATION WHICH CONTAINS PERAMUTTI VER:

1. PERAMUTTI KUDINEER

Dose -1/4 padi
Indication - kalladaippu
Neerkaduppu
Neereriu

-Molligaimarmam .

2. ATTAMOOLA KUDINER

Indication -fever

-Ealiya vaithiya muraigal.

3. SAGAMATHI GIRUTHAM

Dose - 1-1/2 theakkarandi
2 times
Indication - all type of rheumatic disease

4. VALI VEPPU KUDINEER

Indication - Vatha Suram, Nadukkuvatham

5. MAHAVILVA KARPA LEGIYUM

Dose - Pakkalau (2 times a day) 45 days
Indication - Visa Pandu, Sakthikunmam, Rakthapitham, Maradaippu, Kirani, Kulaiervu, Ruthravaiyu, Asthisuram, Mayakkam, Kirukirupu, Soolai.

3.4.2 BOTANICAL ASPECT

Botanical Name - Pavoniaodarata

TAXONOMYICAL CLASSIFICATION

Kingdom	- Plant Kingdom
Class	- Dicotyledons
Subclass	- Polypetalae
Series	- Thalami florae
Order	- Malvales
Family	- Malvaceae
Genus	- Pavonia
Speries	- Odorata

HABIT:

Sub shrub up to 1m

FLOWER

In axillary, solitary, pink (or) white, flowering throughout the year.

FRUIT

A globose schizocarp, pubescent, seed 1 per mericarp, reniform, fruiting throughout the year.

LEAF

Alternate, simple (or) palmate, ovate.

HABITAT

Found in open woods and waste places in the Deccan peninsula parts of West Bengal, Bihar, Orissa, Uttar Pradesh, and Rajasthan also cultivate in garden.

USES

Plant – preparation internally in skin eruptions and disease due to vitiated blood. In the form of medicated Ghirta (purified butter) it was prescribed in phthisis, asthma, emaciation, debility and neurological disorders.

Externally a paste of the herb was applied in erysipelas. During 16^{th} century the drug entered into a number of compound preparations for fever of various origins, diarrhea, dysentery, arthritis, gout, cough, and asthma.Preparation of the root with aeglemarmeloes is useful in dysentery the root used for their refrigerant, antipyretic, stomachic and astringent properties.

Pavonia odarata(Sugandha Bala) essential oil is used as an important ingredient in cosmetics, shampoos, conditioners, pomades, hair tonics, massage oils for rheumatism and much more. You can use this essential oil as gentle massaging oil after blending it with mild carrier oils like olive oil for treating digestive disorders, pain, inflammation, infections, skin problems and rheumatism. Adding 2 to 3 drops of this oil to your bath tub can also grant you similar health benefits and alleviate pain. Using it in diffusers, air fresheners, burners and vaporizes can protect you from the invasion of harmful micro- organisms

ACTIVE PRINCIPLES

Root gave an essential oil containing isovaleric acid, isovalealdehyde aromadendrene, pavonene, alphaterpinene, azulene and pavonenal.Biological activity of the plant was found to be spasmolytic, anti protozoal and antipyretic.

-Indian herbal remedies western therapy ayuredic and other.

LATERAL RESEARCH OF PAVONIA ODARATA LINN

Antimicrobial and anti-inflammatory activity of bioactive. Components of pavonia odarata root. CHCL3 root extract of pavania odarata has better anti microbial activity compare to ethyl acetate and methanol extracts. The anti inflammatory effect of ethyl acetate and chloroform fractions may have related to different chemical composition presented in the plant nature which has proved as anti inflammatory activity compared with the standard drug.

American journal of phytomedicine and clinical therapeutics. www.ajpct.org

3.5 PHARMACEUTICAL REVIEW

KASAYAM (SIDDHA ASPECT)

OTHER NAME:

Marunthu Neer, Unner, Vaikudithidum Punal, Kudineer.

PROCESS:

This is also called as medicinal water (or) decoction.

Dry (or) wet herbs are made in coarse powder and water is added in ratio of 2:1, 4:1, 6:1,8:1, 16:1 (or) 24:1. It is then boiled and filtered. The filterate is known as KASAYAM .

SHEIF LIFE:

3 hours (1 samam)

SPECIAL METHOD:

Thogai kudineer.

Example: pancha moola kudineer.

குடிநீர் வினையற மோதிடு குடிநீரினி தறைவாம்

குதிர் பஞ்ச மூலக் குடிநீர் வகை கேள்

குறிமுக் கடுகரி குடசவேர் மணிவேர்

குல்லிவை யடுவது குடிநீர் பருகிடீர்.

(தே.கரிசல்).

KASAYAM PREPARATION METHOD:

Kasayam prepare in following method the efficacy, purity and safety of kasayam increase.

Use wide mouthed vessel – Always, while preparing Kashayam, use a wide mouthed vessel. This will help in early and easy evaporation of water and makes the process of boiling a lot quicker.

Mild fire – To make kashayam, always use mild heat. Intense heat may cause charring of herbs inside the vessel. Slow transfer of herbal active principles into water is required for maximum efficacy.

Size of herbs – The size of herbs should be neither too large nor too fine. Coarse powder of herbs is best to prepare good quality kashayam.

Use only dry herbs - except for a few herbs, most of the herbs used in preparing kasayam are used in dry form only.

Do not cover with lid – while making kasayam, the vessel should not be covered with lid. If lid is covered, the boiled water will get condensed and will again fall back into the vessel. We need to boil the herbs in open air.

Continuously stir and watch – throughout the procedure, the herbs coarse powder should be continuously stirred and watched. This will avoid charring of herbal powders.

Proportion of herb and water and reduction – for any kasayam, generally herb is to water ratio is 1:4 or 1:8

The kasayam should be boiled till the total water quantity in the vessel reduces to a quarter (1/4)

Take care while filtering – use a clean cloth to filter kasayam. Filter the kasayam when it is still mild hot.

Use it before 4 hours – once prepared kasayam should be used within 4 – 5 hours after preparation. If it is kept for too much long time, then it may get spoilt.

Do not reheat kasayam – once prepared kasayam should not be re-heated. This may spoil the active herbal principles in the kasayam.

Do not re-use the same herbs, once used for kasayam – the herbs used to prepare kasayam should be thrown out after preparation. It should not be re-used.

Table no: 1 ANALYTICAL SPECIFICATIONS OF CURNA/CHOORNAM (FINE POWDER)/KVATHA CURNA/KUTINIR CHOORNAM (COARSE POWDER FOR DECOCTION)

Sl.No	TESTS
1.	Description
2.	Macroscopic, Microscopic
3.	Loss on drying at 105 C
4.	Total – ash
5.	Acid – insoluble ash
6.	Water-soluble extractive
7.	Alcohol – soluble extractive
8.	Particle size (80-100 mesh for Churna; 40-60
9.	mesh for Kvatha churna)
10.	Identifications, TLC/HPTLC-with marker (wherever
11.	possible)
12.	Test for heavy/Toxic metals
13.	Lead
14.	Cadmium
15.	Mercury
16.	Arsenic
17.	Microbial contamination
18.	Total bacterial count
19.	Total fungal count
20.	Test for specific Pathogen
21.	E. coli
22.	Salmonella spp.
23.	S.aureus
24.	Pseudomonas aeruginosa

3.6 DISEASE REVIEW

3.6.1SIDDHA ASPECTS

In Siddha system urinary disease are classified into

1. Neerinai Perukkal Noi

2. Neerinai Arukkal Noi

In Siddha kalladippu disease is mentioned by yugi munivar in yugi vaidhya chinthamani 800. It is one of the urinary diseases which come under Neerinai Arukkal Noi.

VERUPEYAR (synonym):

Ashmari rogam

EYAL (Definition):

தானென்ற மூத்திரத்தில் நறநறவென்று
தங்கியதோர் பொடியெனும் மணல்தானப்பா
வானென்ற சிறிதொரு கல்லாவதப்பா
வளமாக வந்து விழும் நோய்க்கு தானே.
ஏனென்ற அஸ்மரி ரோகம் என்ற பேராம்

- AGASTIYAR GUNAVAGADAM

According to Agastiyar Gunavagadam crystals which look like sand deposited in urine followed by small size stones which is excreted in urine and also sudden obstruction in flow of urine, pain in the base of penis in males and clitoris in females,

burning micturition, loin and groin pain, passing of small sand like stones along with urine are the features of this disease.

IN JEEVARATCHAMIRTHM

Kalladaippu is defined as pain present in abdominal region. Particularly around the umbilicus, fever, dysuria, urine smell like that of goat urine.

NOI VARUM VAZHI (AETIOLOGY)

In Siddha system cause of this kalladaippu disease according to MAANMURUGIYAM are mentioned

கருநீரடக்கல் விரைவில் அடிபடல்
நீரியந்தாக்கல் சிறு நீரடக்கல்
வளிநோய் மிருக்கு முணவும் ஒழுக்கமும்
கடைப் பிடித்திடுதல் மேகமுதற் பல
பிணியுறல் எழுமிவை யடிப்படையாகக்
கல்லடைப் பென்னுங் கடும்பிணி விளையும்
வளியது மீறியை யொடு மல்லாது
கருற்றொடுங் கலந்துற நீரடகத்துச்
சிறுநீர்க் கழிவு தொகுத்த லாதும்
அன்னகை கல்லெனத் திரளும் என்ப

The above poem describe that derangement in human blood, excessive indulgence in sexual activity, trama on tests, sexual pervertion, suppression of urine and semen.

Inflammation of bladder, syphilis, stagnation of urine in urinary tract, dryness of semen causes the formation of stones. Increase intake of food that cause flatulence.

IN SIDDHA MARUTHUVANGA CHURUKKAM:

“நீரினை தருத்தல் செய்யின் நீர்கட்டு துவாரம் புண்ணாகும்
பாரிடும் சந்து தன்னில் பண்புறு நோவதாகும்
நேரிலங் கயரும் காமியம் நிச்சயம் நோதல் செய்யும்
பாரில் அபானவாயு பண்புற சேருமன்றோ”
“சுக்கிலம் தன்னை அடக்கின் சுரமுலடன் நீர்கட்டாகும்
பக்கமாய் கைகால் சந்து பாரமாய் வழியிறங்கும்
மிக்கமோர் நோயுண்டாகும் மிகுந்திடும் பிரமேகந்தான்
தக்கதோர் போதுமாகின் தரித்திடும் வாயுவின் கூறே”

The natural urges of our body are urine and semen suppression of excretion of urine or semen are one of the important causes for the production of stone in bladder.

ACCORDING TO YUGI VAIDHYA CHINTHAAMANI

Chronic mega noi (syphilitic disease) the semen will stagnate for a long time in the urinary tract so it will obstruct the urinary flow so urinary constituents will easily deposit on the urinary tract and form the stone. At that time vitiation of vatham and pitham these small stones become larger in size and block the urinary passage.

கலங்கிணதோர் தண்ணீர்கள் குடித்த போக்கும்
கல்லெறும்பு மயிர் மண் தான் கலந்தன்னத்தில்
அலங்கினதோ ரன்னங்கள் அருந்தலாலும்
மலங்கிணதோர் மாப்பண்ட மருந்தாலும்
மந்தத்தில் வாய்வான பதார்த்தந் தன்னை
துலங்கினதோர் ருசிதன்னதிற் சுவைத்தலாலும்
சுருக்காக் கல்லடைப்பு வந்து தோன்றும்.

-YUGI VAIDHYA CHINTHAMANI

POTHU KURI KUNANGAL

உந்தி தன்னினும் அதன் கீழ் மருங்கினும்
விரை நரம் பிடித்தும் நோவு தோன்றல்
சிறுநீர் நெறியில் கல்லுரத் தடுப்பின்
முரித்து முரித்து நீர் வீழ்ந்திடுதல்
கல்லது விலகி நின்றிடின் சிறுநீர்
தெளிந்தின மஞ்சள் நிறத்தி லொழுதல்
எனுமிவை கல்லடை பொது குறி யென்ப.

Gradual (or) sudden obstruction to flow of urine, agonizing pain in the penis, colicy pain, radiating from loin to groin, lower abdomen, urethra, genitatia. If the calculus is irregular shape. It will burn and scanty micturation and hamaturia.

NOI ENN (Classification)

In Siddha system various types of Kalladippu are mentioned.

ACCORDING TO YUGI VAIDHYA CHINHAAMANI

Four types

1. Valikalladaippu.
2. Azhal kalladaippu.
3. Iyya kalladaippu.
4. Mukkutra kalladaippu.

ACCORDING TO NOIVILAKKAM

வளி முதல் மூன்றினு தோன்றலாலும்

கருநீர் தன்னிற் தோன்றலாலும்

கல்லடை நால்வகை படுமென் மொழியே

Four types

1. Vali kalladaippu.
2. Analaka kalladaippu.
3. Iyya kalladaippu.
4. Karuneer kalladaippu.

ACCORDING TO SIDDAR ARUVAI MARUTHUVAM

Four types

1. Vali kalladaippu.
2. Azhal kalladaippu.
3. Iyya kalladaippu.
4. Veneer kalladaippu.

ACCORDING TO NOIVILAKKAM

KURI GUNANGAL-4

VALI KALLADAIPPU

படர்மிகப் படுதல் பற்கள் கடித்தல்

நடுங்கல் உந்தியுங் குறியும் பிசைதல்

கசடுகீழ் வளியொரு கழலல் அழுதல்

சிறுநீர் துளித்தல் என்பவும் பிறவும்

வளியின் கல்லடைக் குறியென மொழியே.

Tongue biting, palpitation, shivering, crushing pain of the lower abdomen and genital organ, dribbling of urine, the stone are blackish red in colour.

ANALKALLA DAIPPU

சுட்டென நிரியம் மிகவெம் பிடுதலும்
நோதலும் அலைக் கல்லடைக் குறியே
சிவந்துங் கறுத்து மஞ்சளாகியும்
சேங்குரு வடிவில் கல்லது தோன்றும்.

Burning micturition,dysuria the stone are reddish black (or) yellow in colour and passing small stones.

IYYA KALLADAIPPU

நீரியங் குத்தல் திணித்தல் குளிர்தல்
எனுமிவை ஐயக் கல்லடைக் குறியே
வெளுத்தும் தேனிற மாகியு மொளிர்ந்தும்
பெருவடி வுடைத்தாம் ஐயக் கல்லடை.

Pricking pain, forceful pain with severe intensity when passing urine, fever with rigor, white (or) honey coloured, shining (or) laminate larger size stone expelled.

KARUNEER KALLADAIPPU

கருநீரட்க கலின் வளி சினந்தெளுந்து
விரைனளி னடுவில் அதுதனை தடுத்தலின்
கருநீர் கல்லடை மருவிடு

KURIGUNANGAL

ACCORDING TO YUGI VAIDHYA CHINTHAAMANI

VALI KALLADAIPPU

Acute pain in lower abdomen, swelling in abdomen, urinary flow is uncontinuous so pain present in penis, sometime mucous discharge in urine, patient unable to sit.

AZHAL KALLADAIPPU

Obstruction of urinary flow, burning sensation and acute pain in urethra, excretion of small red coloured stones.

IYYA KALLADAIPPU

Severe pain present in umbilicus, pain radiating towards the thigh, burning micturation, excess sweating and small white colour stone sometimes comes with urine.

MUKKUTRA KALLADAIPPU

It is also called Venneer Kalladaippu (or) Manarkalladaippu.

Pain in tip of urethra, irregular urinary output and small stone come along with urine and semen.

IN AYURVEDA KALLADAIPPU

Four types

1. Vataja ashmari.
2. Pittaja ashmari.
3. Kaphaja ashmari.
4. Sukraja ashmari.

VATAJA ASHMARI

- The pain will be excruciating. The patient constantly under severe pain presses his umbilical region.
- Burning sensation is experienced in the peinis, and while urination, belching and defecation become difficult and painful.
- Hematuria may seen.
- Ashmari are found to be a dusty colour, rough, uneven shape, etc.
- In ayruveda texts the shape of the stone described as hard faceted and nodular like kadamba flower.
- Resembles uric acid stone.

PITTAJA ASHMARI

- Sucking, drawing, burning sensation of pain experienced in the condition.
- Stone is found to be reddish, yellowish black, like seed of the Bhallathaka fruit or like the color of the honey.
- Resembles to calcium oxalate, uric acid and cystine stone.
- Urine- color yellowish red.
- Sometime chills, fever also associated.

KAPHAJA ASMARI

- Pain in the bladder region like that of "suchi birava", like needle prick.
- Have the pain but it is not sever as vataja type. Kind of crushing pain, bursting in the organ which becomes cold and heavy. May feel stiffness on that region.
- Urine color is white.
- Stone is white, glossy and attain to large size to that of hens egg. And has the color of madhuka flower.Resembles to calcium phosphate stones.

SUKRAJA ASMARI

- It is seminal origin due to sudden or abrupt stoppage of sexual act or excessive coitus tends to discharge the semen and sediment in abnormal place. Leads to formation of stone.
- Associated with pain in the bladder, dysuria, swelling in the scrotum.

