PRECLINICAL STUDY OF SIDDHA DRUG KATTUVAI MATHIRAI'S ANTI-DIARRHOEAL, ANTI-PYRETIC 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

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PALAYAMKOTTAI
DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"Pre clinical study of herbo mineral drug *KATTUVAI MATHIRAI* for its anti-diarrhoeal, anti-pyretic and anti-spasmodic activities"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. A. Kingsly M.D(s)., Reader, Head of the Department,** Post Graduate Department of *Gunapadam*, Govt. Siddha Medical College, Palayamkottai and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

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ABBREVIATIONS

KM	-	KATTUVAI MATHIRAI
CPCSEA	-	Committee for the purpose of control and supervision of experimental animals.
DC	-	Differential Count
ESR	-	Erythrocyte Sedimentation Rate
FTIR	-	Fourier transform infrared spectroscopy
Hb	-	Haemoglobin
IAEC	-	Institutional Animal Ethical Committee.
ICP-OES	-	Inductively coupled plasma optical emission spectrometry
Ig E	-	Immunoglobulin E
LDH	-	Lactate Dehydrogenase
MCV	-	Mean Corpuscular Volume
OECD	-	Organisation for Economic Co-operation and Development
PCV	-	Packed Cell Volume.
PGE	-	Prostaglandin E
RBC	-	Red Blood Corpuscles
SEM	-	Scanning electron microscope
CCD_S	-	Charge coupled devices.
SPME	-	Solid phase micro extraction
TCD	-	Thermal conductivity detector
FID	-	Flame Ionization detector
CCD	-	Catalytic combustion detector
LD	-	Low dose
Mg		Milligram
Kg		Kilogram
LD_{50}		Lethal Dose $_{50}$
p.o		peros
ML		Milliliter
%		percentage
R&D		Research and Development

EDTA	Ethylene Diamine Tetra Acetic Acid
M	Male
g%	Gram percentage
g	Gram
NOAEL	No-Observed-Adverse-Effect-Level
MLD	Minimum Lethal Dose
MTD	Maximum Tolerated Dose

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Certified that the following plant drugs used in siddha in the siddha formulation ***KATTUVAIMATHIRAI*** internal for the treatment of ***BEDHI*** taken up for the post graduate dissertation study by **Dr.M.ELAKKIYA M.D (s)** (Reg.No :321612001) P.G Department of gunapadam are correctly identified and authenticated through Visual inspection /Experience, Education and training / Organoleptic character / morphology / Micromorphology / taxonamical / microscopic method.

Tamil Name	Botanical Name	Family	Part Used
Ilavampisin	Bombax malabaricum	Bombacaceae	Gum
Athividayam	Aconitum heterophyllum	Ranunculaceae	Root
Saathikkaai	Myristica fragrans	Myristicaceae	Nutmeg
Sathipathiri	Myristica fragrans	Myristicaceae	Arillus of nutmeg
Vilvam	Aegle marmelos	Rutaceae	Fruit
Kaaichukkatti	Acacia catechu	Mimosaceae	Extract
Kirambu	Syzigium aromaticum	Myrtaceae	Bud
Oomathai	Datura metal	Solanaceae	Leaf juice
Neermulli	Hygrophila auriculata	Acanthaceae	Seeds

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TAMIL NAME	ENGLISH NAME	CHEMICAL NAME
LINGAM	CINNABAR	RED SULPHIDE OF MERCURY

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1. INTRODUCTION

Siddha system of medicine is a form of traditional medicine which originated from south India. It is the only medical system that is said to be bestow immortality. This system not only deals with medicine but with spirituality, righteous way of living, rejuvenation and its main aim is attainment of perfection.

The siddha system of medicine is one of the pillars of indian system of medicine. It is dedicate bequest of Siddhars.

Siddha system is the first system to emphasize health as a perfect state of physical, psychological, social and spiritual component of a human being. This explanation is quoted in "Thirumanthiram" which is pioneer word by saint Thirumoolar as follows.

மறுப்பதுடல் நோய் மருந்தென லாகும்
மறுப்ப துளநோய் மருந்தெனச் சாலும்
மறுப்பதினி நோய் வராதிருக்க
மறுப்பது சாவை மருந்தென லாமே"

- திருமந்திரம்.

Siddha system that insist that the physician should enquire the nature of the disease, its cause and its method of cure and treat it faithfully Envagai Thaervugal (the eight methods of clinical examination) are used to determine the diagnosis aetiology treatment and prognosis of diseases.

Our unique system of Tamil medicine is based upon two main theories. They are the Panchabootha theory and Mukkutra theory. According to Panchabootha theory the universe is formed of five elements. Namely, Pirithvi (Earth), Appu (water), Theyu (fire), vaayu (wind) and is formed of it in definite proportion.

This is explained as thiruvalluvar as,

"நிலம் நீர் தீவளி விசும் போடைந்தும்
கலந்த மயக்கம் உலகம் ஆதலின்

According to Mukkutra theory, the three vital humours namely vatham, pitham and kabam. When in equilibrium keep the body in homeostasis but when vitiated either single or in combination bring about disease. It is quoted by thiruvalluvar as follows,

"மிகினும் குறையினும் நோய் செய்யும் நூலோர்
வளிமுதலா எண்ணிய மூன்று"

In the present human era, many diseases threaten the life of human beings, due to the modern life style, food habits, etc. Infectious diseases most commonly affects all age group peolpe from children to adults. One of the most important common and infectious

disease is diarrhoea, which is compared to *Bedhi* in siddha literatures . *Bedhi* is a disease of Gastro intestinal tract. It is an infectious disease occurs due to poor sanitation. The description of the disease is clearly depicted in Siddha Maruthuvam.

In siddha system of medicine, *Bedhi* is also known as *Athisaram*, *kazhichal*, *kirahani*.

As per literature evidence from, sikitcha rathna deepam yennum vaidhiya nool indicated for all types of *Bedhi*. Hence I have selected my dessertation topic is *KATTUVAI MATHIRAI* to evaluate Anti-diarrhoeal, Anti-pyretic, Anti-spasmodic activities.

2. AIM AND OBJECTIVES

AIM

The aim of this study is to do a scientific review to validate the safety and efficacy of *"Kattuvai Mathirai"* for Anti-diarrhoeal, Anti-pyretic and Anti-spasmodic activities.

OBJECTIVES

- The main objective of the present study is to high light the safety and efficacy of *Kattuvai Mathirai* in the treatment of *Bedhi*, the following methodology was adopted to evaluate the drug and its standardization studies.
- To collect the literature systematically from siddha texts as well as modern science.
- To standardize the preparation of drug according to classical siddha literature.
- To subject the drug to physico chemical and chemical analysis.
- To detect the elements present in the drug by instrumental analysis.
- To study the acute and subacute toxicity profile of *Kattuvai Mathirai* according to OECD 423 and 407 guidelines.
- Evaluation of Anti-diarrhoeal activity of test drug.
- Evaluation of Anti-pyretic activity of test drug
- Evaluation of Anti-spasmodic activity of test drug
- To analyse all the above study results to validate the advantage of *Kattuvai Mathirai*

3.REVIEW OF LITERATURE

3.1. CINNABAR - இலிங்கம்

(Mercury sulphide)

3.1.1.GUNAPADAM ASPECT

General character

"பேதிசுரஞ்சந்நி பெருவிரண நீரொடுத
காதகடி காசங் கரப்பான்புண் -ணோத
வுருவிலிங்க சங்கதமா யூறுகட்டி யும்போங்
குருவிலிங்க சங்கமத்தைக் கொள்".
"ஆதி யிரதவுக் காதலாற் சாதிலிங்க
மோதி லிரதகுண முற்றூடலிற் -றீதுபுரி
குட்டங் கிரந்தி கொடுஞ்சூலை வாதமுத
லுட்டங் நோய்களையோட் டும்."

- குணபாடம் தாது சீவம்

It is effective in the treatment of diarrhoea, pyrexia, delirium, utricaria, diuresis, tuberculosis, scabies, unknown insect bites, syphilis, leprosy, eczema, skin diseases, throbbing pain (*Soolai*), and vatha diseases.

Synonyms

Aankuri, Inkuligam, Irraasam, Kadaivanni, Karppam, Kalikkam, Kaanjanam, Kaaranam, Saaniyam, Sandagam, Samarasam, Chendooram, Maniraagam, Milecham, Vani and Vanni.

Vernacular Names:

Tamil	:	*Lingam*
Eng	:	Cinnabar
Tel	:	Ingileekam
Canarose	:	Inglika
Malayal	:	Chayilyam
Urudu	:	Singraff

Properties:

Colour	:	Bright red colour
Appearance	:	Red crystal
Potency	:	Hot
Taste	:	No taste

Action:

Antipyretic

Tonic

Anti diarrhoeal

Anti *vaadha*

Method of Preparation:

Purified mercury	:	280gm
Sulphur	:	70gm
Pottasium Nitrate	:	70gm

Mercury is thoroughly mixed and triturated with sulphur. Pottasium nitrate is then added. Placed in a conical flask and burnt for 18 hours. After cooling, the red sulphide of mercury is collected out.

It is hard when it is put into fire it becomes smoke not soluble in water. It has no smell and taste and has not potency. It has properties of a tonic. This preparation is effective in the treatment of diarrhoea, Pyrexia

Method of Purification:

Alangium bark (Alangium saulifolium) – 1400gm is powdered and added with vinegar is powdered and added with vinegar 5.2litres and placed indews in the night. The next day it is rubbed and mixed well 35gm of cinnabar is tied well in a cloth and put into the above liquid. The pot is covered with another pot sealed with mud pasted cloth, dried and exposed in dew for one day. It is heated with low intensity fire (Flame) until the liquid is dehydrated for 24 hours. Then the cinnabar is taken out and cleaned well . This procedure is repeated using the vinegar soaked individually with the whole plant of vitis lanata (*pulikarunai*) and Indian *sarasaparilla* root as stressed in the following Tamil verses.