(In anubhava vaidya deva ragasiyam.,roga nirnaya saram and jeeva ratchamirtham kalladaippu classified in to 5 types)

MUKKUTRA VERUPADUGAL

(Pathology)

In siddha maruthuvam

During alternation in once diet and water intake vatha and pitha.Dhosa will alter (or) increase in the body it will affect the abana vayu. So urine will be stagnated in the urinary tract so salt deposit easily anywhere in the urinary tract and forms the stone.

In Ayurvedha,

The disease process can be explained in the following ways,

1. Vadha dosha aggravated in urinary bladder. The vadha has the qualities like rough the virtue of these qualities it dries up the locally available urine. Pitha dhosa convert them into sarkara or gravels.
2. Kapha dosha bind those gravels together to make a soft stone like structure.

DIET AND LIFE STYLE

WHAT CAN HAVE?

- Increase fluid intake upto 2-3 liters per day.
- Consume food rich vitamin A, Mg, etc
- Limit salt intake.
- Consume diet rich in fiber, etc.
- Proper exercise and work management.

WHAT DON'T?

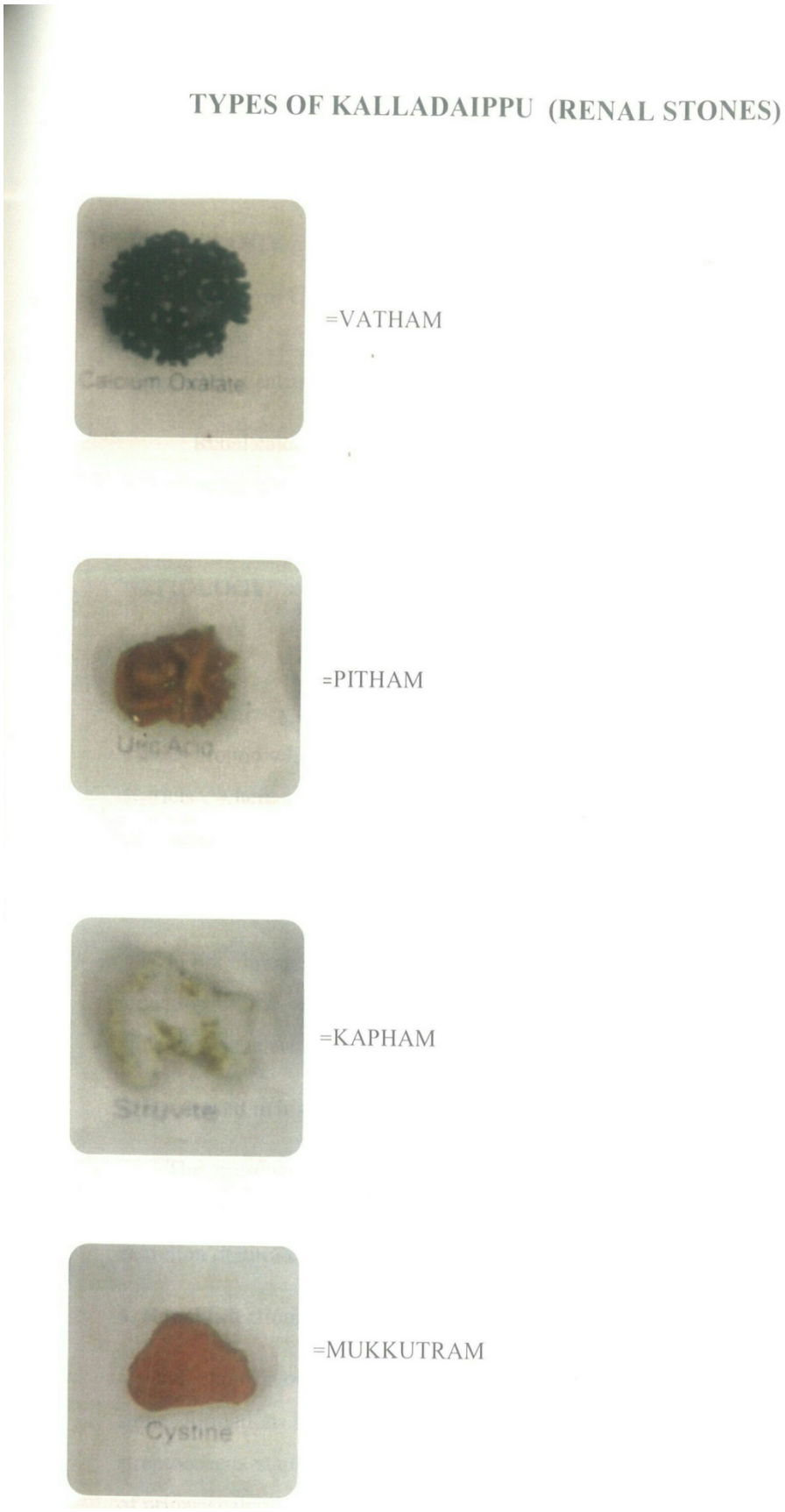
- Avoid milk, ghee and other milk products.
- Leafy vegetables like spinach, tomato, cauliflower, cabbage, peas, etc.
- Avoid excess animal protein, red meat.
- Getting exposed to heat.
- Withholding physical urges like urine, stool, semen, etc.
- Beer, chocolate, soft drinks, etc.

YOGA FOR URINARY CALCULAI

It is advisable to practice yoga along with medicines to relieve the symptoms of calculai. Some of the asana useful to reduce the conditions are,

1. Dhanurasana (bow pose)
2. Pawanamuktasana (wind relieving pose)
3. Uttana padasana (raised leg pose)
4. Matsyasana (fish pose)
5. Vajrasana
6. Pranayama

Figure no:1


TYPES OF KALLADAIPPU (RENAL STONES)
Calcium Oxalate
=VATHAM
Uric Acid
=PITHAM
Struvite
=KAPHAM
Cystine
=MUKKUTRAM

3.6.2 MODERN ASPECT

Urolithiasis :

Urolithiasis (or) formation of Urinary Calculi at any level of the Urinary tract is a common condition. It comprises nephrolithiasis(the formation of kidney stones)

Ureterolithiasis (the formation of stones in the ureter and cystolithiasis. (The formation of bladder stones).

Urinary Calculi are worldwide in distribution but are particularly common in some geographic locates such as in parts of the United States, South Africa, India and South-East Asia. It is estimated that approximately 2% of the population experiences renal stone disease at sometime in their life with male-female ratio of 2.1. The peak incidence is observes in 2^{nd} to 3^{rd} decades of life.

CAUSES

- Decrease Urine Volume.
- Inadequate Urine drainage which may lead to stasis.
- 50% of patients with calcium stones gave idiopatic hypercalciuria, without hypercalcaemia.
- 10% cases are associated with hypercalcaemia and hypercalciuria most commonly due to hyperparathyrodism (or) a detect in the bowel.(i.e. absorptive hypercalciuria) (or) in the kidney (i.e. renal hypercalciuria).
- Infection of urinary tract with Urea splitting, organisms that produce Urease such as by species of Proteus, Streptococcus, Staphylcoccus, Klebsiella, Pseudomonous.

- Uricacid stones are formed frequently in case with hyperuricaemia and hyperuricosuria such as due to primary gout (or) secondary gout.

- Acidic Urinary(below) PH and Low Urinary Volume.

- Cystine stones are associated with Cystinuria due to genitically determined defect in the transport of cystine and other aminoacids across the cellmembrane of the renal tubules.

- Decreased in Urinary Citrate Levels leading to deposition of Calcium.

- Deficiency of Vitamin A and C

- HYPEROXALATE

  High dietary intake of the fruit and vegetable

  Low Calcium diet.

TYPES

1. Calcium Stones – 75%

   Calcium Oxalate.

   Calcium Phosphate.

2. Mixed (struvite) Stones- 15%

3. Uric acid Stones – 6%

4. Cystine Stones – 2%

5. Other type (xanthin calculi) - <2%

MORPHOLOGY OF STONES

1. CALCIUM STONES

Calcium stones are usually small(less than a centimeter). Ovoid, hard with granular rough surface. They are dark brown due to old blood pigments deposited in them as a result of repeated trama caused to the urinary tract by these sharp-edged stones.

2. STRUVITE STONES

Yellow –white (or) grey. They are trend to be soft and triable and irregular in shape.

3. URIC ACID STONES

Smooth, Yellowish-brown, hard and often multiple on cut section, they show laminated structures.

4. CYSTINE STONE

Small, rounded, smooth and often multiple. They are yellowish and waxy.

SIGNS AND SYMPTONS

- In the kidney fixed renal pain, (flank pain)is common.
- In the ureter (according to Localisation of stone)
- If in upper 1/3 of the ureter pain radiates to the perineum if the pelvic brim- pain radiates to the inner aspect of thigh.

- If present in middle 1/3 of ureter –pain radiates to the iliac fossa.

- If the stone is Localised in the bladder neck (or) Urethra.
  -pain may presence as tip of penis.

- Frequently of Urination.

- Oliguria.

- Dribbling of Urine.

- Hematuria.

PATHOGENESIS:

- The mechanism of Calcium stone formation is explained on the basis of supersaturation of ions of forming the stone and the concentration of inhibitors in the urine most likely site where the crystal of Calcium Oxalate and /or Calcium Phosphate are precipitated is the tubular lining (or) around some fragement of debris in the tubule acting as nidus of the stone. The stone grows, as more and more crystals are deposited around the nidus. A number of other predisposing factors contributing to formation of Calcium stones are alkaline Urinary PH decreased Urinary Volume and increased excreation of oxalate and uric acid.

- The solubility of uric acid at PH of 7 is 200mg/dl while at PH of 5 is l5mg/dl. Thus as the urine becomes more acidic, the solubility of uric acid in urine decreases and precipitation of uric acid crystals increases favouring the formation of uricacid stones hyperuricosuria is the most important factor in the production of uricacid stone, while hyperureamia is found in about half the case.

- Excessive excretions of cystine stones are associated with cystinumia due to genetically determined defect which is least soluble of the naturally-occuring aminoacid leads to formation of crystals and eventually cystine calculi.

COMPLICATION

- Hydronephrosis.
- Pyonephrosis.
- Chronic unilateral (or) bilateral hydronephrosis produce other renal diseases.
- Chronic renal failure
- Hydrouretero nephrosis.
- Chronic retention of urine.
- Pyocystitis.

DIAGNOSIS OF KIDNEY STONES:

Diagnosis of kidney stones requires a complete health history assessment and a physical exam. Other tests include:

- Blood tests for calcium, phosphorus, uric acid and electrolytes.
- Blood urea nitrogen (BUN) and creatinine to assess kidney functioning.
- Urinalysis to check for crystals, bacteria, blood and white cells.
- Examination of passed stones to determine type.

The following tests can rule out obstruction:

- Abdominal X-rays.
- Intravenous pyelogram (IVP)
- Retrograde pyelogram
- Ultrasound of the kidney (this is the preferred study)
- MRI of the abdomen and kidneys
- Abdominal CT scan.

MANAGEMENT

Small stone Less than 5 mm (0.2inch) in diameter may pass spontaneously through urination within four weeks of the onset of symptoms but larger stones 5-10mm(0.2 to 0.4 inch) in diameter the rate of spontaneous passage decrease to less than 53% 50 dietary recommendation to minimize the formation of kidney stones include.

- Increase total fluid intake to more than two litre per day of urine output.
- Increasing citric acid intake, lemon juice is the richest natural source.
- Moderate calcium intake.
- Limiting sodium intake.
- Avoidance of large dose of supplemental Vitamin C.
- Limiting animal protein intake.Limiting consumption of cola soft drink because which contain phosphoric acid.

- Restriction of oxalate rich foods such as leaf,vegetables, soy products and chocolate.

TREATMENT

MEDICATION

Pain relief may require narcotic medications. The presence of infection requires treatment with antibiotics. Other medications include:

- Allopurinol for uric acid stones
- Diuretics
- Sodium bicarbonate or sodium citrate
- Phosphorus solutions.

LITHOTRIPSY

Extracorporeal shock wave lithotripsy uses sound waves to break up large stones so they can more easily pass down the ureters into your bladder. This procedure can be uncomfortable and may require light anesthesia. It can cause bruising on the abdomen and back and bleeding around the kidney and nearby organs.

TUNNEL SURGERY (Percutaneous Nephrolithotomy):

Stones are removed through a small incision in your back and may be needed when:

- The stone causes obstruction and infection or is damaging the kidneys.
- The stone has grown too large to pass
- Pain cannot be controlled.

URETEROSCOPY:

When a stone is stuck in the ureter or bladder, your doctor may use an instrument called a ureteroscope to remove it. A small wire with a camera attached is inserted into the urethra and passed into the bladder. A small cage is used to snag the stone and remove it. The stone is then sent to the laboratory for analysis.

4.MATERIALS AND METHODS

4.1 PREPARATION OF MAVILINGU KASAYAM

DRUG SELECTION:

MAVILINGU KASAYAM has been selected from the Siddha literature Agasthiyar 2000 part 3.

Ingredients of the drug are

1. MAVILINGU VADAKU VER
2. NERUNJIL VER
3. CHIRUPEELAI VER
4. PERAMUTTI VER

COLLECTION OF THE RAW DRUG:

1. **Mavilingu vadaku ver :**

   The raw material of crataeva magna root was identified and authenticated by PG Gunapadam Department, Govt Siddha Medical College Palayamkotttai Tamilnadu.

2. **Nerunjil ver :**

   The raw material of tribulus terrestris root was identified and authenticated by PG Gunapadam Department, Govt Siddha Medical College Palayamkotttai Tamilnadu.

3. **Chirupeelai ver :**

   The raw material of aervalanata root was identified and authenticated by PG Gunapadam Department, Govt Siddha Medical College Palayamkotttai Tamilnadu.

4. **Peramutti ver:**

   The raw material of pavonia odorata root was identified and authenticated by PG Gunapadam Department, Govt Siddha Medical College Palayamkotttai Tamilnadu.

PURIFICATION OF RAW INGREDIENTS:

1. Mavilingu vadaku ver:

It was washed with running tap water until sand, soil and dust removed.

2. Nerunjil ver :

It was washed with running tap water until sand, soil and dust removed.

3. Chirupeelai ver :

It was washed with running tap water until sand, soil and dust removed.

4.Peramutiver:

It was washed with running tap water until sand, soil and dust removed.

PROCESS OF PREPARATION:

All the ingredient are purified and grind into a coarse powder by using machineries, this mixer powder 100 gm mix with 800 ml of water and boil it. It reduced into 100 ml then filters it.

SHELF LIFE:

3 hours.

DOSAGE:

60 ml (1/4 to ½ ALAKKU)

INDICATION:

Kalladaippu

Figure no 2 RAW DRUG

MAVILINGU VADAKU VER

NERUNJIL VER

CHIRUPEELAI VER

PERUMUTTI VER

MAVILINGU KASAYA CHOORANAM

MAVILINGU KASAYAM

4.2 STANDARDIZATION OF DRUG:

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

The physiochemical analysis have been done at Aravindh herbal labs (p) ltd, Rajapalayam, Biochemical analysis were done at Govt Siddha Medical College Palayamkotttai and microbiological analysis were done at inbiotics, William hospitial campus ms road, Nagerkovil,And the instrumental analysis was done at Indian Institute of Technology (IIT),Chennai-36 .

Organoleptic character

The organoleptic characters of the sample drug were evaluated.1gm of the test drug was taken and the colour, texture, particle size and other morphology were viewed by naked eye under sunlight. Then the result is noted

4.2.1 PHYSICO CHEMICAL ANALYSIS

Physicochemical studies of the trial drug have been done according to the WHO guidelines.

Determination of Ash Values:

Total Ash:

3g is accurately weighed and incinerated in a crucible dish at a temperature not exceed 450^{o}C until free from carbon. It is then cooled and weighed. The % w/w of ash with reference to the air-dried powder is calculated.

Water Soluble Ash:

The total ash is obtained as the above method for preparation of total ash. The ash is boiled for 5minutes with 25ml water. The insoluble ashes is collected using filter paper and washed with hot water and then transferred to the silica crucible then ignite for 15minutes at temperature not exceeding 450^{o}C. The silica crucible and residue are weighed until constant weight is attained for determination of weight of insoluble ash. The weight of the water soluble ash is determined by substracting the weight of insoluble ash from the weight of total ash.

Acid insoluble Ash:

The total ash is obtained as the above method for preparation of total ash. The ash is boiled for 5minutes with 25ml 10% Hcl. The insoluble ashes is collected using filter paper and washed with hot water and then transferred to the silica crucible then ignite for 15minutes at temperature not exceeding 450^{o}C. The silica crucible and residue are weighed until constant weight is attained.