"சொல்லக் கேள் புலத்தியனே மகனேயிந்தந்
துறையான சாதிலிங்க சுத்திதானே
வெல்லக்கே லழிஞ்சில் புளிங் கருணையோடு
மேலான நன்னாரிக் காடித் தண்ணீர்
புல்லன் கேளதிலோர் மூன்று வைகல்
புகையாமல் விளக்கிலெரி யமுக்கு நீங்கும்
வெல்லக்கே ளளவுதொடி கொன்றே விசை
வெறுந் தண்ணீர் நாலுபடி வீதமாமே"

"மேகப் பிணி முதல் போல வென்றால்
மேலாம லிங்கம் சுத்தி செய்ய
மாகத் தமிஞ்சி கருணை நாரி
வாய்த்த புளிநீ ரொன்பா நாள்
பாகத் தழலெரி தீவி கைபோற்
பாம்புப் பீடகர் கைப்பண் பிதென
வேகமதை நீக்கென் றாடாய் பாம்பே
மேலாஞ் சரக்கிதென் நாடாய் பாம்பே"

Another method of purification :

1. Lime juice, cow's milk and the indian acalypha juice are mixed in equal proportion and allowed to fuse cinnabar so as to get it in a consolidated potency state
2. When the crude form of red sulphide of mercury is soaked for one day in mother's milk and lemon juice respectively it becomes purified.

முன்னுசாதி லிங்கந் தன்னை
முலைப்பாலி லாறவைத்தெ
பின்னரு நற் சம்பீரத்தின்
பெருங்கனிச் சாற்றிச் சுத்தி

சுரக்குகளுக்கொல்லம் இலிங்கம் இறையெனவும், மேக நோய்களுக்கு நமன்போன்றதெனவும், புகன்றிருப்பதை,

"இங்குலிகச் சரக்கொன்றே சரக்குக்கெல்லா மிறை யாகும்"
"மேகவகை வினைக்கு நமனான லிங்கம்"

தேரன் மருத்துவப் பாரதத்தில் அலறு சந்நிக்கு இலிங்கத்தின் ஆட்சி கூறப்பட்டுள்ளது.

Lingam is the chief dominant among the all drugs as well as its effective in sexually transmitted diseases.

Dosage

Internal	-	650mg
External	-	2.1gm
Reference	-	Baijasa *kalpam*

3. Cinnabar is soaked in mother's milk for 30 *naazhigai* (72 mins). It is removed and again fresh milk is added and the process is repeated above for 2 times.

***LINGAM* IN OTHER MEDICINES:**

1. BAIRAVA MATHIRAI:

Dosage : *Kundrimani alavu* (130mg)

Indications : *Athisaram*

- *Anuboga vaidhiya brahma ragashiyam – 339*

2. SANGUVADI MATHIRAI:

Dosage : *Kundrimani alavu* (130mg)

Indications : *Kirakani*

- *Anuboga vaidhiya brahma ragashiyam – 344*

3. PADIGA LINGA CHENDURAM:

Dosage : 3 – 5 *Kundrimani alavu* (650mg)

Adjuvant : Ghee, Butter

Indications : *Seetha bedhi, Ratha bedhi, Oozhi*

4. SUYAMAKKINI KUMARAN CHENDOORAM:

Dosage : *Kundrimani alavu* (130mg)

Adjuvant : Honey

Indications : *Kirani*

5. SANDA RASA PARPAM:

Dosage : *Panavedai* (488mg)

Adjuvant : Jaggery

Indications : *Bedhi, Kirakani, Oozhi*

TOXIC SYMPTOMS OF LINGAM:

Loss of taste and difficulty in eating and drinking water. Ulcers which in the buccal floor. Uvula(base of the mouth), inner portion of the tongue, larynx and large intestine, foul odor from the mouth, discharge of viscous, whitish saliva, difficult to speak and burning sensation are the toxic features of a red sulphide of mercury.

ANTIDOTE:

Nutmeg (Myristica fragrans), cubeb peper (Piper cubeba), root bark of a red cotton tree (Gossypium arboreum) and sugar candy each 4.2gm are made into a decoction and administered twice a day for 48 days.

CINNABAR

3.1.2. GEOLOGICAL ASPECT

Chemical Name

Natural	-	Cinnabar
Synthetic	-	Vermilion

Scientific Name

Mercuric sulphide (or) Mercuric II sulphide

Colour	-	Cochineal-red toward brownish red and lead gray
Symbol	-	HgS

Molecular formula

Molecular weight	-	232.68
Hardness	-	2.5
Specific gravity	-	8.176
Sublimes at	-	446 °C
Crystal systems	-	Hexagonal (or) Rhombohedral

Character

Cinnabar is practically insoluble in water, not allowed by HNO_3 or cold HCl, but decomposed by Con.H_2So_4 soluble in aquaregia with separation of sulphur and in warm HCl with evaluation of Hydrogen sulphate.

Preparation of cinnabar at Laboratory

One part of the mercury and four parts of the sulphur and to be placed parts of the sulphur and to be placed in an iron pot and heated for sometimes. The amalgam is then to be broken into pieces and put into a glass bottle, previously coated all round with mud and rag one inch deep and dried in shade. The bottle is to be heated for five days continuously by means of the five increasing gradually intensity at a uniform rate. The heating is then to be discontinued and the contents of the glass bottle taken out on the seventh day. The product will be found to be cinnabar.

History

Cinnabar commonly known as *Lingam*, Hingul, Shingara is fine red colour powder.

Alchemy is an art of refurination with using mercurial Indian alchemy probably being about 500 A.D.

Mercury and cinnabar was known and used in Europe, China 100 years before the first mention is Indian medicinal works. Chinese could properly select cinnabar as the best substitute of blood in colour. This made cinnabar soul and its components sulphur and mercury sub souls. Nothing better than cinnabar was found as equal to red colour. Cinnabar is a heavy native one mercury. Extracted all over the world found in all countries, except Antartica.

Occurance:

It is a mineral and important chief of mercury it occurs in minerable deposits in a very few localities, commonly found in veins and impregnation deposited near the surface of recent volcanic rocks and hot springs and most important deposits are Almaden and spine and it has been mined for more than 2500 years from these places.

Other localities are Idria, Italy/Kweichow, China and New Almaden, New Idria, California of USA. It is also mined in Navadautah, Olegon Arkananas and Texas.

Properties

Cinnabar exist in 2 modifications black and red. Both occur in native, artificially prepared cinnabar, however a vivid scarlet substances and is used as an artist pigment called 'vermilion'.

The scarlet red variety occurs as lumps and in Hexagona α – form crystals

Colour	-	Vermilion red
Hardness	-	2.5
Specific gravity	-	8.10

The shortest distance HgS. is 2.52 A and the binding between mercury and sulphur probably ionic in character.

Black merury sulphide (meta Cinnabar) (black Kubic β – form)

Colour	-	grayish black
Hardness	-	3
Specific gravity	-	7.6

The zinc blends structure with 5.82 A the shortest HgS distance in the same as in cinnabar. Black one is found in nature in small amount.

The meta cinnabar is the natural of mercury II sulphide black variety. But it also be synthesized artificially by following methods.

Passing hydrogen sulphide (H_2S) gas into mercurial salt solution.

$Hg^{++} + H_2S \quad HgS+2H^+$

Conversion of mercury II sulphide (black) to mercury sulphide (red)

1. In black variety is heated up to 500 C. It changes to red.
2. When the black powder of mercury II sulphide is sublimated to 446C.
3. It yields red form of HgS.
4. In ancient period it was prepared by *vaidya* using Hg and S in 5:1 ratio
5. Cinnabar on heating in a test tube. It sublimes and fumes of SO_4 and black mercury sulphide are obtained. Cinnabar by heating either in a current of air or with the addition of iron or quick lime giving or yielding mercury.
   - When treated with an a current air.

     $$HgS + O_2 \rightarrow Hg + O_2$$
   - When Erected with Iron

     $$HgS + 2\ Fe \rightarrow Hg + Fe_2S$$
   - With quick lime

     $$HgS + CaO \rightarrow Hg + CaS + CaSo_4$$

According to report of Dr.Chahergy, Cinnabar contains 86.22% mercury and 13.78% of sulphur

It is black in acid medium while in renstral medium. Change of red coloured cinnabar powder to back in presence of 3 myrobalans of fruit juice is due It change in pH value. Neutral form of mercuric sulphide is obtained by reacting sublimated mercury and sulphur in kupi pak vs.apparatus.

3.1.3.LATERAL RESEARCH

Mercury in traditional medicines: Is cinnabar toxicologically similar to common mercurials.

Jie Liu, Jing-Zheng Shi, [...], and Michael P. Waalkes

Abstract

Mercury is a major toxic metal ranking top in the Toxic Substances List. Cinnabar (contains mercury sulfide) has been used in traditional medicines for thousands years as an ingredient in various remedies, and 40 cinnabar-containing traditional medicines are still used today. Little is known about toxicology profiles or toxicokinetics of cinnabar and cinnabar-containing traditional medicines, and the high mercury content in these Chinese medicines raises justifiably escalations of public concern. This minireview searched the available database of cinnabar, compared cinnabar with common mercurials, such as mercury vapor, inorganic mercury, and organic mercury, and discusses differences in their bioavailability, disposition, and toxicity. The analysis showed that cinnabar is insoluble and poorly absorbed from the gastrointestinal tract. Absorbed mercury from cinnabar is mainly accumulated in kidney, resembling the disposition pattern of inorganic mercury. Heating cinnabar results in release of mercury vapor, which in turn can produce toxicity similar to inhalation of these vapors. The doses of cinnabar required to produce neurotoxicity are thousands 1000 times higher than methyl mercury. Following long-term use of cinnabar, renal dysfunction may occur. Dimercaprol and succimer are effective chelation therapies for general mercury intoxication including cinnabar. Pharmacology studies of cinnabar suggest sedative and hypnotic effects, but the therapeutic basis of cinnabar is still not clear. In summary, cinnabar is chemically inert with a relatively low toxic potential when taken orally. In risk assessment, cinnabar is less toxic than many other forms of mercury, but the rationale for its inclusion in traditional Chinese medicines remains to be fully justified.