Determination of Extractive Value:

Alcohol Soluble Extractive Value:

3g of test drug powder is weighed and macerated with 100ml of ethanol in a closed container for 24 hours. The resulting solution is shaken continuously for 6 hours and allowed to stand and soak for 18 hours. The solution is filtered and evaporated of the filtrate in a flat bottomed shallow dish and dried at 105^{o}C then cooled and weighed.

Water soluble Extractive value:

3g of test drug powder is weighed and macerated with chloroform and water, respectively, at 80^{o}C for 24 hrs. The resulting solution is shaken continuously for 6 hours and allowed to stand and soak for 24 hrs then filtered. The solution from both chloroform and water respectively is filtered and evaporated of the filtrate in a flat bottomed shallow dish and dried at 105^{o}C then cooled and weighed.

Loss on Drying:

The powdered drug is dried in the oven at 100- 105°C to constant weight. The result was noted.

THIN LAYER CHROMATOGRAPHY

Thin-layer chromatography(TLC) is a chromatographic technique that is useful for separating organic compounds. Because of the simplicity and rapidity of TLC, it is often used to monitor the progress of organic reactions and to check the purity of products.TLC is a simple, quick and inexpensive procedure that gives how many components are in a mixture.TLC is also used to support the identity of a compound in a mixture when the R_f of a compound is compared with the R_f of a known compound(preferably both run on the same TLC plate).Chromatography works on the principle that different compounds will have different solubilities and adsorbtion to the two phases between which they are to be partitioned. As the solvent rises by capillary action up through the adsorbent, differential partitioning occurs between the components of the mixture dissolved in the solvent stationary adsorbent phase. The more strongly a given component of a mixture is adsorbed on to the stationary phase, the less time it will spend in the mobile phase and the more slowly it will migrate up the plate.

The following are some common uses of Thin-Layer Chromatography

1. To determine the number of components in a mixture.
2. To determine the identity of two substances.
3. To monitor the progress of a reaction.
4. To determine the effectiveness of a purification.

Apparatus

Flat glass plates of appropriate dimensions which allow the application at specified points of the necessary quantities of the solution being examined and appropriate reference solutions and which allow accommodation of the specified migration path-length. The plates are prepared as described below; alternatively, commercially prepared plates may be used.An aligning tray or a flat surface on which the plates can be aligned and rested when the coating substance is applied.

The adsorbent or coating substance consisting of finely divided adsorbent materials, normally 5 μm to 40 μm in diameter, is suitable for chromatography. It can be applied directly to the plate or can be bonded to the plate by means of Plaster of Paris (Hydrated Calcium Sulphate) or with any other suitable binders. The adsorbent may contain fluorescing material to help in visualising spots that absorb ultra-violet light.A spreader which, when moved over the glass plate, will apply a uniform layer of adsorbent of desired thickness over the entire surface of the plate.A storage rack to support the plates during drying and transportation.

A developing chamber that can accommodate one or more plates and can be properly closed and sealed. The chamber is fitted with a plate support rack that supports the plates, back to back, with lid of the chamber in place.Graduated micro-pipettes capable of delivering microlitre quantities say 10 μl and less.

A reagent sprayer that will emit a fine spray and will not itself be attacked by the reagent.An ultra-violet light, suitable for observation at short (254 nm) and long (365 nm) ultra-violet wavelengths.

Preparation of plates:

Unless otherwise specified in the monograph, the plates are prepared in the following manner. Prepare a suspension of the coating substance in accordance with the instructions of the supplier and, using the spreading device designed for the purpose, spread a uniform layer of the suspension, 0.25 to 0.30 mm thick, on a flat glass plate 20 cm long. Allow the coated plates to dry in air, heat at 100º to 105º for at least 1 hour (except in the case of plates prepared with cellulose when heating for 10 minutes is normally sufficient) and allow to cool, protected from moisture. Store the

plates protected from moisture and use within 3 days of preparation. At the time of use, dry the plates again, if necessary, as prescribed in the monographs

Method

Unless unsaturated conditions are prescribed, prepare the tank by lining the walls with sheets of filter paper; pour into the tank, saturating the filter paper in the process, sufficient of themobile phase to form a layer of solvent 5 to 10 mm deep, close the tank and allow to stand for 1 hour at room temperature. Remove a narrow strip of the coating substance, about 5 mm wide, from the vertical sides of the plate. Apply the solutions being examined in the form of circular spots about 2 to 6 mm in diameter, or in the form of bands (10 to 20 mm x 2 to 6 mm unless otherwise specified) on a line parallel with, and 20 mm from, one end of the plate, and not nearer than 20 mm to the sides; the spots should be 15 mm apart. If necessary, the solutions may be applied in portions, drying between applications. Mark the sides of the plate 15 cm, or the distance specified in the monograph, from the starting line. Allow the solvent to evaporate and place the plate in the tank, ensuring that it is as nearly vertical as possible and that the spots or bands are above the level of the mobile phase. Close the tank and allow to stand at room temperature, until the mobile phase has ascended to the marked line. Remove the plate and dry and visualise as directed in the monograph; where a spraying technique is prescribed it is essential that the reagent be evenly applied as a fine spray.

For two-dimensional chromatography dry the plate after the first development and carry out the second development in a direction perpendicular to the first.

When the method prescribed in the monograph specified ‘protected from light’ or ‘in subdued light’ it is intended that the entire procedure is carried out under these conditions.

Visualisation

The phrases ultra-violet light (254 nm) and ultra-violet light (365 nm) indicate that the plate should be examined under an ultra-violet light having a maximum output at about 254 or at about 365 nm, as the case may be.

The term secondary spot means any spot other than the principal spot. Similarly, a secondary band is any band other than the principal band.

Rf. Value

Measure and record the distance of each spot from the point of its application and calculate the Rf. value by dividing the distance travelled by the spots by the distance travelled by the front of the mobile phase.

MICROBIAL LIMIT TESTS:

DETERMINATION OF TOTAL AEROBIC MICROBIAL COUNT:

Dissolve 10 g or dilute 10 ml 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 with the same medium. If necessary, adjust the pH to about 7.

Membrane filtration:

Use membrane filters 50 mm in diameter and having anominal pore size not greater than 0.45 μm the effectiveness of which in retainingbacteria has been established for the type of preparation being examined. Sterilise andassemble the filtration apparatus described under tests for sterility.

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.

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.

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.

Escherichia coli:

Place the prescribed quantity in a sterile screw-capped container, add 50 ml of nutrient broth, shake, allow 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 MacConkey 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 MacConkey 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 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 MacConkey broth. The test is not valid unless the results indicate that the control contains Escherichia coli.

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.

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, indicates 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.

Table no: 2 – Test for Salmonella

Medium	**Description of colony**
Bismuth Sulphite agar	Black or green
Brilliant green agar	Small, transparent and colourless, or opaque, pinkish or white (frequently surrounded by a pink or red zone)
Deoxycholate – Citrate agar	Colourless and opaque, with or without black centers
Xylose-lysine-desoxy-cholate agar	Red with or without black centres

Pseudomonas aeruginosa :

Pretreat the preparation being examined as described above and inoculate 100 ml of fluid soyabean-casein digest medium with a quantity of the solution, suspensionor 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 onthe 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 the description in Table 3 are produced, carry out the oxidase and pigment tests.

Streak representative 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.

Table no: 3- Tests for Pseudomonas aeruginosa

Medium	**Characteristic colonical morphology**	**Fluorescence in UV light**	**Oxidase test**	**Gram stain**
Cetrimde agar	Generally greenish	Greenish	Positive	Negative rods
Pseudomonas agar medium for dedection of fluorescein	Generally colourless to yellowish	Yellowish	Positive	Negative rods
Pseudomonas agar medium for dedection of pyocyanin	Generally greenish	Blue	Positive	Negative rods

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.

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.

Table no:4 – Tests for Staphylococcus aureus

Selective medium	**Characteristic colonical morphology**	**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)
Baired-Parker agar	Black, shiny, surrounded by clear zones of 2 to 5 mm	Positive cocci (in clusters)

4.2.2 BIO CHEMICAL ANALYSIS:

Preliminary Basic and Acidic radical studies:

Preparation of the extract:

5gms of the test drug is weighed accurately and placed in a 250ml clean beaker. Then 50ml of distilled water is added and dissolved well. Then it is boiled well for about 10 minutes. It is cooled and filtered in a 100ml volumetric flask and then it is made up to 100ml with distilled water. This preparation is used for the qualitative analysis of acidic/ basic radicals and biochemical constituents in it.

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.

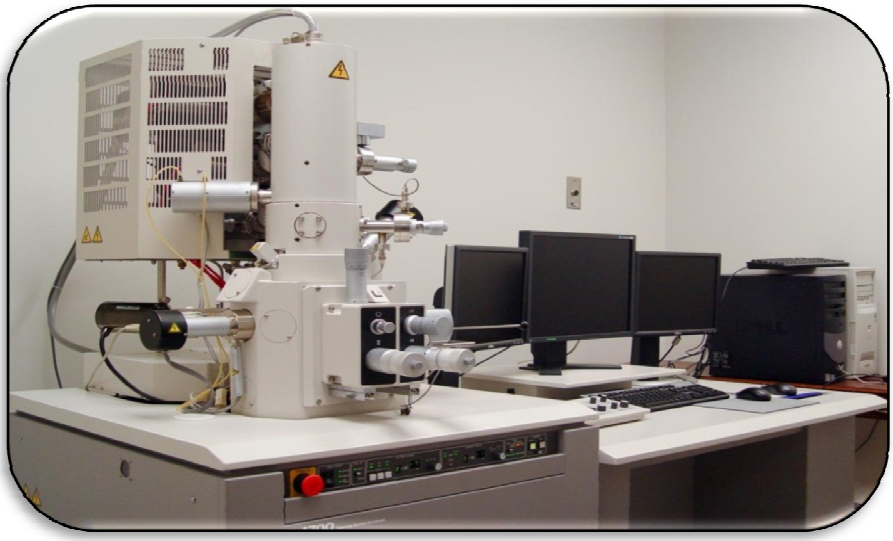
Test for amino acid:

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.

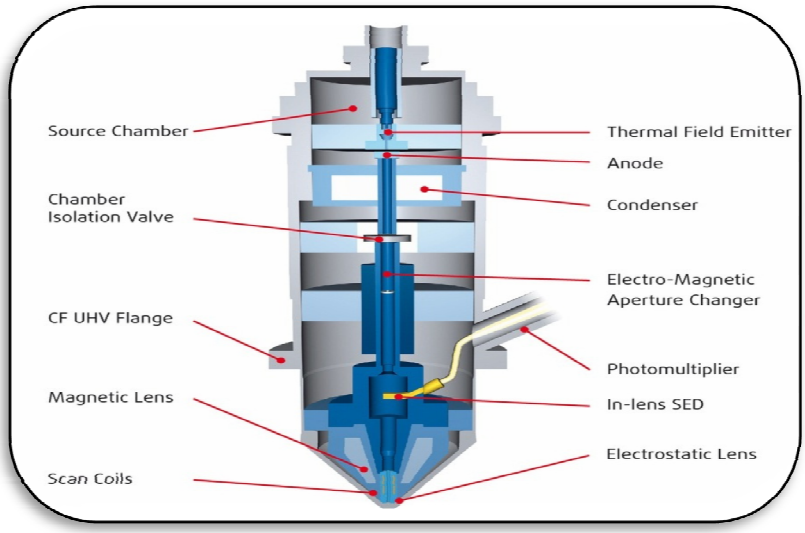
4.2.3INSTRUMENTAL ANALYSIS

SEM *(SCANNING ELECTRON MICROSCOPE)*

SEM INSTRUMENT



SEM - SCANNING ELECTRON MICROSCOPE



MECHANISM

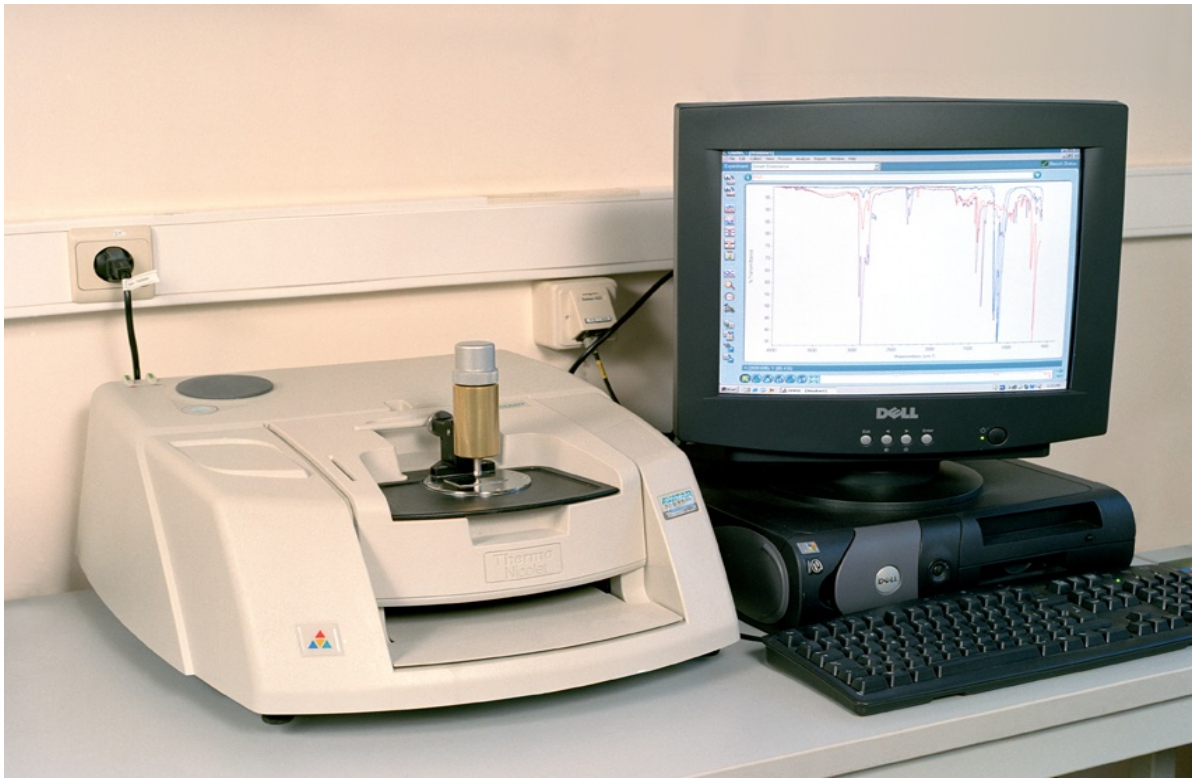
In scanning electron microscope high-energy electron beam is focused through a probe towards the sample material. Variety of signals was produced on interaction with the surface of the sample. This results in the emission of electrons or photons and it is collected by a appropriate detector.

The types of signal produced by a scanning electron microscope include

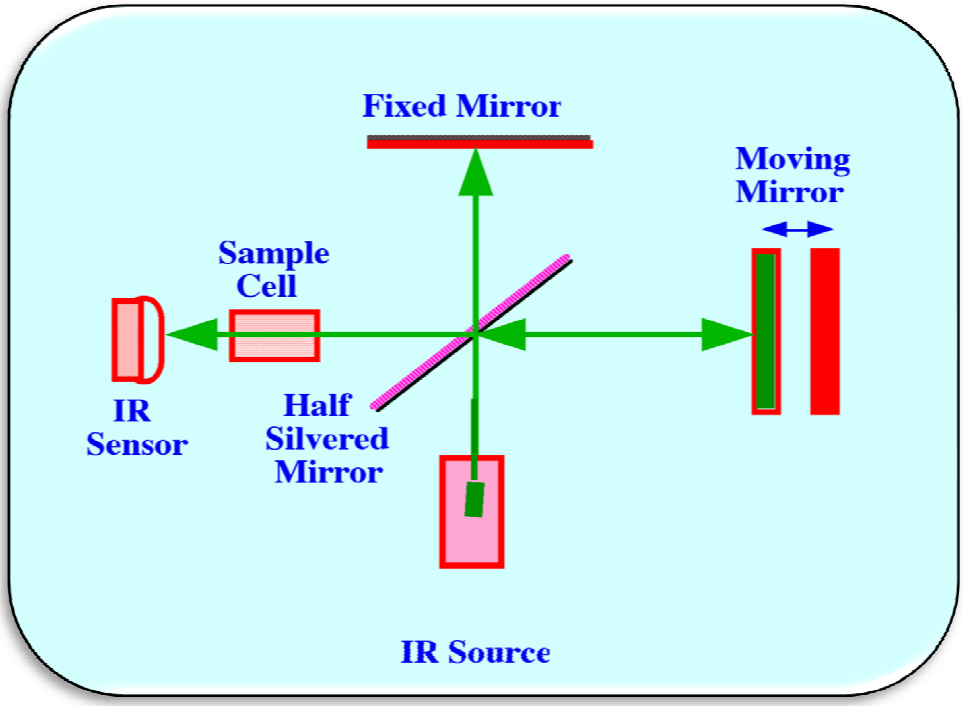
- Secondary electrons
- back scattered electrons
- characteristic x-rays, light
- specimen current
- Transmitted electrons.

This gives the information about the sample and it includes external morphology, texture, its crystalline structure, chemical composition and it displays the shape of the sample.

FT-IR(Fourier Transform Infrared Spectroscopy):



FTIR INSTRUMENT



FTIR MECHANISM

Model **: Spectrum one: FT-IR Spectrometer**

Scan Range **: MIR 450-4000 cm-1**

Resolution **: 1.0 cm-1**

Sample required **: 50 mg, solid or liquid.**

It is the preferred method of infrared spectroscopy. FT-IR is an important and more advanced technique. It is used to identify the functional group, to determine the quality and consistency of the sample material and can determine the amount of compounds present in the sample. It is an excellent tool for quantitative analysis.

In FT-IR infrared is passed from a source through a sample. This infrared is absorbed by the sample according to the chemical properties and some are transmitted. The spectrum that appears denotes the molecular absorption and transmission. It forms the molecular fingerprint of the sample. Like the finger print there is no two unique molecular structures producing the same infrared spectrum. It is recorded as the wavelength and the peaks seen in the spectrum indicates the amount of material present.

FT-IR is the most advanced and the major advantage is its

- Speed
- Sensitivity
- Mechanical simplicity
- Internally calibrated

ICP-OES *(INDUCTIVELY COUPLED PLASMA OPTIC EMISSION SPECTROMETRY)*

ICP-OES *(INDUCTIVELY COUPLED PLASMA OPTIC EMISSION SPECTROMETRY)*

Manufacturer: Perkin Elmer

Model : Optima 5300 DV ICP-OES Inductively Coupled Plasma Spectrometer *(ICP)*

Principle :

An aqueous sample is converted to aerosols via a nebulizer. The aerosols are transported to the inductively coupled plasma which is a high temperature zone *(8,000– 10,000°C).* The analysts are heated *(excited)*to different (atomic and/or ionic) states and produce characteristic optical emissions *(lights)*. These releases are separated based on their respective wavelengths and their strengths are measured *(spectrometry)*. The intensities are proportional to the concentrations of analyses in the aqueous sample. The quantification is an external multipoint linear standardization by comparing the emission intensity of an unknown sample with that of a standard sample. Multi-element calibration standard solutions are prepared from single- and multi element primary standard solutions. With respect to other kinds of analysis where chemical speciation is relevant *(such as the concentration of ferrous iron or ferric iron)*, only total essential concentration is analysed by ICP-OES.

Application:

The analysis of major and minor elements in solution samples.

Objectives:

- Determine elemental concentrations of different metals.
- Learn principles and operation of the ICP-OES instrument
- Develop and put on a method for the ICP-OES sample analysis
- Enhance the instrumental conditions for the analysis of different elements
- probes the outer electronic structure of atoms

Mechanism:

In plasma emission spectroscopy *(OES),* a sample solution is presented into the core of inductively coupled argon plasma *(ICP),* which generates temperature of approximately 8000°C. At this temperature all elements become thermally excited and emit light at their characteristic wavelengths. This light is collected by the spectrometer and passes through a diffraction grating that serves to resolve the light into a spectrum of its essential wavelengths. Within the spectrometer, this deflected light is then collected by wavelength and amplified to yield an strength of measurement that can be converted to an elemental concentration by comparison with standardizationvalues

The Inductively Coupled Plasma Optical Emission Spectrometric *(ICP-OES)* analysis was done in Saif, IIT Madras, and Chennai-36 using Perkin Elmer Optima 5300 DV.

Sample preparation:

Inductively Coupled Plasma Spectroscopy techniques are the so-called "wet" sampling methods whereby samples are introduced in liquid form for analysis.

100 mg “Mavilingu kasaya chooranam*”*was occupied in a clean, dry test tube. To this, 3 ml Nitric acid was added and mixed well and allowedfor few minutes untill the reactions were completed. And then, 25 ml of Refined water, was added to prepare digested solution.

4.3 TOXICOLOGICAL STUDIES

PRECLINICAL TOXICITY STUDIES OF *MKC* ON WISTAR ALBINO RATS

4.3.1ACUTE TOXICITY STUDY IN FEMALE WISTER RATS TO EVALUATE TOXICITY PROFILE OF MKC

OBJECTIVES

The aim of this Study is to evaluate the toxicity of the test substance mavilingu kasaya chooranam, 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) ***The test drug MKC*** was administered at **5 mg/kg, 10 mg/kg,300 mg/kg, 1000 mg/kg, and 2000 mg/kg** body weight of animal as suspension along with water.

3) The results were recorded on day 0, with single oral dosing period of 14 days.

EXPERIMENTAL PROCEDURE

1. ANIMALS

1.1. 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.

1.2. 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 3 rats 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.

2.1. 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.

3.1 Numbering and Identification

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

Table no-5 Numbering and Identification

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

Table no 5 Numbering and Identification

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

3.2 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.

Table no -6 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 14days.

Dose Preparation

MKC 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.

4.3 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.

4.4 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.

4 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 MKC

1. Objective

The objective of this '**Sub-Acute Toxicity Study of MKC ON Wister Rats**' was to assess the toxicological profile of the test item when treated as a single dose daily. 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.

2.Test Guideline Followed

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

3. Test Item Detail

Name: **MAVILINGU KASAYA CHOORANAM**

4.Test System Detail

The study was conducted on 5 male 5 female Wister rats for each group. 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.

5. Acclimatization

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

6.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 5 male and 5 female animals per group.

7.Numbering and Identification

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

Table no-5 Numbering and Identification

Group No(CONCENTRATION/DOSE)	Animal Marking
1. CONTROL	**H,B,T,HB,NM (MALE)** **H,B,T,HB,NM (FEMALE)**
2. LOW DOSE OF MKC 2ml/kg	**H,B,T,HB,NM (MALE)** **H,B,T,HB,NM (FEMALE)**
3. MIDDLEDOSE OF MKC 5ml/kg	**H,B,T,HB,NM (MALE)** **H,B,T,HB,NM (FEMALE)**
4. HIGH DOSE OF MKC 10ml/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:

Cage No	Group No CONCENTRATION/DOSE	Animal Marking	Sex
1	1. CONTROL	H,B,T,HB,NM H,B,T,HB, NM	Male Female
2	2. LOW DOSE OF MKC 2ml/kg	H,B,T,HB,NM H,B,T,HB, NM	Male Female
3	3. MIDDLEDOSE OF MKC 5ml/kg	H,B,T,HB,NM H,B,T,HB, NM	Male Female
4	4. HIGH DOSE OF MKC 10ml/kg	H,B,T,HB,NM H,B,T,HB ,NM	Male Female

8. Husbandry

8.1 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 10 rat of the differnt sex and treatment group.

8.2 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 ± 3^{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.

8.3 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.

8.4 Water

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

8.5 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.

Table no -6 Dose level

TEST GROUP	C0NCENTRATION/DOSE TO ANIMALS (ml/kg body-weight/day)	NUMBER OFANIMALS
Group-1	**1. CONTROL**	10 (5MALE and5 FEMALE)
Group-II	**2. LOW DOSE OF MKC 2ml/kg**	10(5MALE and 5 FEMALE)
Group-III	**3. MIDDLEDOSE OF MKC 5ml/kg**	10(5MALE and 5 FEMALE)
Group-IV	**4. HIGH DOSE OF MKC 10ml/kg**	10(5MALE and 5 FEMALE)

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

Dose Preparation

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

8.6 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.

9. 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.

9.1. 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.

9.2. 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.

9.3. 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.

9.4 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 rats 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 etc……...

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

4.4PHARMACOLOGICAL STUDY

4.4.1 LITHOTRIPTIC ACTIVITY

EVALUATION OF LITHOTRIPTIC(ANTILITHIATIC) EFFECT OF MKC ON 1% ETHYLENE GLYCOL INDUCED LITHIASIS IN ALBINO RATS

Introduction

Urinary stone disease has afflicted humankind since antiquity and can persist, with serious medical consequences, throughout a patient's lifetime. In addition, the incidence of kidney stones has been increased in western societies in the last five decades, in association with economic development. Most calculi in the urinary system arise from a common component of urine, e.g. calcium oxalate (CaOx), representing up to 80% of analyzed stones (51).Currently, open renal surgery for nephrolithiasis is unusual and used only rarely since the introduction of extracorporeal shockwave lithotripsy (ESWL), which has revolutionized urological practice and almost become the standard procedure for eliminating kidney stones. However, in addition to the traumatic effects of shock waves, persistent residual stone fragments and the possibility of infection, suggest that ESWL may cause acute renal injury, a decrease in renal function and an increase in stone recurrence (52,53).

A number of vegetable drugs have been used in India and elsewhere which claim efficient cure of urinary stones (54). In the indigenous system of medicine, the mavilinga kashayam is reported to be useful in the treatment of urinary stones. However, so far no systematic study has been reported regarding the antiurolithiatic property of mavilinga kashayam. In the present study, an effort has been made to establish the scientific validity for the antiurolithiatic property of MKC using ethylene glycol induced hyperoxaluria model in rats.

Materials and methods

Animal selection

For acute toxicity studies, Wistar albino rat of either sex weigthing between 25 and 30g were selected and healthy adult male Wistar albino rats weigthing between 150 and 200g were selected for the antiurolithiatic activity. The animals were acclimatized to standard laboratory conditions (temperature: 25±2°C) and maintained on 12-h light: 12-h dark cycle. They were provided with regular rat chow (Lipton India Ltd., Mumbai, India) and ad libitum. The animal care and experimental protocols were in accordance with Institutional Animal Ethical Committee (IAEC).

Acute toxicity studies

The acute oral toxicity study (55) was carried out as per the guidelines set by Organization for Economic Cooperation and Development (OECD) received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). One-tenth of the median lethal dose (LD50) was taken as an effective dose (56).

Ethylene glycol induced urolithiasis model

Ethylene glycol induced hyperoxaluria model (57) was used to assess the antilithiatic activity in albino rats. Animals were divided into six groups containing six animals in each.

TREATMENT PROTOCOL

The grouped animal's received the treatment as follows

Group I Received normal diet and served as controls.

Group II Lithiatic control: The animals were given normal diet and 1% Ethylene glycol in drinking water for 28 days.

Prophylactic Study:

Group III Received 1% ethylene glycol in drinking water and then treated with MKC at a dose of 100 mg/kg orally for 28 days

Group IV Received 1% Ethylene glycol in drinking water and then treated with MKC at a dose of 200mg/kg orally for 28 days.

Group V Received 1% Ethylene glycol in drinking water andthen treated with MKC at a dose of 300mg/kg orally for 28 days.

Group VI Received 1% Ethylene glycol in drinking water and then treated with cystone at a dose of 500mg/kg orally for 28 days.

Collection and analysis of urine

All animals were kept in individual metabolic cages and 24 h urine samples were collected on 14th, and 28th day of calculi induction treatment. The volume and calcium content of urine were measured. Calcium in urine was estimated using kit by "COBAS MIRA PLUS" auto analyzer. Urine was analyzed for oxalate (58), magnesium (59,60), phosphate (61), uric acid (62), citrate(63) and total protein(64).

Serum analysis

The blood was collected from the retro-orbital sinus under anesthetic condition and serum was separated by centrifugation at 10,000g for 10 min and analyzed for creatinine and uric acid. The creatinine kit (Reckon Diagnostics Pvt. Ltd., India) and uric acid diagnostic kit (Span Diagnostics Ltd., India) were used to estimate serum creatinine and uric acid levels respectively.

Statistical analysis

The results were expressed as mean ± standard error mean (SEM). The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Newmann keul's multiple range tests and $p < 0.05$ was considered significant.

4.4.2 DIURETIC ACTIVITY

DIURETIC ACTIVITY OF SIDDHA PREPARATION MAVILINUKASAYACHOORANAM

Introduction

Medicinal plants can be important sources of unknown chemical substances with potential therapeutic effects. Besides, the World Health Organization has estimated that over 75% of the world's population still relies on plant-derived medicines, usually obtained from traditional healers, for basic health-care needs (92). The study of plant species with diuretic effects is still a fruitful research in search of new diuretics. Diuretics are the drugs that increase the rate of urine flow; clinically useful diuretics also increase the rate of excretion of Na+ (natriuresis) and an accompanying anion, usually Cl-. Most clinical applications of diuretics aim to reduce extracellular fluid volume (edema) by decreasing total body Nacl content. Although continued administration of diuretic causes a sustained net deficit in total Na+, the time course of natriuresis is finite because renal compensatory mechanisms brings Na+ excretion in line with the Na+ intake, a phenomenon known as diuretic braking. Diuretics alter the excretion of other cations (e.g. K+, H+, Ca2+, Mg2+), anions (e.g. Cl-, HCo3- and H2Po4) and uric acid. In addition diuretics may alter renal hemodynamics indirectly mediated by local prostaglandins synthesis(93).

Despite the popular use of this species as a medicinal plant, there are no data about the pharmacological effect of MKC on diuretic activity. The aim of the present study was to evaluate the potential diuretic and natriuretic activities of MKC in experimental animal.

Material and Methods

Experimental animals

Healthy male albino rats weighing 180-200 g were used for the study. The animals were maintained in polypropylene cages of standard dimensions at a temperature of 37 ± 1°C and standard 12h : 12h day/night rhythm. The animals were fed with standard rodent pellet diet (Hindustan Lever Ltd.) and water *ad libitum*. Prior to the experiment, the animals were acclimatized to the laboratory conditions. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) constituted under CPCSEA.

Drug Treatment

MKC at the dose levels of 100, 200 and 300 mg/Kg body wt., p.o. was administered once daily for three consecutive days. Fursemide (20 mg/Kg; p.o.) was used as standard for diuretic activity. Control group of animals (n=6) received normal saline (10 ml/Kg)

Experimental design

The animals were divided into 5 groups of 6 rats each as follows; Group I: received only 10ml/kg normal saline Group II: received Fursemide 20 mg/kg, Group III: received MKC 100 mg/kg body weight p.o., Group IV: received MKC 200 mg/kg body weight p.o. and Group V: received MKC 300 mg/kg body weight p.o.

Diuretic activity

Rats were fasted overnight and treated with vehicle, Fursemide and mavilinga kashayam as stated above along with normal saline (10 ml/kg). The rats were placed in metabolic cages and the urine samples were collected for 24h, measured using a

standard measuring cylinder. The amount of urine (in ml) collected for 24 h was compared and tabulated (94).

Natriuretic activity

Estimation of Sodium and Potassium content of the urine samples of all groups of animals were done by using a laboratory model flame photometer. The ratio of Na+/K+ is calculated for Natriuretic activity. A value greater than 2.0 indicates a favorable Natriuretic effect. Ratio greater than 10.0 indicates a potassium sparing effect(95).

Statistical analysis

The results were expressed as mean ± S.E.M. Statistical comparisons were made by means of newmann keuls multiple range tests. p values smaller than 0.05 was considered as significant.

4.4.3 ANTISPASMODIC ACTIVITY

IN-VITRO ANTISPASMODIC ACTIVITY OF MKC 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.

5. MICROBIOLOGICAL ANALYSIS

ANTI - MICROBIAL STUDIES

Aim

To study the Anti-microbial action of ***MAVILINGU KASAYAM***

Components of Muller Hinton agar medium

Beef extract - 300gms/lit

Agar - 17 gms/lit

Starch - 1.5 gms/lit

Casein Hydrolysate - 17.5 gms/lit

Distilled water - 1000 ml

PH - 7.

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.

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.

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

Methodology:

Muller Hinton Agar plates are prepared and Pseudomonas, Staphylococcus, Enterococcus, Escherichia coli, Streptococcus,klebsiella is inoculated separately.

The prepared disc of *maviligukasayam* are placed over the incubated plate using sterile forceps and incubated for 24 hours at 37 degree celcius. The plates after 24 hours incubation are observed for the zone of inhib

6. RESULTS AND DISCUSSION

The Siddha herbal drug *mavilingu kasayam* had been subjected to various studies to establish the works of Siddhars to be true. Literary collections, physicochemical and Elemental analysis, toxicological study, pharmacological study and clinical study are done to prove the activity of *mavilingu kasayam in* lithotriptic, diuretic, anti-spasmodic activity.

Standardisation of the test drug

Standardisation of the drug is more essential to derive the efficacy and potency of the drug, which was analysed by the various methods. The results of physicochemical and biochemical analysis have been done and tabulated. Pharmacological activity and toxicological results of the drug were derived. The results reveal the effectiveness of the trial drug *mavilingu kasayam* has been proved by the following scientific parameters.