3.2. *ACONITUM HETEROPHYLLUM* – அதிவிடயம்

3.2.1. GUNAPADAM ASPECT

Synonyms

Atthiranam, Pangurai, Mathiri

Vernacular names

English: Indian Atis Root

Tel : Ati-Vasa

Mal : Athividayam

Kan : Bhangura, upavishankan

Sans : Ativisha

Pers : Vajje-turki

Hind : Atlis

Duk : Atvika, vajje-turki

Part used: Root

Oraganoleptic character

Taste (*Suvai*) : Bitter *(Kaippu),* Pungent *(Kaarppu)*

Potency (*Thanmai*) : Hot *(Veppam)*

Biotransformation (*Pirivu*): Pungent *(Kaarppu)*

Actions

Stomachic *(Pasithethoondi)*

Astringent *(Thuvarppi)*

Febrifuge *(Veppagatri)*

Aphrodisiac *(Aanmai perukki)*

Tonic *(Uramakki)*

Anti periodic *(Murai veppagatri)*

General properties

அதிவி டயம்சர்க்க ராற்புதநோய் வெப்பு
கொதிமருவு பேதியொடு கோழை – எதிர்வாந்தி
என்றுரைக்கும் நோய்கூட்டம் இல்லா தகற்றிவிடும்
குன்றை நிகர்முலையாய்! கூறு

- குணபாடம் மூலிகை வகுப்பு

Indian atis root is used to treat wounds, fever, diarrhoea, cough, vomitting & subsides fever. It is used as anti-periodic and febrifuge by allopathic doctors.

Traditional uses

- Indian atis root powder 1300mg to 19 grams mixed with honey is given per periodic fever.
- 3200 gram to 6500 gram Indian atis Root powder is given thrice a day for weakness developed after fever.
- Dicoction for diarrhoea with fever

  Athividayam, chukku, Kudasapallai pattai, muthakasu, seenthilkodi, each 1 ¼ varagan add it to uzhaku water dehydrated to 4:1 ratio.

***ATHIVIDAYAM* IN OTHER MEDICINES**

1. GANTHAGA SINTHAMANI CHOORANAM

Indication : *Bedhi*

Dosage : *Verukadi alavu* (1250 – 1500mgm)

2. AANANDHA CHOORANAM

Indication : *Bedhi*

Adjuvant : Honey

3. PATTAI CHOORANAM

Indication : *Bedhi*

Dosage : *Verukadi alavu* (1250 – 1500mgm)

Adjuvant : Honey (or) Ghee

4. DHARMAR MATHIRAI

Indication : *Bedhi*

Dosage : *Thoothulangai alavu* (0.5803gm)

Adjuvant : Honey

5. MADHULAIYATHI KULIGAI

Indication : *Bedhi*

Dosage : *Sundaikkayalavu* (0.798gm)

Adjuvant : Buffalo curd

6. VIDAIYATHI MATHIRAI

Indication : *Bedhi*

Adjuvant : Decoction of cumin seeds

ACONITUM HETEROPHYLLUM

3.2.2. BOTANICAL ASPECT

Taxonomical classification

Kingdom	:	Plantae
Division	:	Angiosperms
Class	:	Eudicots
Order	:	Ranunculales
Family	:	Ranuculaceae
Genus	:	Aconitum
Species	:	Heterophyllum
Botanical name	:	Aconitum heterophyllum linn

Habitat

Usually found on humus – rich soils in the alpine and subalpine zones and in forests 2300-2900 meters.

Description

Leaves

Heteromorphous, glabrous, lowest on long panicle blade-orbicular-cardate (or) ovate-cardate in outline with a usually narow sinus, 5-lobed to middle, amplexicual.

Flower

Inflorescence slender raceme (or) lax, leafy panicle, cripso-pubescent, sepals bluish (or) violet, navicular obliquely erect, shortly (or) obscurely beaked 18-20 mm high, 8-9mm wide, carpels-5, elliptic oblong.

Root

Biennial paired, tuberous, whitish (or) grey.

Stem

Erect, simple (or) branched from 15-20 cm high glabrous below.

Seeds

Pyramidal 3-4mm long, blackish brown.

Chemical constituents

Aconitum heterophyllum contains

Alkaloids, amide alkaloids, flavonoids, flavonol glycosides, diterpenoid and norditerpenoid compounds.

Atisine, Heteratisine, heterophyllisine, heterophylline and heterophyllidine.

Therapeutic uses

- The root powder of ativisha with honey is prescribed for cough irritations and bronchitis.
- It is a good bitter remedy against gastroenteric fevers amongst infants and children.
- It is also useful in rheumatism, nervous pains as an analgesic.
- Inhalation of roots by nose in beneficial in headache, especially migraine.

3.2.3. LATERAL RESEARCH

A REVIEW ARTICLE ON AYURVEDIC/ HERBAL PLANT "ARUNA" (ACONITUM HETEROPHYLLUM). in International Journal of Advanced Research Int. J. Adv. Res. 5(2(1 FEB):319-325· February 2017 with 404 Reads DOI: 10.21474/IJAR01/3150

Abstract

In modern days there are Allopathic medicines are most widely used for general treatments. Herbal medicine are major remedy of Traditional and Ayurvedic medicine systems. Herbal or Ayurvedic medicine are less toxic or side effects. Medicinal plants are playing an important role in the drug discovery and development of new molecules. There are a number of herbs which are used from ancient time Aruna (Aconitum heterophyllum) is important herbs because in this medicinal plant there are a lot off pharmacological activity like Anti-inflammatory, hepatoprotective , Digestive activity. But some species of Aconitum plant is poisons in nature. This review should be of interest to readers in the areas of Pharmacognostical and Pharmacological activity and indication of Aconitum heterophyllum. And Aconitum Heterophyllum is one of the herbs which are used for its medicinal properties. Aconitum species belongs to the family Ranunculaceae . It's about 300 species are found in all over the world but in India, only 24 species are found in the Himalayas sub-alpine and alpine region, suits required about 2400-3600 m altitude above sea level. Seed germination micro propagation method is used for the cultivation of Aconitum plants. Aconitum heterophyllum has a lot of pharmacological use like in the treatment of urinary infection, diarrhoea, as an expectorant, Anti-inflammatory, hepatoprotective activity. It is a good source of diterpene alkaloids, flavonoids. There are some indication occurs in Aconitum heterophyllum roots part: Nausea, Bleeding piles, Periodic fever, Dyspepsia, Diarrhea, Dryness of mouth. When taken in larger doses may cause constipation.

3.3. *MYRISTICA FRAGRANS* – சாதிக்காய்

3.3.1.GUNAPADAM ASPECT

Synonyms

Kulakkai, Jathikkai

Vernacular names

Sanskrit	-	Jati – Phalam
English	-	Nutmeg
Hindu.Duk & Ber	-	Jayphal, Jaiphal, Jaepatri
Telungu	-	Jejikaya
Tamil	-	Jadikkay, Jathikkai
Malayalam	-	Jathika
Can	-	Jajikai
Burm	-	Zadu-phu
Malay	-	Bush-pala

Partused : Unriped fruit

Organoleptic Characters

Taste (*Suvai*) : Astringent *(Thuvarppu),* Pungent *(Kaarpu)*

Potency (*Thanmai*) : Hot *(veppam)*

Bio Transformation (pirivu) : Pungent *(Kaarpu)*

செய்கை

- Stimulant *(Veppamundakki)*
- Carminative *(Agattuvaivagatri)*
- Narcotic *(Moorchaiundakki)*
- Aromatic *(Manamooti)*
- Aphrodisiac *(Kamam peruki)*
- Tonic *(Uramakki)*

General properties

தாதுநட்டம் பேதி சருவாசி யஞ்சிரநோய்
ஓதுசுவா சங்காசம் உட்கிரணி – வேதோ
டிலக்காய் வரும்பிணிபோம் ஏற்றமயல் பித்தங்
குலக்கா யருந்துவர்குக் கூறு.

- குணபாடம் மூலிகை வகுப்பு

Nut meg is useful in the cases of oligospermia, diarrhoea, headache, asthma, cough, stomachache bloated abdomen.

Traditional uses

- Decoction of nutmeg is used for dehydration in the case of vomitting and diarrhoea
- Nutmeg paste is applied around the eyes to promote eye sight.
- Nut meg oil is used for tooth ache.
- Nutmeg powder 130mg, dry ginger powder 130 mg and cumin seed powder 130mg taken internally before meals for indigestion
- For diarrhoea 2 grams of nutmeg powder is given with milk.