PHYSIOCHEMICAL ANALYSIS

The following characters have been noted in *MKC*

Organoleptic characters

Table no-7

Colour in day light	Greenish yellow
Smell	Pleasant odour
Taste	Bitter, L.Pungent
Appearance	powder
Touch	Nice

Table no-8 Physicochemical properties

Table S.NO	Parameter	Result
1	Loss on drying	5.2%
2	Ash content	8.1%
3	Acid insoluble ash	1.3%
4	Water sol.matter	21.5%
5	Alcohol sol.matter	18.9%
6	PH	6
7	Nice	coarse powder

Interpretation:

Total Ash: Total ash value of plant material indicated the amount of minerals and earthy materials present in the plant material. The total inorganic content (ammonium, potassium, calcium, chloride, iron, etc.,) present in the drug is measured through the Total ash value. The total ash value of the drug was determined as 8.1%.

Acid insoluble ash: The acid insoluble ash value of the drug denotes the amount of siliceous matter present in the plant. The quality of the drug is better if the acid insoluble value is low. It is 1.3% for the drug.

Water soluble ash: Water-soluble ash is the part of the total ash content, which is soluble in water. It is 21.5% for the drug

Alcohol soluble ash: Alcohol-soluble ash is the part of the total ash content, which is soluble in alcohol. It is 18.9% for the drug

- These are indicating the approximate measure of chemical constituents of crude drug.
- The percentage of soluble matters present in the drug is determined by the values of water extractive and ethanol extractive.
- Water-soluble extractive value plays an important role in evaluation of crude drugs
- The alcohol-soluble extractive value was also indicative for the same purpose as the water-soluble extractive value

Loss on drying:

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

MICROBIAL LIMIT TESTS:

Table No: 9 Results for Microbial limit test :

S.No	Microbes	Colony measurements
1.	Total viable aerobic count	1.6 $x10^4$ col/g
2.	Total *Enterobacteriaceae*	Nil
3.	Total fungal count	3.5 $x10^2$ col/g
	Test for specific pathogen	
1.	*Salmonella sp*	Nil
2.	*Staphylococcus aureas*	Nil
3.	*E.coli*	Nil
4.	*Pseudomonas aeruginosa*	Nil

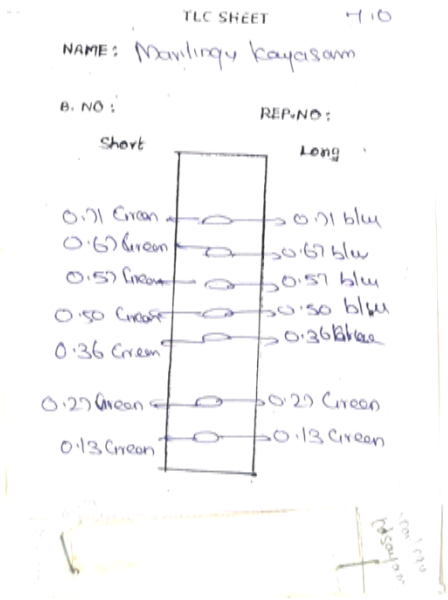
Interpretation:

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, Pseudomonas aeruginosa and Staphylococcus aureus were found to be completely absent in the drug

THIN LAYER CROMATOGRAPY (TLC):

Figure no:3



Interpretation:

Spots of green, blue colours were detected on the chromatoplates under UVlight.The R_f value of spots in the sample was approximately green(0.71,0.67,0.57,0.50,0.36,0.27,0.13) blue(0.71,0.67,0.57,0.50,0.36).it indicates the presence of active compound in the sample which is responsible for the therapeutic effect.

BIO-CHEMICAL ANALYSIS OF "MAVILINGU KASAYAM"

Table No: 10 Preliminary tests for basic and acidic radical:

S.NO	EXPERIMENT	INFERENCE
1.	TEST FOR CALCIUM:	Absent
2.	TEST FOR SULPHATE:	**Present**
3.	TEST FOR CHLORIDE:	Absent
4.	TEST FOR CARBONATE:	Absent
5.	TEST FOR STARCH:	Absent
6.	TEST FOR IRON FERRIC:	Absent
7.	TEST FOR IRON FERROUS:	**Present**
8.	TEST FOR PHOSPHATE:	**Present**
9.	TEST FOR ALBUMIN:	Absent
10.	TEST FOR TANNIC ACID:	Absent
11.	TEST FOR UNSATURATION:	**Present**
12.	TEST FOR THE REDUCING SUGAR:	Absent
13.	TEST FOR AMINO ACID:	Absent
14.	TEST FOR ZINC:	Absent

Interpretation:

Sulphate:

- Sulphate may prevent the occurrence of any infection
- Sulphate is potent anti oxidant activity in human body[46]
- The main function of sulfate is to stabilize protein structure.
- Sulphate ion is used as counter ion for some cationic drug
- Sulphate has anti bacterial activity and it is one of the macronutrient of cells. It inhibits growth of yeasts and moulds in low pH and inhibits growth of enterobacteriae and other gram- negative bacteria in high pH

Iron:

- Iron improves the haematological level in the body and also it is essential for growth, reproduction, healing and immune function.
- In this the iron is present in ferrous form, which is soluble and readily absorbed in the intestinal lumen.
- The heme containing enzymes such as catalase and peroxidase protect cell against potentially damaging highly reactive species

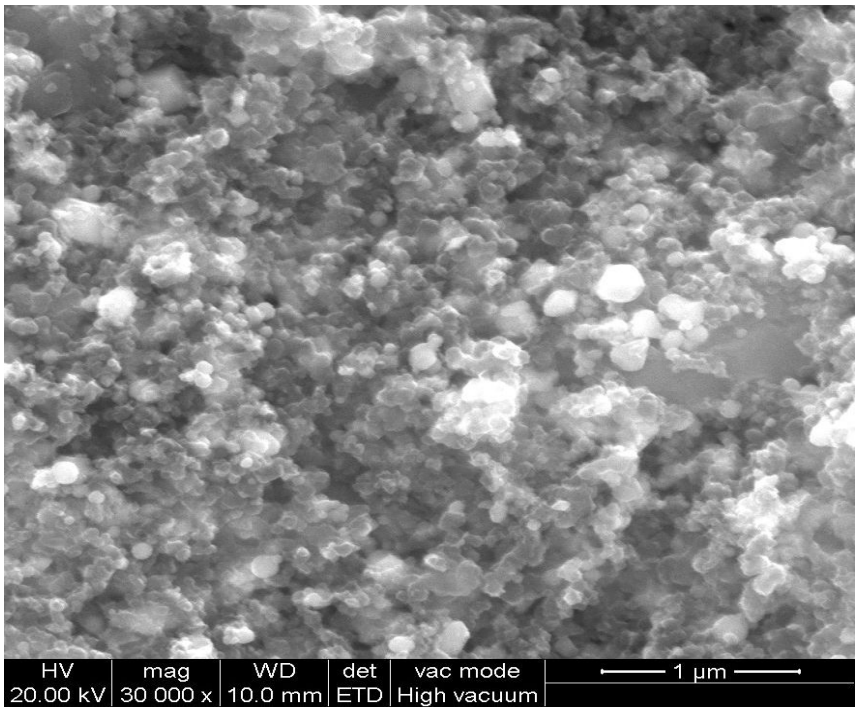
- Iron is needed for transporting oxygen and carbon dioxide. It also has other important roles in the body.

PHOSPHATE:

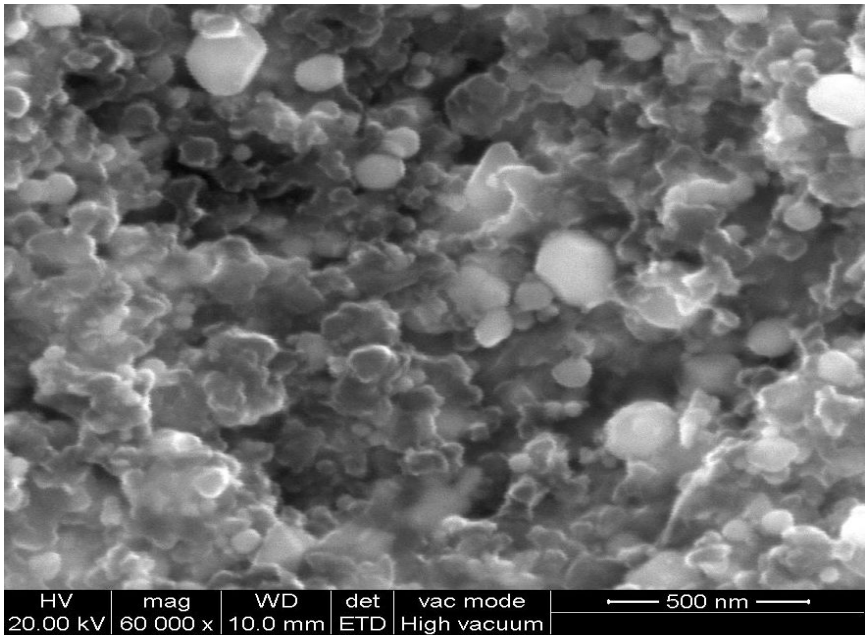
- The recommended dietary allowance of phosphate is based on the intake of calcium, the of **Ca:P** of 1:1 is recommended
- Phosphate filters out waste in your kidneys.
- Phosphate growth, maintains, and repair tissue and cells.
- Phosphate balance and use vitamins such as vitamins B and D, as well as other minerals like iodine, magnesium, and zinc.
- It assists in muscle contraction.

INSTRUMENTAL ANALYSIS

SEM RESULT:Figure no:4



SEM picture 30,000 magnification



SEM picture 60,000 magnification

Interpretation for SEM

The morphology of the Mavilingu kasayam sample 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. We have observed from SEM photographs that particles are spherical in shapes and sizes are in the range from 0.5 micron to 1 micron. Although the particle sizes of different batches showed similarity, it seems that these particles are aggregates of much smaller particles. When dispersed in an aqueous medium, these preparations form a negatively charged hydrophobic particle suspension. This hydrophobicity gives these particles a tendency to aggregate together to form larger particles. Mavilingukasayam 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.

FTIR RESULTS OF MKC:

Figure No: 5 FTIR Result Graph

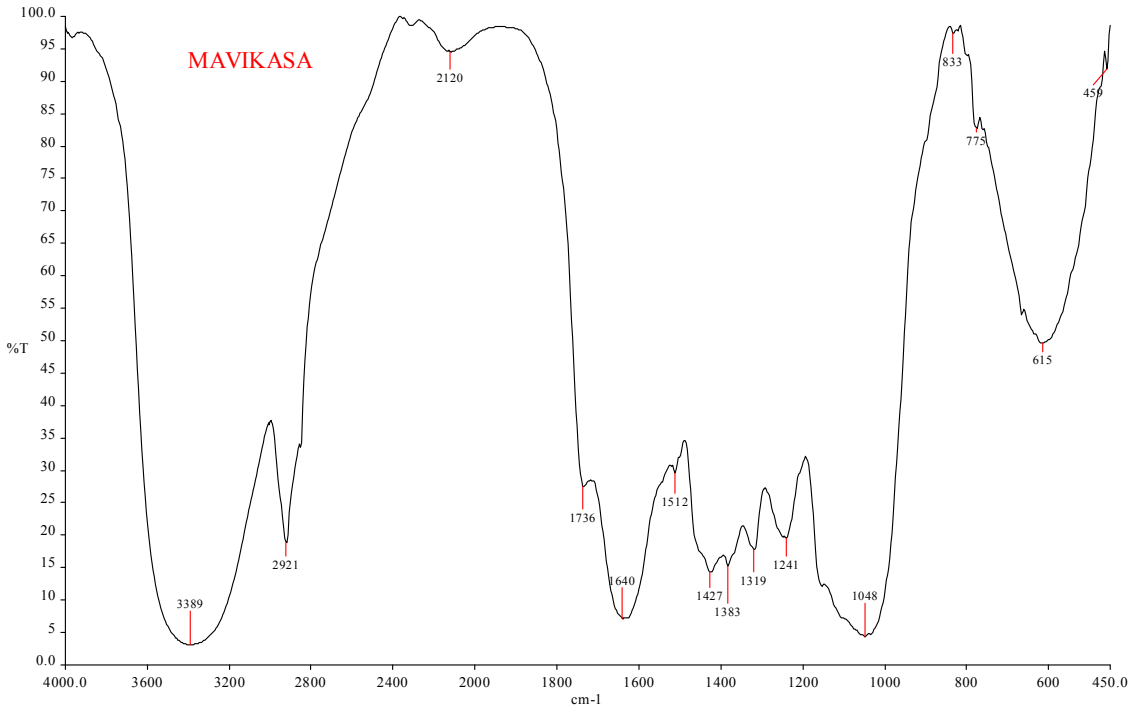


Table no - 11 Table showing represented functional groups

Absorption peak cm^{-1}	Stretch	Functional group
3389	O-H stretch,H-Bonded	Alcohols,phenols
2921	C-H stretch	Alkenes
2120	C(triple bond)C- stretch	Alkynes

1736	C=O Stretch	Esters,saturated aliphatic
1640	-C=C-	Alkenes
1512	N-O asymmetric stretch	Nitro compound
1427	C-C stretch(in ring)	Aromatics
1383	C-H bend	Alkenes
1319	C-N stretch	Aliphatic, amines
1241	C- N stretch	Aliphatic, amines
1048	C-N stretch	Aliphatic, amines
833	C-Cl stretch	Alkyl halides
775	C-H “oop”	Aromatics
615	C-Br stretch	Alkyl halides

Interpretation:

In FTIR the wave numbers between 4000cm^{-1}-400cm^{-1}. 4000 cm^{-1}-1500 cm^{-1} is known as functional group area.1500 cm^{-1}-600cm^{-1} is known as finger print area. The identity of FTIR of two compounds is much more characteristic than the comparision of their many physical properity. The corresponding absorption frequency by FTIR shows the presence of alcohols, phenols, alkenes, amines, aliphatic amines, alkyl halides, nitrocompound, aromatics esters, saturatedaliphatic, alkynes.

Amines groups:

- Amines groups act as neurotransmitters.
- It is involved in protein synthesis.
- This group of substances has analgesic activity.

Alcoholic groups:

- Alcoholic group of substances act as antimicrobial and antiseptic agents.

Phenolic groups:

- Phenolic group act as neurotransmitters.
- This group of substance has antimicrobial, antiseptic and antioxidant activities.

Alkanes groups:

- Alkanes have little biological activity.
- It is predominate in plants. They protect against bacteria and fungi.[45,46,48]

Alkyl Halides:

- These are group of compounds derived from alkanes containing one or more halogens.Some are used as anesthetics and antiseptic agents. Some of them are used in medicine for the elimination of hook worms

AROMATICS:

- Aromatics are good pain relievers. Auto-immune activities.

Ester:

- Esters are organic compound formed when an acid combine with an alcohol and release water. Ester of Sitostanol decrease in cholesterol absorption

Alkyne:

- Alkyne conjugated probes which plays an important role in inflammation

ICP-OES (Inductively Coupled Plasma Optical Emission Spectroscopy):

The drug *MKC* sample was analysed by the Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) to detect the trace elements and other elements quantitatively. The result of ICP-OES is given on the **Table No: 12.**

Sample ID	Elements Symbol	Wavelength(nm)	Concentration (A)P
1.	Al	1396.152	BDL
2.	As	188.979	BDL
3.	Cd	228.802	BDL
4.	Ni	231.604	BDL
5.	Pb	220.353	BDL
6.	Cu	327.393	BDL
7.	Hg	253.652	BDL
8.	Ca	315.807	72.180mg/l
9.	K	766.491	83.821 mg/l
10.	Fe	238.204	22.376mg/l
11.	Mg	285.213	02.104 mg/l
12.	Na	589.592	74.320 mg/l
13.	P	213.617	106.341mg/l
14.	Zn	206.200	02.228mg/l
15.	S	180.731	01.314 mg/l

BDL:Below Detectable Limit (Normal – 1 ppm)

1% = 10000ppm,

1ppm = 1/1000000 or 1ppm = 0.0001%

The toxic metals and the permissible limits

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

Plants are the rich source of elements. These elements have vital function. They were significant role against a variety of diseases. Hence they were used for several health problems.

The MKC showed presence of Ca,Na, K, Mg, Zn,P,S,Fe. In *mavilingu kasayam,* the heavy metals like As, Hg, Cd, Pb,Cu and trace element like Ni,Al were below detectable level. The result indicate that the formulation is extremely safe as it contains heavy metals within specified limits.

Interpretation:

- **Sodium :**sodium is an impartant component in the humen body.The human diet has requirement of 1.5grams of sodium intake everyday.sodium regulate the blood pressure and regulate blood volume.it help to transmit impulses for nerve and muscle contrction. It regulat the acid balance.Too much of sodium can be harmful to kidney disease.because kidney cannot eliminate excees sodium.so sodium restriction is recommended if blood presser is high and ckd condition.
- **Potassium:** In the presence of Sodium and Potassium regulate the acid-base balance of the body fluids.They regulate the water balance of the body fluids.They help to preserve the neuromuscular irritability by maintaining a state of equilibrium on account of their relative proportion in the Extra cellular fluid and Intra cellular fluid. Potassium is an electrolyte critical in maintaining proper acid-alkaline balance. Potassium is highly present inintra cellular fluid(ICF), while its partner, sodium, is highly present in extra cellular fluid(ECF).It is their perfect ratio that maintains fluid and electrolyte

balance. Potassium dilates the arteries and relaxes the smooth muscles and increases the blood flow (F.J. Haddy, 2012).

- **Calcium:** Calcium is an important component of cellmembrane. It controls i permeability and electrical properties of the cellmembrane. Calcium necessary for the maintenance and regulation of acid –base balance and water balance in the body. Calcium from food can help prevent kidneystone formation & help maintain bone density. Decreasing calcium intake; this will increases oxaluria
- **Magnesium**:Magnesium inhibits the formation of calcium-oxalate crystals in the urine. It also inhibit the stone formation by inhibition of growth of crystals as well as aggregation. Inhibition of crystal attachment of calcium oxalate appears to require supra-physiologic concentrations.Magnesium reduces the formation of stones by reducing the calcium oxalate in the blood that makes up the stones.

- **Zinc:** Mg, Zn which are considered to be stone inhibitors and anti microbial activity against E.coli which in the commonest urinary tract infection among patients with urolithiasis[57]. Zinc is a micro nutrient and is required for wound healing.

- **Phosphorus:** Phosphorus is an important constituents of phosphate buffers in the blood and urine.It is required for the formation of certain physiologically important phosphorus containing compounds like phospholipids, coenzymes and enzymes of intermediary metabolism

- **Iron:**In this the iron is present in ferrous form, which is soluble and readily absorbed in the intestinal lumen.The heme containing enzymes such as catalase and peroxidase protect cell against potentially damaging highly reactive species. Iron is needed for transporting oxygen and carbon dioxide. It also has other important roles in the body.Iron is essential for oxygen transport, energy production, and cellular growth and proliferation.Iron deficiency may be determined by measurement of iron levels within the body,

mainly serum ferritin levels, which may also help distinguish between iron deficiency

- Most of the iron in the body is found in the hemoglobin of red blood cells and in the myoglobin of muscle cells and anemia associated with chronic disease, such as chronic kidney disease (CKD)

- **Sulphate**: Sulphate may prevent the occurrence of any infection. Sulphate is potent anti oxidant activity in human body. The main function of sulfate is to stabilize protein structure. Sulphate ion is used as counter ion for some cationic drug. Sulphate has anti bacterial activity and it is one of the macronutrient of cells. It inhibits growth of yeasts and moulds in low pH and inhibits growth of enterobacteriae and other gram- negative bacteria in high Ph

TOXICITY STUDIES

EVALUATION OF ACUTE TOXICITY OF *MKC*

Effect of Acute Toxicity (14 Days) of *MKC*

RESULT Table no –13 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 no-14 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
Tail pinch response	Normal	3	3	3	3	3

Table no-15 Hand held observation

Functional and Behavioral observation	Observation	Control	5 mg/kg (G-I)	50 mg/kg (G-II)	300 mg/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 no-16 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 ***MKC*** 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 ***MKC*** is 2000 mg/kg.

INTERPERTATION:

MKC 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 ***MKC*** 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 ***MKC*** on the growth rate. Body weight change in drug treated animals was found normal.

Sub-Acute Toxicity Study in Wister rats to Evaluate Toxicity Profile of MKC

Results and Interpretation of 28-day repeated dose oral toxicity study in rats

Table no-17

EFFECT OF SUB-ACUTE DOSES (28 DAYS) OF MKC ON BODY WEIGHT

GROUP	CONTROL	MKC LOW DOSE	MKC MIDDLE DOSE	MKC HIGH DOSE
1st day	116.5±1.25831	115.833±2.76184	114.667±3.22146	108±2.17562
7th day	126.5±2.17179	121.667±2.69155	121.667±2.24598	118.833±0.980363
14th day	132.833±1.44722	130.667±3.25235	130±2.0166	129±1.89737
21st day	139.167±1.35195	140.5±1.17615	136.333±2.1551	140.333±1.2561
28th day	143.833±1.74005	144±1.98326	145.333±1.72562	141.833±1.01379

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

EFFECT OF SUB-ACUTE DOSES (28 DAYS) OF MKC ON FOOD INTAKE IN gms Table no -18

GROUP	CONTROL	MKC LOW DOSE	MKC MIDDLE DOSE	MKC HIGH DOSE
1st day	39.3333±2.09231	37.8333±3.8159	36.3333±2.76486	38±4.93288
7th day	47.6667±3.13759	47.3333±2.77689	40.8333±1.85143	42.1667±2.57445
14th day	47.5±2.86065	48.6667±2.61619	54.1667±6.62026	50.1667±4.09403
21st day	53±3.06594	54.1667±1.90467	59.6667±3.3731	55.3333±4.55827
28th day	54.1667±3.45848	49.1667±2.68845	51.1667±3.40016	51.6667±2.71621

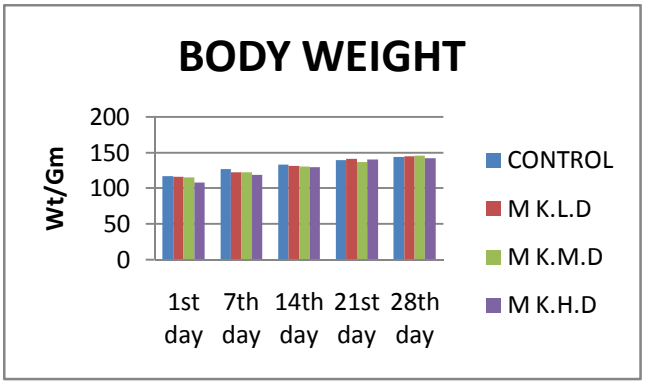
Values are expressed as the mean ± S.D. Statistical significance (p) calculated by one way ANOVA followed by dunnett's. ns- not significant $^{**}P< 0.05$ calculated by comparing treated group with control group

EFFECT OF SUB-ACUTE DOSES (28 DAYS) OF MKC WATERINTAKE IN gms Table no19

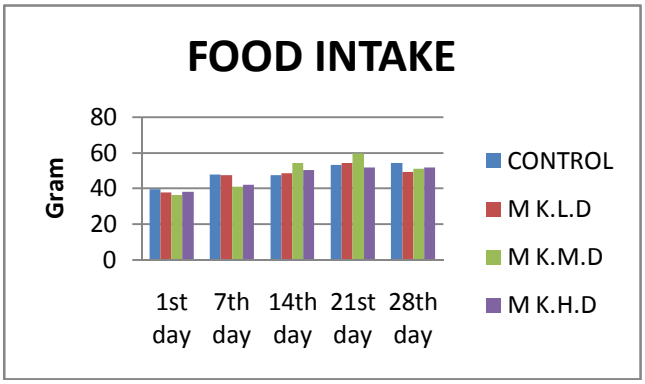
GROUP	CONTROL	MKC LOW DOSE	MKC MIDDLE DOSE	MKC HIGH DOSE
1st day	33.3333±6.08094	39.8333±5.51009	33.5±2.66771	85.8333±5.0492
7th day	43.5±8.65544	56±6.3456	42.5±6.37574	54.8333±6.36352
14th day	65.8333±4.98275	87.6667±2.69155	81.6667±4.86256	65.8333±4.98275
21st day	91.8333±3.43915	94±5.07937	81.3333±1.85592	88.6667±3.10555
28th day	96.3333±2.77689	86.6667±1.68655	91±3.25576	85±5.09902

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

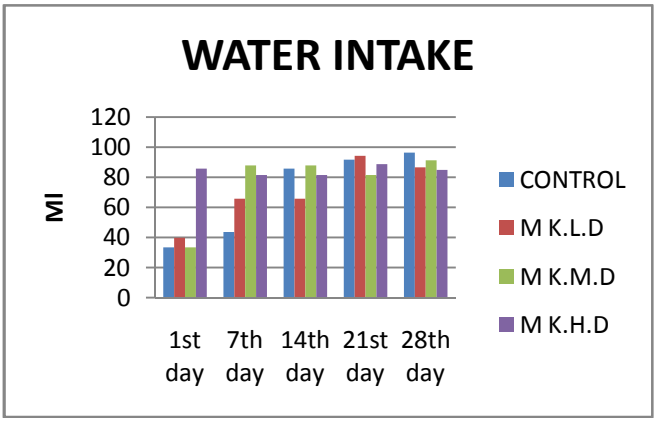
EFFECT OF SUB-ACUTE DOSES (28 DAYS) OF MKC ON BODY WEIGHT IN gms Figure no- 6



EFFECT OF SUB-ACUTE DOSES (28 DAYS) OF MKC ON FOOD INTAKE IN gms Figure no:7



EFFECT OF SUB-ACUTE DOSES (28 DAYS) OF MKC WATERINTAKE IN GMS Figure no:8

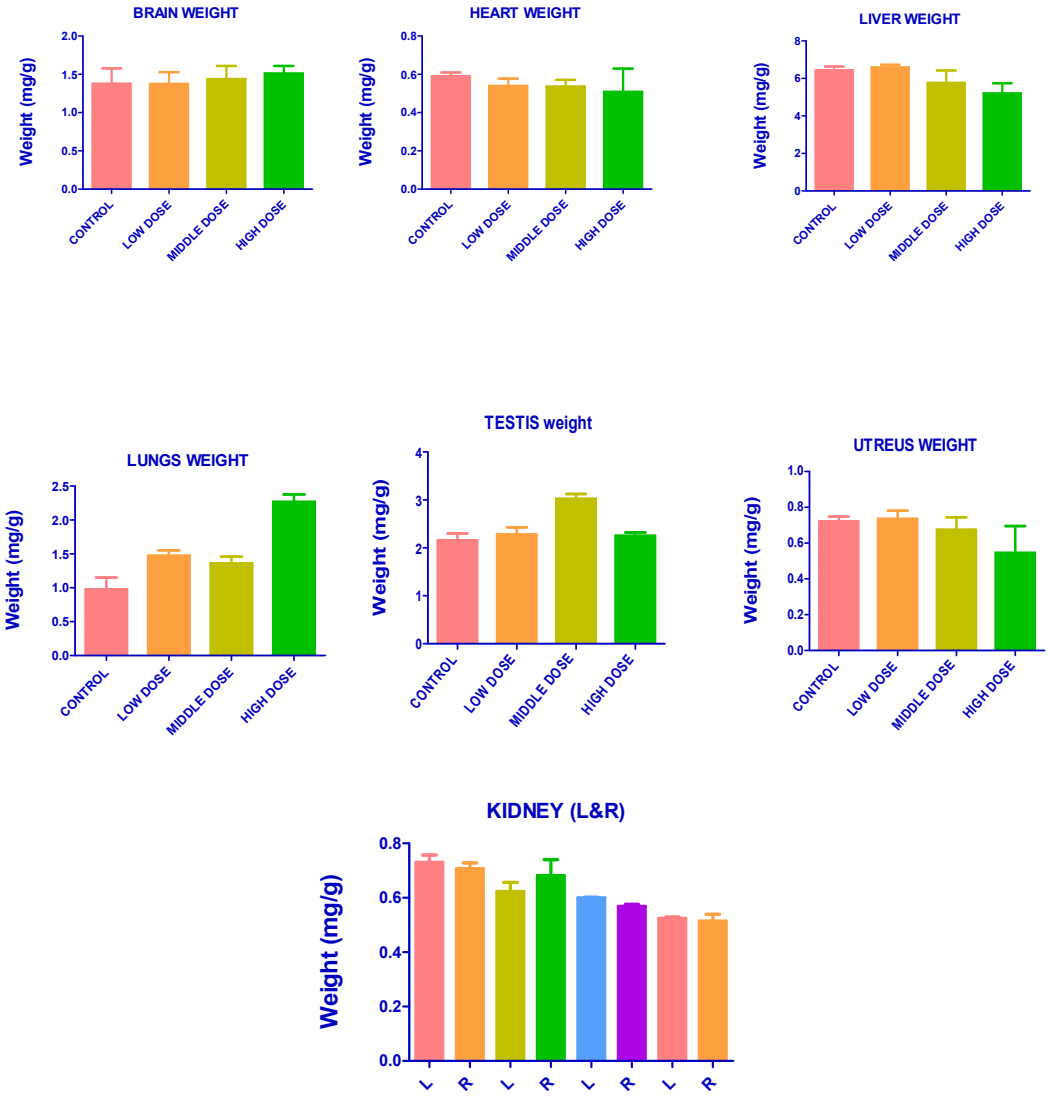


EFFECT OF SUB-ACUTE DOSES (28 DAYS) OF MKC ON ORGAN WEIGHT IN gms Table no 20

GROUP		CONTROL	MKC LOW DOSE	MKC MIDDLE DOSE	MKC HIGH DOSE
BRAIN		1.379±0.198698	1.37633±0.152471	1.43933±0.169412	1.51267±0.095919
HEART		0.590667±0.0189854	0.540667±0.0367892	0.536667±0.0345704	0.509333±0.120257
LIVER		6.44267±0.193168	6.59433±0.133225	5.77067±0.652078	5.211±0.533896
LUNGS		0.979±0.171966	1.47567±0.0764991	1.36167±0.0974241	2.26967±0.108201
TESTIS		2.15867±0.142625	2.281±0.146848	3.02667±0.0977707	2.256±0.0650231
UTRES		0.722±0.0262742	0.736±0.0454899	0.675333±0.0692082	0.546±0.149055
KIDNEY	L	0.576±0.0394	0.6287±0.07753	0.5847±0.09359	0.4657±0.081578
	R	0.5477±0.05045	0.5203±0.08391	0.5553±0.0452	0.523±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

EFFECT OF SUB-ACUTE DOSES (28 DAYS) OF MKC ON ORGAN WEIGHT IN gms Figure no:9



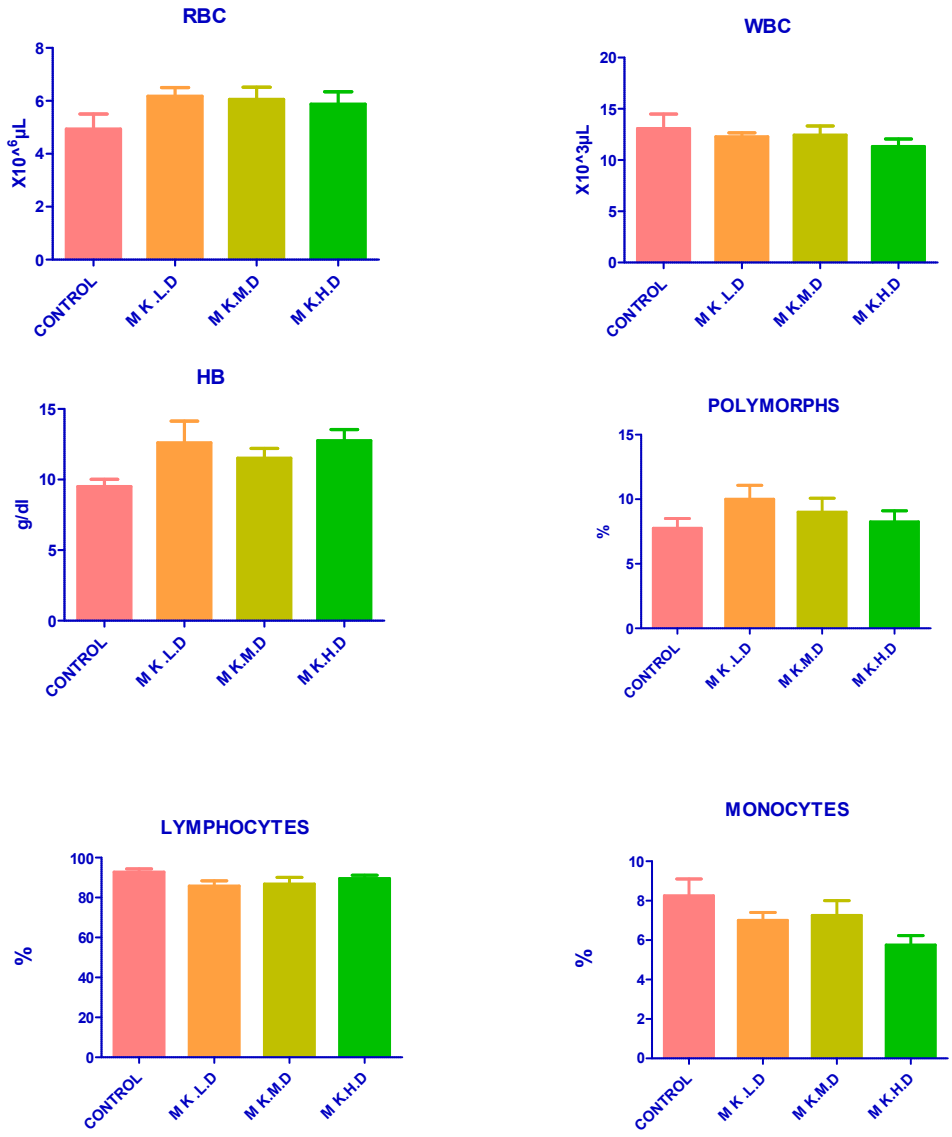
EFFECT OF SUB-ACUTE DOSES (28 DAYS) OF MKC ON HAEMATOLOGICAL PARAMETERS

Table no-21

GROUP	CONTROL	MKC LOW DOSE	MKC MIDDLE DOSE	MKC HIGH DOSE
RBC (X10^6µL)	4.935±0.569408	6.1775±0.328085	6.055±0.460534	5.875±0.471478
WBC(X10^3µL)	13.075±1.40971	12.275±0.377216	12.45±0.870345	11.325±0.733002
HB (g/dl)	9.5±0.514782	12.605±1.5332	11.525±0.682367	12.75±0.790042
POLYMORPHS %	7.75±0.75	10±1.08	9±1.08	8.25±0.8539
LMPHOCYTES%	92.75±1.65202	85.75±2.59406	86.75±3.37577	89.5±1.70783
MONOCYTES %	8.25±0.853913	7±0.408248	7.25±0.75	5.75±0.478714