***SATHIKKAI* IN OTHER MEDICINES**

1. SATHIKKAI LEGIUM

Indication : *Ratha bedhi, Seetha bedhi*

Dosage : *Kalarchikkai alavu* (3 gms)

2. VAJIRAKANDI LEGIUM

Indication : *Athisara bedhi, Ratha bedhi*

Dosage : *Sundaikkai alavu* (0.798gm)

3.KIRANI MATHIRAI

Indication : *Gunmam, Sobai, Kirani*

Dosage : *Uchi karandi alavu* (16ml)

4. PULIYARAI KIRUTHAM

Indication : *Gunmam, Sobai, Kirani*

Dosage : *Uchi karandi alavu* (16ml)

5. JATHIBALATHI CHOORANAM

Indication : *Kirani, Ratha bedhi, Seetha bedhi*

Dosage : *Thirikadi alavu* (800 – 1000mgm)

Adjuvant : Honey

6. KATTUVAI CHOORANAM

Indication : *Bedhi*

Dosage : *Thirikadi alavu* (800 – 1000mgm)

Adjuvant : Honey

7. KABADA MATHIRAI

Indication : *Bedhi*

Dosage : *Ilanthai vithai alavu* (790mgm)

Adjuvant : Honey

MYRISTICA FRAGRANS

3.3.2. BOTANICAL ASPECT

Classification

Taxonomical Classification

Kingdom	:	Plant kingdom
Division	:	Flowering plant
Class	:	Dicotyledonae
Subclass	:	Monopetalae
Series	:	Microembryeae
Family	:	Myristicaceae
Genus	:	Myristica
Species	:	Fragrans

Habitat

Nutmeg tree is indigenous to the Malay, Peninsula, Moluccas and Penang, now cultivated in many tropical countries of both hemispheres. In India, it is grown in Madras state (Nilgiris, Coimbatore, Salem, Ramanathapuram, Tirunelveli, Kanyakumari and Madurai Districts) a few trees are found invarious localities in kerala, Assam and other states. Preliminary trials have shown that Araku Valley (Andrapradesh), wynaad (Madrasstate) are well suited for its cultivation.

Description

Habit:

Moderate sized usually dioecious evergreen aromatic tree, upto 12mm tall.

Leaves

Alternate, quite entire exstipulate, often pellucid-punctate.

Flowers

Dioecious, small, regular fascicled umbelled or panicled. Bracteoles persistent or caducoas.

Male Flowers

Perianth-3 (2-4) lobed, valvate in bud. Anthers 3 or more connate in a sessile or stropitate column head ring or disk, 2-celled.

Female flowers:

Perianth of the male, staminodes 0. ovary superior, free sessile, 1-celled style short or a stigma capitate, discoid or lobed, ovale, basal, erect, anatropous.

Fruit

Fleshy at length 2 rarely 4 – Valved.

Seed

Erect, enclosed in a thin or fleshy entire or lacerate often highly coloured aril, testa thin or crustaceous, albumen hard densely ruminate, embroyo basal, small, cotyledons rounded spreading often wrinkled, radicle short inferior.

Macroscopic Description of seed

Seed ellipsoid, 20-30 mm long and about 20mm broad, externally greenish brown sometimes marked with small irregular, dark brown patches or minute dark points and line slightly focused reticulately, a small light coloured area at one end indicating the position of the radicle. A grove running along the line of raphe to the darker chalza at the opposite end, surrounded by a thin layer of perisperm with infoldings appearing as dark ruminations in the abundant greyish brown endosperm, embryo in an irregular cavtiy, small with two widely spreading crumpled colyledons and a small redicle, odour, strong and aromatic, taste, pungent and aromatic.

Microscopic Description of seed

Transverse section of endosperm show peripheral perisperm, of several layers of strongly flattened polyhedral cells with brown contents or containing prismatic crystals, inner layer of perisperm of thin walled parenchyma about 40 µ thick, infolding into the tissue of the endosperm to form the raninations containing numerous very large oil cells with brown cell walls, vascular strands, in the peripheral, region numerous small spiral vessels, large called, endosperm parenchymatous with occasional tannin idioblasts, with the brown walls, containing numerous simple rounded and compound starch grains, with upto about 10 componenets usually 2-8, individual grains, upto 2 µ in diameter present most of the cells with crystalline fat and often a large aleurone grain in each cell, containing a rhombic protein crystal upto 12 µ and small aleurone grains with less regular crystalloids, embryo of shrivelled and collapsed parenchyma.

Chemical constituents

The main chemical compounds are

- a-pinene, camphene, b-pinene
- Sabinene, myrcene, a-phellandrene

- a-terpinene, limonene, 1, 8 cinede, y-terpinene, linalool, terpinen-4-ol
- Safrole, methyl legend and myristicin.

Therapeutic uses

- Oil of nutmeg is employed for flavouring food products and liquorus
- It is used for scenting, soaps, tobacco and dental cream.
- It has been recommended for treatment of inflammation of bladder and urinary tract.
- Alcoholic extract of nutmeg shows anti-bacterial activity against micrococcus pyogenes, aqueous decoctions are toxic to cockroaches.

3.3.3.LATERAL RESEARCH:

ANTI-INFLAMMATORY ACTIVITY OF *MYRISTICA FRAGRANS* (NUTMEG) USING HRBC MEMBRANE STABILISING METHOD INT. J. PHARM. SCI. REV. RES., 44(1), MAY - JUNE 2017; ARTICLE NO. 11, PAGES: 40-42

ABSTRACT : The aim of the study is to determine the anti-inflammatory activity of *Myristica fragrans* (Nutmeg) using HRBC membrane stabilising method. Inflammation being a common symptom for various diseases has to be treated properly. The anti-inflammatory drugs come into role here in decreasing the inflammation. Nutmeg spice is a good source of minerals like copper, potassium, calcium, manganese, iron, zinc and magnesium. Since it's a natural drug and has a lot of anti-inflammatory properties having no side effects, therefore it is better than synthetic drugs. Nutmeg being a natural drug with least side effects, in comparison with the other drugs can be used in the future to produce an efficient anti-inflammatory drug.

3.4. *MYRSTICA FRAGRANS, GRONOV* - சாதிபத்திரி

3.4.1. GUNAPADAM ASPECT:

Synonyms:

Vasuvaasi, Jathipathiri.

Vernacular names:

Eng – Arillus of the nut.

Tel – Japtri.

Mal – Jatipatri.

Kan – japtri.

Sans – Jatipattriri.

Arab – Bisbasah.

Pers – Bazbaz.

Hind – Javatri.

Puk – Joutri.

Parts used:

Arillus of the nut.

Organaoleptic Charactes:

Taste *(Suvai)* - pungent *(Kaarpu).*

Potency *(Thanmai)* - hot *(Veppam)*

Bio-transformation - pungent *(Kaarpu).*

Actions:

Aphrodisiac *(Kamamperukki)*

Carminative *(Agattuvaivagatri)*

Stimulant *(Veppamundakki)*

Hypnotic. *(Urakkamundakki)*

General Properties:

சாதி தரும் பத்திரிக்குத் தாபச் சுரந்தணியும்
ஓதுகின்ற பித்தம் உயருங்கான் - தாதுவிர்த்தி
யுண்டாங் கிரகணியோ டோதக் கழிச்சலறும்
பண்டாங் குறையே பகர்.

- குணபாடம் மூலிகை வகுப்பு

Sathipathiri cures thabasuram, dysentery, diarrhea. It also strengthen the body. It induces the azhal.

Therapeutic Uses:

Oil extract checks gastric trouble and diarrhoea.

This increases the semen secretion.

Fever, tuberculosis, chronic stomach ache are treated by sathipathiri.

It gives strength to uterus. When this powder is inhaled, it relieves headache and epileptic fits.

This is also used in low stages of fever, chronic diarrhoea, asthma and chronic bowel complaints.

***SATHIPATHIRI* IN OTHER MEDICINES**

1. *KATTUVAI MATHIRAI*

Indication : *Bedhi*

Adjuvant : Mother's milk

- *Anuboga Vaidhiya Brahma Ragasiyam*

2. BAIRAVA MATHIRAI

Indication : *Gunmam, bedhi.*

Dosage : *Kundri alavu* (130mg)

- *Anuboga Vaidhiya Brahma Ragasiyam*

3. IMPOORAL ILAGAM

Indication : *Kuruthi kazhichal*

Dosage : 1-2 gm

- *Koshayi Anuboga Vaidhiya brahma Ragasiyam*

4. KESARI ILAGAM

Indication : *Kuruthi kazhichal*

Dosage : 2-4 gm

5. KADUKKAI ILAGAM

Indication : *Athisaram*, *Kirani*

Dosage : *Punnaikkayalavu (3-5 gms)*

MYRSTICA FRAGRANS

3.4.2. BOTANICAL ASPECT:

Taxonomical classification:

According to Bentham and Hooker's classification, Myrstica fragrans is classified as follows:

Kingdom	:	Plant kingdom
Division	:	Flowering plant
Class	:	Dicotyledonae
Sub-class	:	Monopetalae
Series	:	Microembryeae
Family	:	Myristicaceae
Genus	:	Myristica
Species	:	fragrans

Distribution:

Nutmeg tree is indigenous to the Malay, Peninsula, Moluccas and Penang, now cultivated in many tropical countries of both hemispheres. In India it is grown in Madras, Nilgiris, Coimbatore, Salem, Ramanathapuram, Tirunelveli, Kanyakumari and Madurai district. A few trees are found in various localities in Kerala, Assam and other states. Preliminary trials have shown that araku valley (Andhra Pradesh), waynnad (Madras state) are well suited for cultivation

Description:

Mace , spice consisting of the dried aril, or lacy covering, of the nutmeg fruit of Myristica fragrans, a tropical evergreen tree. Mace has a slightly warm taste and a fragrance similar to that of nutmeg. In the processing of mace, the crimson-coloured aril is removed from the nutmeg that it envelops and is flattened out and dried for 10- 14 days; its colour changes to pale yellow, orange or tan. Whole dry mace consists of flat pieces-branched or segmented, smooth, horny and brittle-about 40mm (1.6 inches) long.

Chemical constituents:

Mace and mace oil have the same constituents as nutmeg and nutmeg oil but with a higher concentration of myristicin and less amount of fixed oil.