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

EFFECT OF SUB-ACUTE DOSES (28 DAYS) OF MKC ON HAEMATOLOGICAL PARAMETERS Figure no:10



EFFECT OF SUB-ACUTE DOSES (28 DAYS) OF MKC ON BIOCHEMICAL PARAMETERS Table no-22

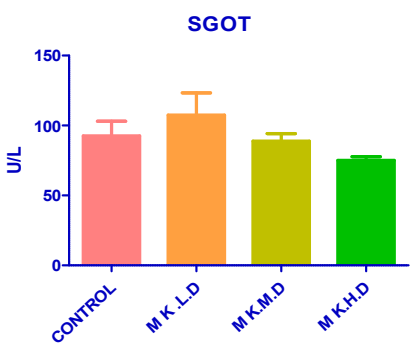
GROUP	CONTROL	MKC LOW DOSE	MKC MIDDLE DOSE	MKC HIGH DOSE
SGOT (U/L)	92.6±10.416	107.48±15.8746	88.8033±5.32485	75.0333±2.70883
SGPT(U/L)	36.44±4.64251	93.6667±19.7425	53.5±4.05956	37.0333±3.96414
ALP (U/L)	129.667±2.18581	137±6.08276	122.333±2.96273	151.4±27.1048

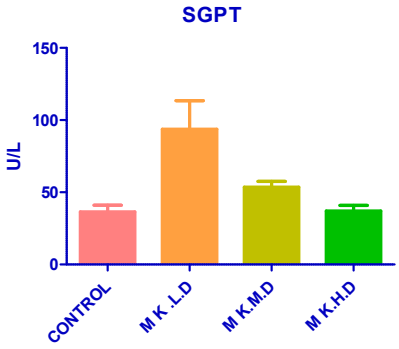
Table no-23 TOTAL BILURUBIN

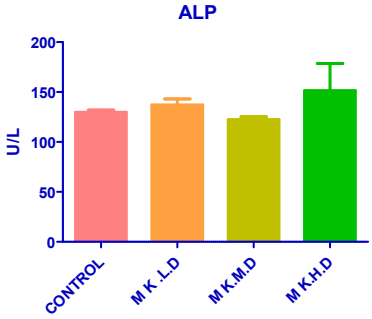
GROUP	CONTROL	MKC LOW DOSE	MKC MIDDLE DOSE	MKC HIGH DOSE
TOTAL BILURUBIN (g/ml)	1.323±0.0409	1.44±0.09165	1.777±0.193	1.51667±0.095

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

EFFECT OF SUB-ACUTE DOSES (28 DAYS) OF MKC ON BIOCHEMICAL PARAMETERS Figure no:11







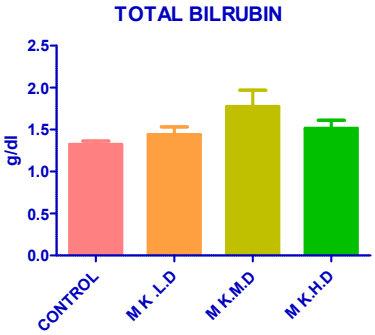
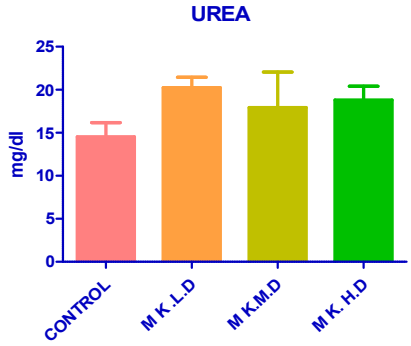
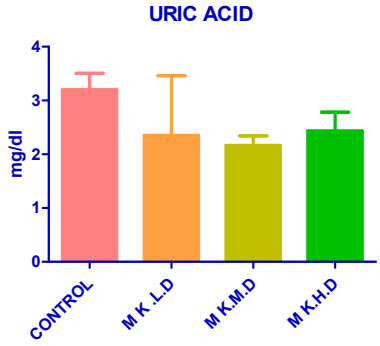


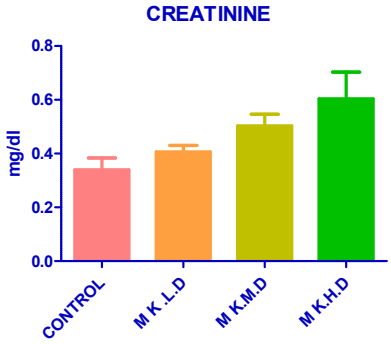
Table no-24 UREA,URIC ACID,CREATININE

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	MKC LOW DOSE	MKC MIDDLE DOSE	MKC HIGH DOSE
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	0.4066±0.0233	0.5033±0.04333	0.603333±0.09955







EFFECT OF SUB-ACUTE DOSES (28 DAYS) OF MKC ON ELECTROLYTES Table no 25

GROUP	CONTROL	MKC LOW DOSE	MKC MIDDLE DOSE	MKC HIGH DOSE
SODIUM (mg/ml)	4.275±0.278014	6.625±0.278014	6.175±0.298259	5.485±0.455952
CALCIUM (mg/ml)	1.5275±0.097145	4.425±0.579332	3.555±0.100125	5.475±0.426956
PHOSPHORUS (U/L)	0.45±0.0426224	1.23±0.740282	0.6175±0.033757	0.635±0.062249

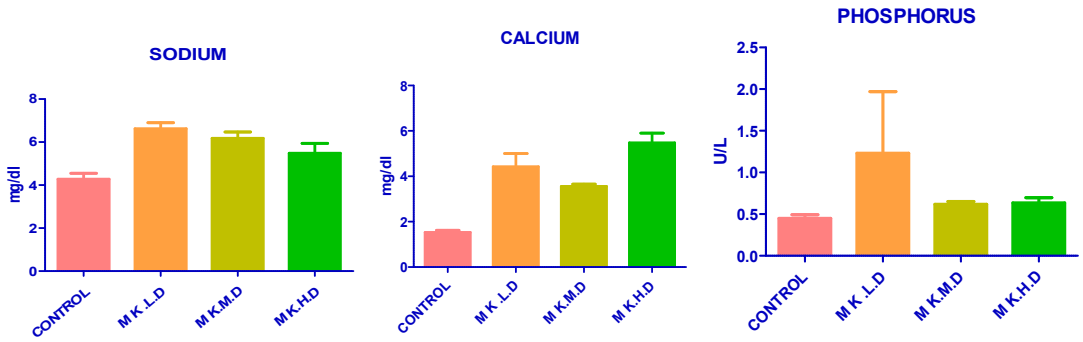


Figure no:12

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.

6.0 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 no-17 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.20 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 no- 21 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 the day 29th and recorded in Table no 22 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.

INTERPERTATION:

1) All the animals from control and all the treated dose groups up to 15ml/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 29th, revealed no abnormalities attributable to the treatment.

6) Biochemical analysis conducted at the end of the dosing period on day 29^{th}, 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.

PHARMACOLOGICAL STUDY

LITHOTRIPTIC ACTIVITY

Table no-26 Effect on urinary output in urolithiasis induced rats

Days	GP1	GP2	GP3	GP4	GP5	GP6
0	7.65± 0.42	7.42± 0.40	7.84± 0.76	7.60± 0.78	8.55± 0.92	8.32± 0.88
14	7.85± 0.66	6.95± 0.42**a	8.40± 0.95**b	9.12± 1.34**b	10.35± 1.55**b	11.32± 1.66**b
28	8.36± 0.72	6.36± 0.32**a	8.66± 1.25**b	10.32± 1.44**b	11.45± 1.62**b	11.92± 1.72**b

GP_1- Normal; **GP_2**- Lithiatic Control; **GP_3**- MK (100mg/kg); **GP_4**-MK (200mg/kg); **GP_5**-MK (300mg/kg); **GP6**-Cystone herbal tablets (500mg/kg)

- Values are expressed as mean ± SEM
- Values were found out by using ONE WAY ANOVA Followed by Newman keul's multiple range tests.
- **(a) Values were significantly different from normal control (GP_1) at $P< 0.01$
- **(b) Values were significantly different from Lithiatic control (GP_2) at $P<0.01$

Table.no:27

EFFECT ON URINARY BIOCHEMICAL PARAMETERS ON THE DAY 14

GP	Protein (mg/dl)	Magnesium (mg/dl)	Calcium (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
GP_1	71.92± 2.95	4.30± 0.52	5.68± 0.50	8.28± 0.68	0.82± 0.10	17.86± 1.45	35.94± 2.85
GP_2	152.32 ± 5.30**(a)	1.15 ± 0.20**(a)	25.20± 1.90**(a)	16.60 ± 1.60**(a)	1.61 ± 0.14**(a)	30.72 ± 3.20**(a)	76.64 ± 4.30**(a)
GP_3	88.35 ± 3.92 **(b)	2.60 ± 0.30**(b)	19.30 ± 2.15**(b)	10.60 ± 0.95**(b)	0.99 ± 0.09**(b)	24.30 ± 2.45**(b)	45.60 ± 3.75**(b)
GP_4	84.52 ± 3.55**(b)	2.82 ± 0.40**(b)	13.45 ± 0.80**(b)	12.35 ± 0.84**(b)	0.92 ± 0.11**(b)	22.28 ± 2.32**(b)	40.82 ± 3.22**(b)
GP_5	85.15± 3.70**(b)	2.66 ± 0.44**(b)	15.70 ± 0.60**(b)	9.95 ± 0.85**(b)	0.89 ± 0.09**(b)	23.22 ± 1.92**(b)	36.25 ± 2.30**(b)
GP_6	81.30± 2.85**(b)	3.33 ± 0.58**(b)	17.68 ± 0.42**(b)	8.92 ± 0.76**(b)	0.84 ± 0.10**(b)	20.22 ± 1.88**(b)	35.25 ± 2.30**(b)

GP_1- Normal; **GP_2**- Lithiatic Control; **GP_3**- MK (100mg/kg); **GP_4**- MK (200mg/kg); **GP_5**- MK (300mg/kg); **GP_6** - Cystone herbal tablets (500mg/kg)

- Values are expressed as mean ± SEM
- Values were found out by using ONE WAY ANOVA Followed by Newman keul's multiple range tests.
- **(a) Values were significantly different from normal control (GP_1) at P< 0.01
- **(b) Values were significantly different from Lithiatic control (GP_2) at P<0.01

Table no-28 EFFECT ON URINARY BIOCHEMICAL PARAMETERS ON THE 28TH DAY

GP	Protein (mg/dl)	Magnesium (mg/dl)	Calcium (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
GP_1	74.85 ±3.80	4.30 ±0.45	7.15 ±0.70	3.42 ±0.50	0.92 ±0.30	17.45 ±1.36	31.55 ±2.48
GP_2	163.25 ±8.22**(a)	1.84 ±0.50**(a)	21.85 ±1.56**(a)	13.55 ±1.45**(a)	1.60 ±0.55**(a)	50.25 ±4.60**(a)	80.60 ±4.70**(a)
GP_3	90.20 ±5.60**(b)	2.15 ±0.38**(b)	14.68 ±1.05**(b)	8.54 ±0.58**(b)	1.20 ±0.36**(b)	26.60 ±2.65**(b)	47.70 ±3.32**(b)
GP_4	87.50 ±5.40**(b)	3.92 ±0.53**(b)	12.40 ±0.95**(b)	8.30 ±0.45**(b)	1.22 ±0.40**(b)	23.45 ±2.55**(b)	44.30 ±3.16**(b)
GP_5	83.30 ±4.60**(b)	3.66 ±0.50**(b)	11.80 ±0.40**(b)	7.90± 0.42**(b)	1.46 ±0.28**(b)	21.30 ±2.10**(b)	42.30 ±2.75**(b)
GP_6	84.60 ±4.85**(b)	3.50 ±0.48*(b)	11.18 ±0.32**(b)	7.18± 0.34**(b)	1.24 ±0.50**(b)	20.70 ±2.15**(b)	40.75 ±2.25**(b)

GP_1- Normal; **GP_2-** Lithiatic Control; **GP_3-** MK (100mg/kg); **GP_4-** MK(200mg/kg); **GP_5-** MK(300mg/kg); **GP_6** Cystone herbal tablets(500mg/kg)

- Values are expressed as mean ± SEM
- Values were found out by using ONE WAY ANOVA Followed by Newman keul's multiple range tests.
- **(a) Values were significantly different from normal control (GP_1) at $P< 0.01$
- **(b) Values were significantly different from Lithiatic control (GP_2) at $P<0.01$

Table.no:29

EFFECT ON SERUM PARAMETERS ON THE 28th DAY

GP	Magnesium (mg/dl)	Calcium (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
GP_1	4.94 ±0.58	10.56 ±1.50	3.20 ±0.22	0.44 ±0.25	6.2 ±0.70	12.90 ±1.55
GP_2	1.99 ±0.42**(a)	17.45 ±2.46**(a)	9.70 ±1.22**(a)	1.22 ±0.55**(a)	13.55 ±1.70**(a)	29.18 ±3.48**(a)
GP_3	3.80 ±0.55**(b)	12.80 ±1.80**(b)	4.28 ±0.48**(b)	0.70 ±0.42**(b)	9.50 ±0.90**(b)	24.22 ±2.80**(b)
GP_4	3.96 ±0.20**(b)	12.80 ±1.55*(b)	4.12 ±0.43**(b)	0.75 ±0.45**(b)	8.80 ±0.85**(b)	22.78 ±2.68**(b)
GP_5	3.85 ±0.50**(b)	11.10 ±1.45**(b)	3.70 ±0.40**(b)	0.60 ±0.32**(b)	8.55 ±0.80**(b)	18.20 ±1.60**(b)
GP_6	4.12 ±0.52**(b)	10.15 ±1.40**(b)	3.15 ±0.40**(b)	0.50 ±0.28**(b)	7.15 ±0.60**(b)	17.28 ±1.56**(b)

GP_1- Normal; **GP_2-** Lithiatic Control; **GP_3-** MK (100mg/kg);

GP_4- MK (200mg/kg); **GP_5-** MK (300mg/kg);

GP_6 - Cystone herbal tablets (500mg/kg)

- Values are expressed as mean ± SEM
- Values were found out by using ONE WAY ANOVA Followed by Newman keul's multiple range tests.
- **(a) Values were significantly different from normal control (GP_1) at $P< 0.01$
- **(b) Values were significantly different from Lithiatic control (GP_2) at $P<0.01$

RESULT:

In the present study, chronic administration of 1% (v/v) ethylene glycol aqueous solution to wistar rats resulted in hyperoxaluria. Urinary concentration of the various ions investigated varied drastically, following ethylene glycol treatment.

EFFECT OF MKC ON URINARY PARAMETERS ON DAY 14 & 2

The oxalate excretion was 24hr on day 14^{th} & 28^{th} respectively for GP_1. It increased significantly ($P < 0.001$) on day 14^{th} & 28^{th} day in GP_2 following ethylene glycol treatment. Treatment at a dose of MKC 100mg/kg, 200mg/kg and 300mg/kg and cystone herbal tablet at a dose of 500mg/kg (GP_3 to GP_6) reduced the oxalate excretion significantly to ($P<0.01$) on 14^{th} day treatment. Likewise on 28^{th} day, treatment with this MK reduced the oxalate excretion significantly to ($P<0.01$) in (GP_3 toGP^6) rats respectively. The results are shown in the table no: 27 & 28.

The urinary calcium excretion was increased significantly on day 14^{th} and 28^{th} day in GP_2 following ethylene glycol treatment. The calcium excretion was significantly reduced to treatment with MK at a dose of 100 200 and 300mg/kg and cystone herbal tablet at a dose of 500mg/kg (GP_3 to GP_6) reduce the calcium excretion significantly to on 14^{th} day treatment likewise on 28^{th} day calcium excretion was significantly reduced to s24hr ($P<0.01$) in (Gp_3 to Gp_6) rats respectively. The results are shown in the table no: 27 & 28.

Likewise phosphate and creatinine excretion values gradually increased in GP_2 on the 14^{th} & 28th day. However in (GP_3 to GP_6) grouped treated animals these elevated values were significantly reduced on 14^{th} and 28^{th} day respectively. However, regarding creatinine in (GP_3 to GP_6) these elevated values were significantly reduced on 14^{th} day and on 28^{th} day respectively. The results are given in table no:27 & 28..

Likewise urinary protein and uric acid concentration increased following ethylene glycol treatment in GP_2 and it reached maximum respectively on the 14^{th} & 28^{th} day. On treatment with MK at a dose of 100,200 and 300mg/kg and cystone herbal tablet at a dose of 500mg/kg (GP_3 to GP_6) the protein and uric acid excretion was restored to near normal limits in (GP_3 to GP_6) for protein on 14^{th}day and on 28^{th} day ($p<0.001$) and for uric acid on 14^{th} day and on 28^{th} day ($P<0.01$). The results are tabulated in table no:27 & 28.

In GP_2 lithiatic control rats, the magnesium level in urine gradually decreased following ethylene glycol treatment on the 14^{th} & 28^{th} day . Subsequent administration of the MK and cystone herbal tablets enhanced the magnesium excretion significantly on 14^{th} day & 28^{th} day.

EFFECT OF MKC ON SERUM PARAMETERS ON DAY 28

In prophylactic study the serum parameters such as calcium, uric acid, creatinine, oxalate, phosphate levels were increased significantly in GP_2 (Lithiatic control) following ethylene glycol treatment, Treatment with MK at a dose of 100,200 and 300mg/kg and cystone herbal tablet at a dose of 500mg/kg (GP_3 to GP_6) reduce the all above mentioned parameters significantly. On the contrary the magnesium levels were decreased significantly in GP_2 (Lithiatic control) following ethylene glycol treatment**.** After treatment with MK at a dose of 100mg/kg, 200mg/kg and 300mg/kg and cystone herbal tablet at a dose of 500mg/kg (GP_3 to GP_6) the magnesium level was restored near to normal levels.

INTREPERTATION:

Results show increased elimination of oxalate,calcium, phosphate, magnesium, uricacid, creatinine which decresed circulating level of those minerals and as prevent calculi formation of kidneys

DIURETIC ACTIVITY

Table no: 30 Diuretic activity of MKC (urine Volume) in 24 h

Group	Treatment	Urine Volume
I	Normal saline 10ml/kg	8.10±0.55
II	Frusemide 20mg/kg	12.95±0.94**
III	MKC 100mg/kg	11.25±0.70**
IV	MKC 200mg/kg	12.35±0.85**
V	MKC 300mg/kg	13.15±0.95**

Values are Mean ± SEM, n=6, **p<0.01.

Table no:31 Natriuretic activity of MKC

Treatment	Na+	K+	Na+/K+
Normal saline 10ml/kg	1.72±0.10	0.72±0.03	2.38
Frusemide 20mg/kg	3.25±0.25**	0.88±0.04**	3.69
MKC 100mg/kg	1.98±0.16*	0.72±0.01ns	2.75
MKC 200mg/kg	2.25±0.18**	0.70±0.02ns	3.21
MKC 300mg/kg	2.30±0.24**	0.71±0.03ns	3.23

Values are Mean ± SEM, n=6, *p<0.05, **p<0.01, NS - not significant

Results:

Table -30 shows the urine volume collected in 24 h for all the groups. It is evident that the MKC treated groups excreted more urine than the control groups. The MKC at 100, 200 and 300 mg/kg exhibited comparable effect with that of the reference drug Fursemide 20 mg / kg and the results were statistically significant. Table -31 shows the sodium and potassium content of the urine for all groups. The amount of Sodium excreted was increased for Fursemide treated group; statistically significant rise in Na+ excretion was also noticed for MKC treated groups. The potassium content excreted in the urine was statistically insignificant for all the groups. The Natriuretic effect was calculated by employing the formula Na+ / K+. It was found that the MKC treated groups possess favorable Natriuretic effect. The present study showed that the MKC significantly increases the urine output and excretion of urinary sodium and had no effect on the urinary potassium excretion. Diuretics have two separate connotations; increase urinary par se and net loss of solute (i.e. electrolyte) and water (i.e. saluretic). These two processes are involved in the suppression of renal tubular reabsorption of electrolytes, water and low molecular weight organic compounds into the blood stream and a consequence; promote the formation of urine (96). An attempt to extrapolate the diuretic action of plant extract from rats to man using the activity of Fursemide in the organism as a guideline has been reported.

INTERPERTATION:

The results clearly shows that the MKC at doses of 100, 200 and 300 mg / kg produced significant dose dependent increase in urinary excretion and urinary sodium loss but no effect on urinary potassium loss with respect to control and standard drug treated groups. The data demonstrates that the MKC has diuretic effect, Natriuretic effect but no potassium sparing effect and is as potent as Fursemide. (97).

ANTI-SPASMODIC ACTIVITY

DoseResponse Relationship Observations of Acetylcholine

Table no-32

Si.No	Concentration/dose	Acetylcholine
		Response (cm)
1	0.1 ml	2.8cm
2	0.2 ml	3.4cm
3	0.4 ml	3.9 cm
4	0.8 ml	4.5 cm
5	1.6 ml	5.2cm

Dose Response Relationship Observations of Atropine

Si.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	-

Dose Response Relationship Observations of Acetylcholine and **MKC**

Si.No	Concentration/dose	Acetylcholine + **MKC**
		Response (cm)
1	0.1 ml +0.1 ml	3.3 cm
2	0.2 ml +0.2 ml	3.7 cm
3	0.4 ml +0.4 ml	4.4 cm
4	0.8 ml +0.8 ml	4.7 cm
5	1.6 ml + 1.6 ml	5.6 cm

Comparative Dose Response of Ach and Ach followed by **MKC**

Table no-33

Si No	Treatment	Dose(ml)	response	% of response
1	Acetylcholine	0.1 ml	2.8 cm	
2		0.2 ml	3.4 cm	
3		0.4 ml	3.9 cm	
4		0.8 ml	4.5 cm	
5		1.6 ml	5.2 cm	
6	Acetylcholine + **MKC**	0.1 ml +0.1 ml	3.3 cm	17.85 %
7		0.2 ml +0.2 ml	3.7 cm	8.82 %
8		0.4 ml +0.4 ml	4.4 cm	12.82 %
9		0.8 ml +0.8 ml	4.7 cm	4.44 %
10		1.6 ml + 1.6 ml	5.6 cm	7.69 %

Figure no:13

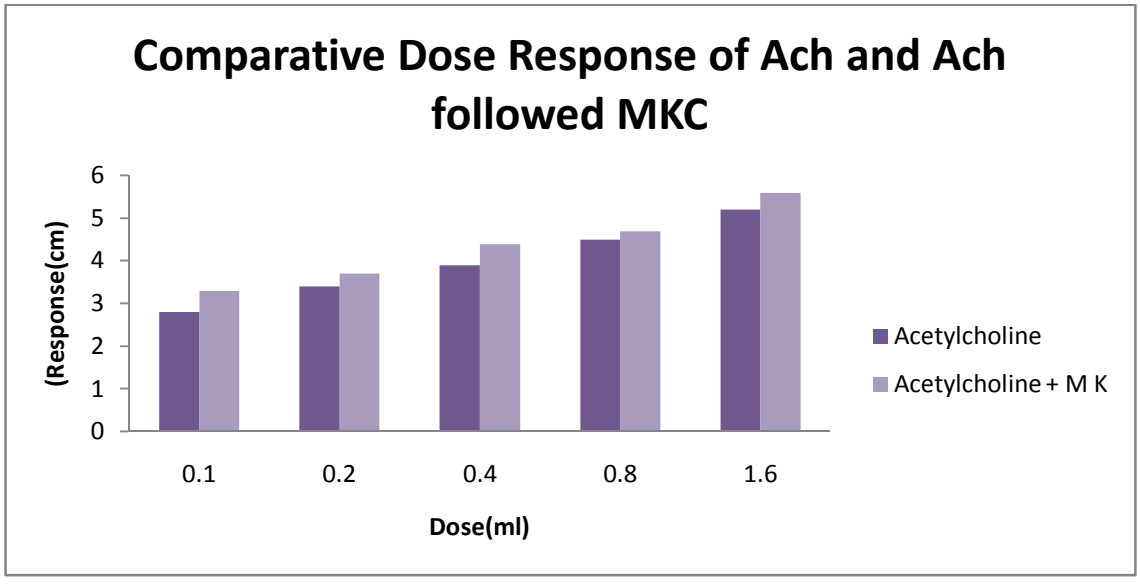


Fig: Comparative dose response relationship of Acetylcholine and **MKC** on excisedrat ileum.

RESULTS:-

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

INTERPERTATION:

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 **MKC** there was a marked decrease in contraction of ileum was observed. This revealed that **MKC** possess a high degree of spasmolytic (anti-spasmodic) activity by blocking cholinergic receptors

ANTI – MICROBIAL ACTIVITY

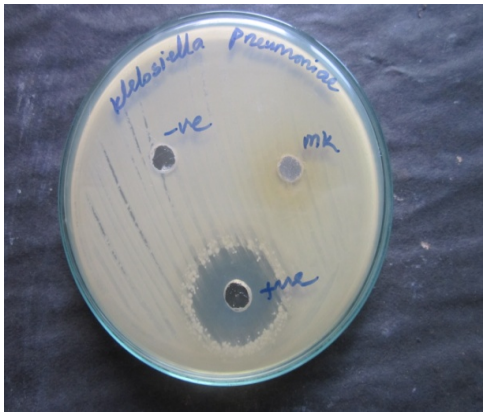
Table No: 34 Anti – Microbial Activity of Mavilingu Kasayam

S.No	Organism (Culture)	Susceptibility	Zone size	
			Streptomycin zone size	Medicine size
1.	E.Coli	Sensitive	12mm	21mm
2.	Klebsiella pnemoniae	Resistant	25mm	-
3.	Pseudomonas aeruginosa	Resistant	17mm	-
4.	Streptococcus muntant	Moderate Sensitive	23mm	17mm
5.	Staphylococcus aureus	Sensitive	14mm	24mm
6.	Enterococcus faecalis	Moderate Sensitive	20mm	11mm

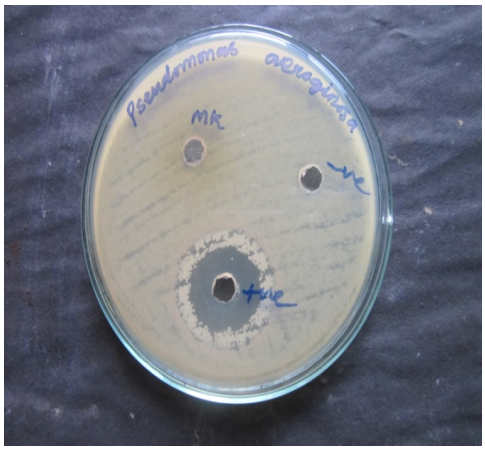
Figure no : 14 **Anti – Microbial Activity of Mavilingu Kasayam**

Ecoli

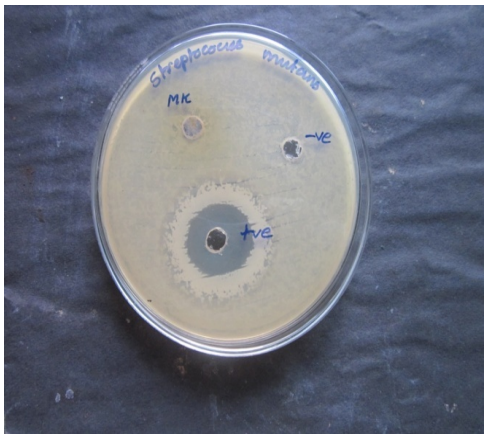
Staphylococcus aureus



Pseudomonas aeruginosa

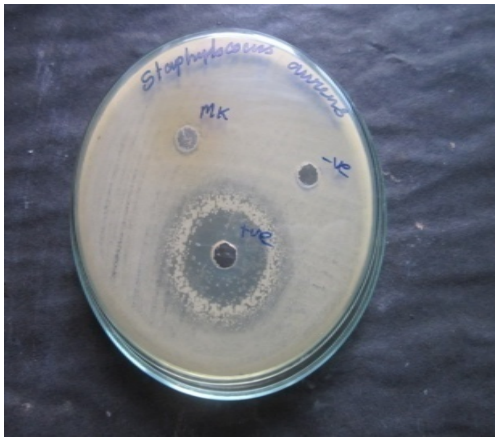


Streptococcusmutant



Staphylococcus aureus

Enterococcus faecalis



Interpretation:

The drug Mavilingu kasayam anti Microbial activity was compared with standard drug Streptomycin for the following pathogens they are E.coli, klebsiella, pseudomonas, streptococcus, staphylococcus & enterrococcus the drug mavilingu kasayam showed the inhibition of growth of micro organism at 100 micro gram/ml concentration for the organism there results represents Mavilingu kasayam potencially inhibit the E. coli, staphylococcus and moderately inhibit streptococcus, enterrococcus.

7. SUMMARY:

- The trial drug MAVILINGU KASAYAM is a herbal formulation selected from the text book of AGASTHIYAR 2000, PART 3 (Page no.31) for the validation of safety, efficacy and therapeutic potency on kalladaippu.

- The raw drug Mavilingu Vadaku Ver was collected from Nagarkovil, nerunjl ver, chirupeelai ver and peramutti ver collected from Tirunelveli. These raw drugs was identified and authenticated by Gunapadam Department, Govt. Siddha Medical College, Palamkottai, Tamil nadu.

- A review of the literatures and lateral research works reveals that all the ingredient of mavilingu kasayam used in treating kalladaippu.

- In siddha literature alteration in diet and water intake increases the vatha and pittha dhosa in body it will affect the abana vayu. So urine will be stagnanted in urinary tract. So salt deposit easily anywhere in the urinary tract and forms the stone.

- The ingredient of mavilingu kasayam paeramutti and nerunjil biotransformed sweet taste. The ingredient mavilingam and chirupeelai biotransformed pungent taste. Sweet taste neutralizes the vatham and pitham at the same time pungent taste neutralize the abana vayu. So these characters very much useful to kalladaippu.

- Physiochemical analysis shows determination of total ash, insoluble ash and moisture content it was within acceptable range. It indicates the longer shelf life period.

- TLC result shows variation of R_f values indicates the presences of various chemical compounds in this drug.

- Biochemical analysis shows the presence of Sulphate, Iron ferrous, Phosphate, Unsaturated compound. All of these possess significant potency in the elimination of renal calculus.

- ICP-OES analysis of these drug shows heavy metals Like Ca, Na, K, Mg, Zn, P,S,Fe. In *Mavilingu kasayam,* the heavy metals like As, Hg, Cd, Pb,Cu and trace element like Ni,Al were below detectable level. This reveals the safety of the drug.

- FTIR analysis of mavilingu kasayam revealed the presence of alcohols, phenols, alkenes, amines, aliphatic amines, alkyl halides, nitrocompound, aromatics esters, saturatedaliphatic, alkynes groups. Alcoholic group of act as antimicrobial activity.Phenolic groups has anti microbial, anti oxidant activities, which are used in the inhibition of Renal stone.

- SEM photographs that particles are rounded in shapes and sizes are in the range from 0.5 micron to 1 micron.The size of the particles enables better absorption which denotes that the trial drug could have potent drug delivery.

- Anti microbial activity of mavilingu kasayam potencially inhibits *Escherchia.coli, staphylococcus* and moderately inhibit *Streptococcus, enterococcus*.

- The pharmacological study revealed that trial drug mavilingu kasayam possesses a lithotriptic (anti lithiatic), diuretic and antispasmodic activity. The experimental evidence obtained in the laboratory model.

- Toxicological study of both acute and sub-acute toxicity study were carried out in animal model Wistar albino rat according to the OECD guidelines. The acute toxicity of test drug there were no physical and behavioral changes observed in female Wister rats 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.

- The sub-acute toxicity after the repeated dose of 28 days was done. The mortality, functional observations, hematological and biochemical investigations were made. There was no significant change seen in the normal values. Thus the toxicological study of the test drug greatly establishes the safety and gives the justification for long time administration.

- Thus the drug mavilingu kasayam was very effective and safe for treating kalladaippu without causing any adverse effects.

8. CONCLUSION

The trial drug ***MAVILINGU KASAYAM*** was selected for the elaborate study of its efficacy on Kalladaippu. From the literature review, physiochemical, pharmacological, microbiological, bio chemical, Instrumental analysis. It has been concluded that Mavilingu kasayam has got a good Lithotriptic (Anti lithiatic), Diuretic, Anti – spasmodic activities and hence effective for Kalladaippu.

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