3.4.3. LATERAL RESEARCH:

Pharmacognostic, phytochemical, physicochemical and TLC profile study Mace (Aril) of Myristica malabarica

Lamk. (Myristicaceae) Seema Yuvraj Mendhekar*, Chetana Dilip Balsaraf, Mayuri Sharad Bangar, S.L. Jadhav, D.D. Gaikwad

ABSTRACT

The plant Myristica malabarica Lamk. is traditionally used as a medicine and spices in food . It is belonging to family Myristicaceae. The plant is native to India and endangered trees are mostly found in western ghat. Extracted with various solvents by successive soxhlet hot extraction processs with increasing order of polarity on phytochemical investigation. The extract has shown alkaloids, saponin, tannin and flavones glycosides. It has important medicinal uses like Ayurvedic Medicines. It is traditionally used as anticancer, antiInflammatory, anti-Oxidant, Sedative hypnotics, Antimicrobial, Antifertility, Hepatoprotective and cytotoxicity. The chemical constituents such as Malabaricones, Malabaricanol, Isoflavones are isolated .Myristica Fragrans also known as fragnant Nutmeg or true Nutmeg. The present study i.e. Pharmacognostic, Phytochemical, Physicochemical and TLC Profile Study of Mace (Aril) Of Myristica malabarica Lamk. is helpful in the characterization of the crude drug. Physiochemical and phyto-chemical analysis of mace confirm the quality and purity of plant and its identification. The information collected is useful for further pharmacological and therapeutical evaluation of mace (Aril) Of Myristica malabarica Lamk. and anthology of quality control of crude drug. Keywords: Myristica fragrans, Malabaricone, Malabaricanol, Phytochemical screening, Microscopical study, TLC

3.5. *BOMBAX MALABARICUM, DC.* - இலவம்பிசின்

3.5.1. GUNAPADAM ASPECT

Synonyms:

Mul ilavam, Sanmali, poorani, pongar, mosam.

Vernacular names

Tamil	:	(Mul) Elavu
English:		Red silk-cotton tree
Hindi	:	Ragai-senbal
Tel	:	Mundla-buraga chettu
Mal	:	Mul-elava
Kan	:	Mullu-buraga Mara
Duk	:	Kantno-ka-sema
Sans	:	Kantaka-shalmali

Part used

Leaf, flower, seed, Bark, gum, root

Organoleptic characters

Taste (*Suvai*)	:	Sweet, Astringent (*Inippu*, *Thuvarppu)*
Potency(*Thanmai*)	:	Coolant (*Inippu*)
Biotransformation (*Pirivu*)		: Sweet (*Inippu*)

Actions

Refrigerant (Kulirchiyundakki)

Styptic (Kuruthi perukkadaki)

Astringent (Thuvarppi)

Tonic (Ullalalaatri)

Diuretic (Siruneer peruki)

General properties

“தந்துமே கஞ்சிறுநீர்த் தாரைவெப் பம்வாயு
வுந்தவரு பேதியிவை யோட்டுங்காண் - முந்திக்
கிளர்வள்ளை பாயும்வரிக் கெண்டை விழியாய்!
வளர்முள் ளிலவு மரம்.”

- குணபாடம் மூலிகை வகுப்பு

Traditional uses

- The leaf paste of red silk cotton tree is given with milk for burning micturation.
- Decoction prepared from flower of Red silk cotton tree releives constipation and anuria.
- Decoction prepared from bark of red silk cotton tree given internally, thrice a day cures chronic wounds, burning micturation, oliguria, anuria, leucorrhoea and diarrhoea.
- 1-2 gram gum powder of Red silk cotton tree with tender coconut for leucorrhoea & diarrhoea.

***ELAVAMPISIN* IN OTHER MEDICINES**

1.INJI NEI

Indication : Diarrhoea

- (Agathiyar 2000-3rd part, P.No.308)

2. VETPALAI NEI

Dosage : 1 teaspoon

Indication : Diarrhoea

- (Agathiyar 2000-3rd part, P.No.306)

3.VILVATHI CHOORANAM

Dosage : *Mooviral alavu* (800mg – 1000mg)

Adjuvant : Honey

Indication : Diarrhoea

- (Agathiyar 2000-3rd part, P.No.165)

4.THURBATHI VADAGAM

Dosage : *Kottaipakku alavu* (6 gms)

Adjuvant : Honey (or) curd

Indication : Diarrhoea, dysentry

- (Agathiyar 2000-3rd part, P.No.198)

5. NARATTHAMPAZHA NEI

Indications : Diarrhoea

- (Agathiyar 2000-3rd part, P.No.304)

6. ASAMODHATHI CHOORANAM

Dosage : *Mooviral alavu* (800mg-1000mg)

Adjuvant : Hot water

Indication : Diarrhoea

BOMBAX MALABARICUM

3.5.2. BOTANICAL ASPECT

Taxonomical classification

Kingdom	:	Plant
Division	:	Tracheophyta
Subdivision	:	Spermatophytina
Class	:	Magnoliopsida
Order	:	Malvales
Family	:	Malvaceae
Genus	:	Bombax
Species	:	Bombax malabaricum, DC.

Habitat

Thrives hot, moist, tropical forest, with high rainfall well distributed throughout year, such as in the hotter regions of india, estern himalayas and bengal.

Description

Habit

Deciduous large tree up to 20-25m tall and spreads 8-15m wide.

Macroscopic character

The pieces of bark of bombax is generally curved fragments, 0.5-1cm thick, freshly collected bark is brownish internally and externally light grey in colour. Bark is covered with hard, sharp, conical prickles.

Leaves

Palmate with about 6 leaflets, radiating from a central point, 7-10cm wide, 13-15cm in length. The leaf's long flexible petiole is up to 20cm long.

Stem

Woody, solid, erect, grey, glabrous bark prickles, delicate branches cylindrical, smooth with distinct nodes.

Flower

Cup-shaped flowers solitary or clustered, axillary or sub-terminal stigma is light red, upto 9cm in length.

Fruit

Brown oval capsule, which, when rip contains white fibers like cotton.

Gum

Gum is light brown in colour resembling the galls and gradually becomes opaque a dark brown.

Chemical constituents: Phytochemicals

Bombax malabaricum contains,

- Taraxeryl acetate
- Squalene
- Taraxerol
- Taraxerone,
- β-sitosterol palmitate
- 1 H-indole-3-carboxylic acid
- Lolilide.

Lupeol isolated from extract of the stem bark.

Therapeutic uses

- Decoction of the bark is given orally for fever.
- Decoction of the heartwood treat diabetes
- Bark juice is given to reduce stomachache.
- Cream is prepared from Bombax along with other plants to treat pimples and skin eruptions.
- Root powder of Bombax malabaricum has been used for heart disease and diabetes.
- Young roots are astringent, stimulant.
- Stem bark extraction with curd is given for bloody dysentry.

3.5.3. LATERAL RESEARCH:

Asian Journal of Pharmaceutical Education and Research Vol -6, Issue-3, July-September 2017 ISSN : 2278 7496 AJPER July-September 2017, Vol 6, Issue 3 (16-27)

Pharmacognostic and Pharmacological Profile of Bombax Ceiba

Abstract

Bombax ceiba Linn. is a tall tree buttressed at the base that is widely distributed throughout India, Ceylon and Malaya, upto 1500 m of altitude. Bombax ceiba is commonly known as silk cotton tree and semal which belongs to family Bombacaceae. Its medicinal usage has been reported in the traditional systems of medicine such as Ayurveda, Siddha and Unani. It has wide range of medicinal and pharmacological application. Many parts of the plant (root, stem bark, gum, leaf, prickles, flower, fruit, seed and heartwood) are used by various tribal communities and forest dwellers for the treatment of a variety of ailments. The plant literature survey shows the plant possesses astringent, cooling, stimulant, diuretic, aphrodisiac, demulcent, and tonic effects and also helps in dysentery. It also possesses important pharmacological activity such as aphrodisiac, anti-inflammatory and hepatoprotective activity in addition to anticancer and anti-HIV activity, anti-Helicobacter pylori, antiangiogenic, analgesic and antioxidant activity and hypotensive, hypoglycemic and antimicrobial activity. It is reported to contain important phytoconstituents such as naphthol, naphthoquinones, polysaccharides, anthocyanins, shamimin and lupeol. This paper provides an overview on pharmacological, phytochemical properties and therapeutic benefits of the plant.

3.6. *SYZYGIUM AROMATICUM, LINN* - இலவங்கம்

3.6.1. GUNAPADAM ASPECT:

Synonyms:

Anjugam, urkadam, karuvai kirambu, sosam, thirali, varangam.

Vernacular names:

Eng	:	cloves
Tel	:	lavangalu, lavanga poo
Mal	:	karambu
Kan	:	lavanga
Sans	:	long
Arab	:	lavangam
Pers	:	aaranful
Hind	:	long
Tamil	:	kirambu.

Parts used:

Flowering bud.

Organaoleptic Charactes:

Taste *(Suvai)* : pungent *(Kaarpu)*

Potency *(Thanmai)* : hot *(Veppam)*

Actions:

- Anti-spasmodic *(Isivagattri)*
- Carminative *(Agattuvaivagatri)*
- Stomachic *(Pasitheethoondi)*

General Properties:

பித்த மயக்கம் பேதியோடு வாந்தியும்போம்
சுத்தவிரத் தக்ககடுப்புந் தோன்றுமோ – மெத்த
இலவங்கங் கொண்டவருக் கேற் சுகமாகும்
முலமங்கே கட்டுமேன வாழ்த்து.
சுக்கிலநட் டங்கர்ண சூர்வியங்க லாஞ்சனந்தாட்
சிக்கில்விடாச் சர்வா சியப்பிணியு – மக்கிக்குட்
டங்கப் பூவோடு தரிபடருந் தோன்றிலில்
வங்கப்பூ வோடுரைத்து வா.

- *Gunapadam Mooligai Vaguppu*

Kirambu cures giddiness, diarrhoea, vomiting, dysentery, chronic diarrhoea, ear diseases, red mole and black mole.

Therapeutic Uses;

Medicinally they are used as to correct gripping caused by purgatives.

It is also used to relieve the presence of excessive gas in the digestive tract, various forms of gastric irritability, colic dyspepsia and increase the flow of saliva.

Lavangathi chooranam made of cloves, dry ginger, black pepper and fried borax taken equal measure is useful in bronchitis.

It is an appetizer and also stops vomiting.

***KIRAMBU* IN OTHER MEDICINES**

1. OOZHI MATHIRAI

Indication : *Athisaram*, *Kirani*

Dosage : *Kundri alavu* (130mg)

2. KABADA MATHIRAI

Indication : *Bedhi*

Dosage : *Ilanthai vithai alavu* (790mg)

Adjuvant : Honey

3. GANTHGA RASAYANAM

Indication : *Bedhi*, *Moolam, Gunmam.*

Dosage : 10 to 15 *kundrimani alavu*

4. KARISALAI LEGIUM

Indication : *Bedhi*

Dosage : *Punnaikkayalavu(3-5gms)*

5. INJI VADAGAM

Indication : *Bedhi*, *Seriyamai*

Adjuvant : Water

6. POORA MATHIRAI

Indication : *Bedhi*

Adjuvant : Honey (or) Mother's, milk

SYZYGIUM AROMATICUM

3.6.2. BOTANICAL ASPECT:

Taxonomical classification:

Kingdom	:	Plantae
Division	:	Magnoliophyta (flowering plants)
Class	:	Magnoliopsida (dicotyledons)
Subclass	:	Rosidae
Order	:	Myrtales
Family	:	Myrtaceae
Genus	:	Syzygium
Species	:	S. aromaticum

Distribution:

Indonesia, Tanzania, Madagascar, Malaysia, Brazil, Srilanka, Huait and India are the major clove growing countries. The islands of Zanzibar and Pemba are the world's largest producer of clove, which supplies 90 percent of the total demand followed by Indonesia.

Description:

Flowers occur in terminal clusters. Unopened flower buds are green first, then slowly change to pink or red or crimson, then finally bright red when is full blown. Each flower is about 6mm wide. Flowering occurs when the tree reaches 5-7 years. Cloves are harvested at 1.5-2 cm long, and consist of a long calyx that terminates in four spreading sepals, and four unopened petals that form a small central ball.

Chemical constituents:

Eugenol composes 72-90% of the essential oil extracted from cloves and is the compound most responsible for clove aroma. Other important essential oil constituents of clove oil induce acetyl eugenol, beta-caryophyllene and vanillin, crategolic acid, tannins such as bisornin, gallotannic acid, methyl salicylate, the flavanoids eugenin, kaempferol, rhamnetin and eugenitin, triterpenoids such as oleanolic acid, stigmasterol and campesterol and several sesquiterpenes.

3.6.3. LATERAL RESEARCH:

In vitro controlling of selected human diarrhea causing bacteria by clove extracts (Syzygium aromaticum L.) ISSN: 2221-1063 (Print) 2222-503X

Abstract

Antibacterial activity of clove extracts (*Syzygium aromaticum* L.) was proven against five diarrhea causing bacteria. This was further confirmed when compared with commonly used three commercial antibiotics (ciprofloxacin, tetracycline and erythromycin) as a positive control. Significant differences ($P<0.0001$) were observed in the effect of the antimicrobial agents (clove extracts and antibiotics), and in the sensitivities of the bacterial species ($P<0.0001$) to the antimicrobial agents. Clove extracts had significant ($P<0.001$) activity with the acetone extract demonstrating highest activity followed by antibiotics and other extracts against tested bacteria. The zone of inhibition of clove extracts was ranged from 7.33 to 12.00 mm whereas in antibiotics, it was 0.00 to 11.67 mm. Of all the bacteria, *Salmonella typhimurium* was the most susceptible against all of the extracts as well as concentrations of clove, while low MIC (180 mgml-1) and MBC (680 mgml-1) of the extracts were observed against *Shigella dysenteriae*. Consequently, clove has a significant antidiarrheal activity and it could be used as an effective antibacterial agent, alternative to the use of antibiotics.

3.7.*ACACIA CATECHU (Lin.f)wild* – காய்ச்சுக்கட்டி

3.7.1.GUNAPADAM ASPECT

Vernacular names

Tamil	:	Karungali
English	:	Black catechu, Cutch tree
Tel	:	Chandra
Mal	:	Karingali
Kan	:	Khadira
Sans	:	Khandira
Hind	:	Katha
Duk	:	Kher

Part used

Bark, Gum, Root.

Organoleptic characters

Taste *(suvai)*	:	Astringent (*Thuvarppu)*
Potency (*Thanmai*)	:	Coolant (*Inippu*)
Biotransformation *(pirivu)*	:	Pungent *(Kaarpu)*

Actions

Astringent *(Thuvarppi)*

General properties

குட்டங் கயரோகங் குன்மம் பெருவயிறு
நெட்டைப் புழுதிமிரு நீரிழிவும் - விட்டே
யருங்கான கத்தேகு மஞ்சுகமே! நல்ல
கருங்காலிப் நீரதனைக் கண்டு.

- குணபாடம் மூலிகை வகுப்பு

Black catechu is used to treat diseases of leprosy, ascitis, diabetes and anaemia.

Traditional uses

- Acacia catechu bark decoction is used to treat diabetes.
- For fistula, the bark dicoction is given with buffalo ghee and embelia powder.
- A bark of Acacia catechu, Azadiracta indica and Eugenia jumbolana are made into paste with the help of cow's urine are used to heal wounds.
- Because of its astringent taste it used to treat diarrhoea and bleeding gums.

KAICHUKKATTI IN OTHER MEDICINES

1. ARITHAGI CHOORANAM

Dosage	:	*Verukadi alavu* (1250-1500mg)
Adjuvant	:	Honey
Indication	:	Diarrhoea

2. KAICHUKKATTI CHOORANAM

Dosage	:	Diarrhoea
Adjuvant	:	500mg - 1 gm
Indication	:	Ghee

3. KIRANI KABADAM

Dosage	:	Diarrhoea
Adjuvant	:	*Ilanthai vithai alavu* (790mg)
Indication	:	Curd

ACACIA CATECHU

3.7.2. BOTANICAL ASPECT

Taxonomical classification

Kingdom	:	Plant
Division	:	Tracheophyta
Class	:	Magnoliopsida
Order	:	Fabales
Genus	:	Acacia
Species	:	Acacia catechu (L.f) wild
Family	:	Fabaceae
Botanical name	:	Acacia catechu (L.f) wild

Habitat

It grows throughout India from the Himalayas to the south of India.

Description

Habit

Is a deciduous, thorny tree which grows upto 15m in height.

Leaf

Leaves bipinnate, altermate, stipulate, stipular spines slightly infra axillary, paired, 3-10mm long, straight or hooked.

Flowers

Flowers pale yellow, sessile, in long solitary or in groups of 2-4 axillary spikes, bracts cauducous.

Fruit

A pod 5-10 x 1-1.6 cm, flat, straight, unlobed or sinuate along margins, thin walled, beaked at apex.

Macroscopic characters

Black or dark brown in colour, irregular masses or cubes, rough, dull or slightly glossy and porous.

Microscopic characters

Bark shows numerous uni to bi-seriate medullary rays, vessels occuring isolated or in small groups of two to four. Xylem fibers with narrow lumen occupying major portion of wood.

Chemical constituents

- It contains tannins like catechins, catechu tannic acid.
- Flavonoids like quercetin and it derivatives.
- Catechu red, gum
- Catechin
- Epicatechin
- Kaempferol
- Mesquitol, ophioglonin, aromadendrin 4-hydroxybenzoic acid.

Therapeutic uses

- It is a valuable astringent given in doses of 5-15 gms, in diarrhoeas and haemorrhages.
- A small piece of catechu with cinnamom held in tooth ache, bleeding, ulcerations and sponginess of the gums.
- It is useful for strengthening gums.
- Gum is an astringent, demulcent and haemostatic.
- Gum is useful in diarrhoea, dysentry, menorrhagia and other affections.

3.7.3. LATERAL RESEARCH

PHYTOPHARMACOLOGY OF *ACACIA CATECHU* WILLD: A REVIEW *ejpmr, 2019,6(1), 216-223*

ABSTRACT

Khadir (Acacia catechu Willd) beloning *to* Family-Fabaceae and subfamily-Mimosoideae has a great importance due to its medicinal properties and is commonly known as Katha. It is a historical plant; widely used in traditional medicine especially in Asia. In *charka Samhita*, *vimansthan* chapter 8 *Khadira* (*Acacia catechu* Willd) included in *kashay skandha. Kashay rasa* plays an important role in *kledashoshan* (Absorbing *Kleda*). *Dhatushaithilyanashan* (destroys *Dhatushaithilyanashan*) beside this *kashay rasa* has *vranropan* (wound healing) property. *Khadir (Acacia catechu* Willd) possess predominant *kashay rasa*, *sheet veerya* and *katu vipak*. The useful part of *Khadira* is bark. The main chemical constituents of Black catechu are flavanoids (catechin, (-) epicatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate, rocatechin, phloroglucinol, procatechuic acid, catecutannic acid, quercetin, quercitrin), alkaloids (kaempferol, dihydrokaempferol, taxifolin, afzelchin gum), glycosides (poriferasterol, poriferasterol acylglucosides), tannins (gallic acid, phlobatannins), sugars (d-galactose, d-rhamnose and larabinose). It has been shown to possess multifarious medicinal properties such anti-bacterial, anticancer, antidiarrhoeal, anti-inflammatory, antimicrobial, antioxidant, antipyretic, anti-ulcer, antisecretory, hepatoprotective, hypoglycaemic, sore throat and wound healing and anti-obesity etc.

3.8. *AEGLE MARMOLOS.LINN* – வில்வம்

3.8.1. GUNAPADAM ASPECT

Synonyms

Kusabi, Koovilam, Koovilai, Sivatthurumam, Ninmali, Maathuram.

Vernacular names

Tamil	:	Vilvam
English	:	Bael (tree) Holy fruit tree
Sans	:	Bilva
Hindi	:	Bel
Tel	:	Bilvamu
Mal	:	Kuvalam
Kan	:	Beta
Pers	:	Shul

Part used

Leaf, flower, unripe fruit, Root, Bark.

Organoleptic characters:

Taste *(suvai)* : Astringent *(Thuvarpu),* slightly Bitter *(siru kaipu)*

Potency (*Thanmai*) : Coolant (*Inippu*)

Biotransformation : Pungent *(Kaarpu)*

Actions

Diaphoretic *(Viyarvai perukki)*

Aphrodisiac *(Kamam perukki)*

Febrifuge *(Veppagattri)*

Astringent *(Thuvarppi)*

Laxative *(Malamilakki)*

Stomachic *(Pasithethoondi)*

General Properties

பல்லவம்பூ பிஞ்சின் பழநிரியம்சம் முறையே
வல்வவம் மேகமந்த மாகுன்மம் - செல்லுகின்ற
நோக்கமருள் விந்துநட்ட நூறு மடுத்தவர்கட்
காக்கமருள் வில்லுவத்தி லாம்.

- குணபாடம் மூலிகை வகுப்பு

- Young leaves of vilvam are used to treat secondary syphilis, flower – used to treat indigestion, unriped fruit- used to treat gastric ulcer. Fruit – used to treat eye diseases. Resin – used to treat oligospermia.
- 2-6 gram of unriped fruit of vilvam paste mixed with buffalo curd is used to treat gastric ulcer, diarrhoea.
- Manappagu prepared with the help of vilvam fruit is used to treat diarrhoea.
- 2-4mg powder of dried fruit flesh is used to treat diarrhoea.
- Leaf juice of vilvam with piper nigrum powder used to treat jaundice and dropsy.

***VILAVA PAZHAM* IN OTHER MEDICINES**

1. THADI MATHI CHOORANAM

Dosage : *3 viral alavu* (800-1000mg)

Adjuvant : Honey (or) curd

Indication : *Kirani*

- *Agathiyar* – 2000 – 3rd part.

2. KORAIKIZHANGU NEI

Dosage : *1 Kasu edai alavu* (800mg)

Indication : Diarrhoea, Fever

- *Agathiyar* – 2000 – 3rd part

3. KIRANI KABAPAM

Dosage : *Elanthai alavu* (790mg)

Adjuvant : Curd

Indication : *Kirani*

- *Thanjai Vaidhiya raja sinthamani*

4. MADHULAI CHOORANAM

Dosage : *Verukadi alavu* (1250-1500mg)

Adjuvant : Gingelly oil

Indication : Diarrhoea, indigestion.

- *Anupoga Vaidhiya Murai*

5. MATHA KAJA KANDEERAVAM

Dosage : *Milagu alavu* (56mg)

Indication : Diarrhoea

- *Siddha vaidhiya thirattu*
- ***Aegle Marmelos***

AEGLE MARMELOS

3.8.2. BOTANICAL ASPECT

Taxonomical classification

Kingdom	:	Plantae
Sub kingdom	:	Tracheobionta
Division	:	Magnoliaphyta
Class	:	Magnoliopsida
Sub class	:	Rosidae
Order	:	Sapindales
Family	:	Rutaceae
Genus	:	Aegle
Species	:	Marmelos

Habitat

Aegle marmelos is a deciduous shrub or small to medium sized tree, upto 13m tall with slender drooping branches and rather shappy crown. Is native across the indian subconfinent and southeast asia and is cultivated throughout srilanka.

Description

Macroscopic characters

Leaves : Are alternate, pale green, trifoliate, terminal leaflet. 15.7cm long, 2.8cm broad, having a long petiole.

Flowers

Greenish white, sweetly scented, bisexual, actinomorphic, stalk 8mm long

Fruit

Yellowish green, with small dots on the outer surface, oblong to globose, 5.3cm to 7.2cm in diameter weight 77.2gm; yellow & mucilaginous.

Microscopic characters

Presence of characteristic stone cells, compact parenchyma cells and oil globules phloem cells were characteristically dark pinkish colour, radially flattened cork cells. Presence of brownish granules, laticiferous ducts were unique.

Chemical constituents: Phytochemicals

Bael tree contains,

- Furocoumarins,
- Xanthotoxol

- Flavonoids
- Rutin and marmesin
- Aegeline is a constituent that can be extracted from bael leaves.
- Agelin, marmeline, aegelenine present
- Aegle marmelosine.
- Methyl ester of alloimperatorin
- A number of essential oil
- a fargarine
- O-methylhafordinol
- Luvangetin, aurapten, Marmelide and tannin

Therapeutic uses

- Bael fruits are used in the treatment of chronic diarrhoea, dysentry and peptic ulcers.
- It is gastroprotective, anti-ulcerative.
- Dry powder of this fruit with mustard oil for the treatment of burns.
- Fruits are also used in gastric troubles, tonic, digestive, cardiac tonic, antiviral, gonorrhea, epilepsy.
- The ripe fruit is helpful in treating inflammation of rectum.

3.8.3. LATERAL RESEARCH:

Studies on the antidiarrhoeal activity of *Aegle marmelos* unripe fruit:Validating its traditional usage

Abstract

Background: *Aegle marmelos* (L.) Correa has been widely used in indigenous systems of Indian medicine due to its various medicinal properties. However, despite its traditional usage as an antidiarrhoeal there is limited information regarding its mode of action in infectious forms of diarrhoea. Hence, we evaluated the hot aqueous extract (decoction) of dried unripe fruit pulp of *A. marmelos* for its antimicrobial activity and effect on various aspects of pathogenicity of infectious diarrhoea. **Methods:** The decoction was assessed for its antibacterial, antigiardial and antirotaviral activities. The effect of the decoction on adherence of enteropathogenic *Escherichia coli* and invasion of enteroinvasive *E. coli* and *Shigella flexneri* to HEp-2 cells were assessed as a measure of its effect on colonization. The effect of the decoction on production of *E. coli* heat labile toxin (LT) and cholera toxin (CT) and their binding to ganglioside monosialic acid receptor (GM1) were assessed by GM1- enzyme linked immuno sorbent assay whereas its effect on production and action of *E. coli* heat stable toxin (ST) was assessed by suckling mouse assay.

Results: The decoction showed cidal activity against *Giardia* and rotavirus whereas viability of none of the six bacterial strains tested was affected. It significantly reduced bacterial adherence to and invasion of HEp-2 cells. The extract also affected production of CT and binding of both LT and CT to GM1. However, it had no effect on ST. **Conclusion:** The decoction of the unripe fruit pulp of *A. marmelos*, despite having limited antimicrobial activity, affected the bacterial colonization to gut epithelium and production and action of certain enterotoxins. These observations suggest the varied possible modes of action of *A. marmelos* in infectious forms of diarrhoea thereby validating its mention in the ancient Indian texts and continued use by local communities for the treatment of diarrhoeal diseases.

3.9. *DATURA METEL* – ஊமத்தை

3.9.1. GUNAPADAM ASPECT

Synonyms

Ommatthai

Vernacular names

Tamil	:	Oomatthai
English	:	Dhatura (White flowering) Thorn –apple
Tel	:	Ummeth-tha
Sans	:	Datura, unmattha
Mal	:	Ummath-tham
Hind	:	Dhatura
Kan	:	Ummatte-gida

Part used

Leaf, flower, unriped fruit, Seed

Organoleptic character

Taste (*Suvai*) : Bitter *(Kaippu)*

Potency (*Thanmai*) : Hot *(Veppam)*

Biotransformation (*Pirivu)* *:* Pungent *(Karppu)*

Actions

Emetic *(Vaanthiundakki)*

Antispasmodic *(Isivagattri)*

Anodyne (*Thuyaradakki)*

Narcotic *(Moorchaiundakki)*

General properties

நாய்க்கடியால் வந்து நலிசெய் விரணமும்போம்
வாய்குழிப்புண் கட்டிகளு மாறுங்காண் - தீக்குணத்தைச்
சேமத்தில் வைத்திலிடந் தீருமுத்தோ டங்களறும்
ஊமத்தை யின்குணத்தை யுன்னு.

- குணபாடம் மூலிகை வகுப்பு

- Dried leaf powder of dhatura, 32-100mg given internally for bronchial asthma
- Fumigation produced with dhatura leaf act as expectorant and also releives breathing difficulty.
- 1-3 drops of dhatura leaf juice is given with jaggery for dog's bite.
- 1-2 drops of dhatura leaf juice is used as ear drops for earache.
- Seed paste prepared with cow's ghee applied externally for hemorrhoids.

OOMATTHAI IN OTHER MEDICINES

1. MATHAKAJA KANDEERAVAM

Indication : Diarrhoea

Dosage : *Milagalavu* (56mg)

- Siddha Vaithiya Thirattu, P.No. 69

2. LAGUVATHA VIDHVAMSA MATHIRAI RASAM

Indication : *Kabam, Vadham, Akkinimantham, Soolai, Kirani.*

- Anubava vaidhiya deva ragasiyam, P.No. 414

TOXIC SYMPTOMS OF OOMATHAI

Symptoms :

Dryness of mouth, difficulty in speaking, Dilatation of cutaneous blood vessels, Dilatation of the pupil, Dull vision, Delirium, Drowsiness, Dysphagia.

Fatal Dose:

50 – 75 Seeds

Antidote:

- The tuber of Nelumbo nucifera is grounded with fermented rice water and made into paste its given internally.
- Fer emetic effect, indigofera tinctoria bark grounded with fermented rice water made into paste it is given internally. After vomiting, Alangium salvifolium root bark is grounded with lemon juice made into paste taken internally.

DATURA METEL

3.9.2. BOTANICAL ASPECT

Taxonomical classification

Kingdom	:	Plant
Division	:	Tracheophyta
Subdivision	:	Spermatophytina
Class	:	Magnolipsida
Order	:	Solanales
Family	:	Solanaceae
Genus	:	Datura L.
Species	:	Datura metel L.

Habitat

A herb grows in the waremer parts of the world, such as india.

Description

Habit

Shrub-like, annual herb with purple stem.

Leaves

Simple, alternate, petiolate, entire or deeply lobed, glabrous showing unicostate reticulate venation and exstipulate.

Stem

The stem is hollow, green and herbaceous with strong odour.

Flower

Large, greenish white, bracteate, ebractiolate, pedicellate, complete, dichlamydeous, pentamerous, regular, actionomorphic, bisexual and hypogynous.

Inflorescence

Solitary and axillary cyme

Fruit

Spinescent capsule opening by four apical valves with persistant calyx.

Seed

Endospermous.

Root

Branched tap root

Chemical constituents: Phytochemicals

Datura metel contains

- Tropane alkaloids – Hyoscyamine, Scopolamine
- Fastudine and fastunine.
- Fastusic acid, alantoin
- Ascorbic acid
- Scopatone, Daturadiol, G-sitosterol, vanillin, N-trans-feruloyl-tyramine, scopoletin and Hyoscyamilactol.

Therapeutic uses

- The paste of roasted leaves is applied over the area to releive pain.
- Dhatura seeds and leaves are used as antiasthmatic, antispasmodic, hypnotic and narcotic
- Excess doses cause giddiness, drymouth, hallucinations
- Hyoscyamine is used to provide symptomatic relief of various gastro intestinal disorders.
- Atropine dilates the pupils and is used in eye surgery.

3.9.3. LATERAL RESEARCH:

Medical importance of Datura fastuosa (syn: Datura metel) and Datura stramonium - A review IOSR Journal Of Pharmacy www.iosrphr.org (e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219 Volume 7, Issue 2 Version. 1 (Feb 2017), PP. 43-58

Abstract:- The preliminary phytochemical investigation was performed on methanolic and hydroalcoholic extract of Datura fastuosa (syn: Datura metel) revealed the presence of alkaloids, tannins, cardiac glycosides, flavonoids, carbohydrates, amino acids and phenolic compounds, while, phytochemical analysis of Datura stramonium showed that it contained alkaloids, saponins, tannins, steroids, flavonoids, phenols and glycosides. The previous pharmacological studies of Datura fastuosa showed that it possessed antimicrobial insecticidal, antidiabetic, cytotoxic, antioxidant, antiinflammatory, analgesic, antipyretic, neurological, wound healing, reproductive and antispasmodic, while Datura stramonium exerted Antiepileptic, Anti-asthmatic, analgesic, antioxidant, antimicrobial, insecticidal, repellent and organophosphate protective effects.

3.10. *HYGROPHILA AURICULATA* – நீர்முள்ளி

3.10.1. GUNAPADAM ASPECT

Synonyms

Ikkuram, Kagandam, Thuragathamoolam, Mundagam.

Vernacular names

Tamil : Neermulli

English: Long leaved barleria

Tel : Nirugobbi

Sans : Kokilaksha

Mal : Vayalchulli

Hindi : Talmakhana

Kan : Kollavalike

Part used

Whole plant, flower, seed

Organoleptic character

Taste (*Suvai*) : Sweet (*Inippu*), slightly bitter *(Sirukaippu)*

Potency (*Thanmai*) : Coolant (*Inippu*)

Biotransformation (*Pirivu*): Sweet (*Inippu*)

Actions

- Refrigerant *(Kulirchiundakki)*
- Diuretic *(Siruneer perukki)*
- Aphrodiasiac *(Kamam perukki)*
- Tonic *(Uramakki)*

General properties

விந்துவுமாம் தாதுவுமாம் மேகரோகந்தொலையும்
உந்து மதிசாரம் ஒழியுங்காண் - வந்துடலில்
ஏறியநீர் வீக்கம் இறங்கும் இளைப்புமறும்
கூறிய நீர்முள்ளி விதைக்கு

- குணபாடம் மூலிகை வகுப்பு

Barleria seed cures swelling, leucorrhoea, diarrhoea and also enhances spermatogenesis.

Traditional uses

- The whole plant of barleria is used to treat anaemia, dropsy, sinusitis and swelling.
- The plant was extensively used in traditional system of medicine for various ailments like rheumatism, inflammation, jaundice, hepatic obstruction & pain.
- It is antitumor, diuretic, free radical scavenging anti-inflammatory and antipyretic.
- In ayurvedic system of medicine is used for the treatment of a number of conditions including premeham (diabetes) and athisaram (dysentry)
- The entire plant and its ashes and roots can be used as cooling medicine and due to its diuretic properties, is very useful against cases of rheumatism dropsy, hepatic obstruction.
- The seeds of the plant are diuretic and demulcent.

***NEERMULLI VIDHAI* IN OTHER MEDICINES.**

1. SINTHAMANI MEVA

Indication : Diarrhoea

- Thanjai Vaidhiyaraja Sinthamani

2. KABADA ILAGAM

Indication : *Seelkirani, Neerkirani, Moolakirani*

- Bogar vaidhiyam – 700

3. MAHA VILVATHI ILAGAM

Dosage : 5-10gm

Indication : Diarrhoea, Indigestion

- Agathiyar Vaidhiya rathina churukam

4. MAGUDATHI CHOORANAM

Dosage : *Verukadi alavu* (1250-1500mg)

Adjuvant : Honey, Curd, Buttermilk

Indication : Diarrhoea, dysentry.

HYGROPHILA AURICULATA

3.10.2. BOTANICAL ASPECT

Taxonomical classification

Kingdom	:	Plant
Division	:	Tracheophyta
Subdivision	:	Spermatophytina
Class	:	Magnoliopsida
Order	:	Lamiales
Family	:	Acanthaceae
Genus	:	Hygrophila
Species	:	Hygrophila auriculata

Habitat

A herb growing in wet places.

Description

Habit

This is an annual herb or under shrub it grows to a height of about 60cms.

Leaves

The leaves are elliptic- lanceolate and sessile. The outer leaves of the whorl larger than others, each one with a yellow straight spine in its axil.

Stem

Square and thickened at nodes, hairy.

Flowers

4 pairs at each node, bracts leaf like, lower portion ciliate with long white hairs. Corolla purplish –blue, stamens -4, didynamous, filaments unequal.

Fruit

Capsule oblong, seeds 4-8, with hygroscopical white hairs.

Chemical constituents : Phytochemicals

Hygrophila auriculata contains

Phytosterols, fatty acids, flavonoids, terpenoids and glycosides

Lupenone, β- carotene, apigenin-7-o-glucuronide ascorbic acid, glucose, mannose, rhamnose, arbinose, maltose, myristic acid, oleic acid, palmitic acid, stearic acid, β-sitosterol, histidine, phenylalanins, lysine, apigenin-7-o-glucoside, 3-methylnonacosane, asteracanthine, luteolin-7-rutinoside.

Two alkaloids, asteracanthine and asteracanthicine were reported from the seeds.

Therapeutic uses

- The seeds are ground into a paste and given in buttermilk for diarrhoea.
- The seeds are used in treatment blood disorders gonorrhea and fever.
- The tincture of the whole plant is beneficial in urinary affections, dysuria and painful micturation.
- The leaves are tonic, aphrodisiac and useful in the treatement of diarrhoea, thirst, urinary calculi.
- The oil extracted from the whole plant is antibacterial.
- The roots are used in the form of decoction in rheumatism, gonorrhoea and hepatic obstruction.

3.10.3. LATERAL RESEARCH

Evaluation of cytotoxic, analgesic, antidiarrheal, and phytochemical properties of *Hygrophila spinosa* (T. Anders) whole plant DOI 10.1515/jbcpp-2016-0103

Abstract

Background: Synthetic drugs are going to be replaced by plant-derived traditional drugs due to their cost effectiveness, relatively less harmfulness, and efficacy against multidrug resistance organisms. *Hygrophila spinosa* (Acanthaceae) has been used in a wide range of ailments including flatulence, diarrhea, dysentery, gonorrhea, and menorrhagia. Therefore, we investigated the cytotoxic, antinociceptive, and antidiarrheal effects of *H. spinosa* ethanol extract (EExHs). **Methods:** Preliminary phytochemical screening was accomplished by established methods modified in experimental protocol. EExHs was undertaken for cytotoxic

assay by Brine shrimp lethality bioassay, antinociceptive action by acetic acid induced writhing test, and antidiarrheal activity by castor oil induced antidiarrheal test. Data were analyzed by GraphPad Prism 6.0 software using Dunnett's test for multiple comparisons. **Results:** Reducing sugar, steroid, glycoside, tannin, alkaloid,saponins, and flavonoids were found to be present in EExHs. Lethal concentration (LC50) of EExHs for brine shrimps was 50.59 µg/mL which was relatively lower than that of the standard drug vincristine sulfate. In acetic acid induced writhing test, oral administration of EExHs at three different doses (125, 250, and 500 mg/kg) decreased writhing in dose-dependent manner while the highest dose (500 mg/kg) achieved the maximum percentages of pain inhibition (58.8%). Diclofenac sodium (25 mg/kg) was used as a reference antinociceptive drug. The antidiarrheal action of EExHs was not found to be very promising for further use; however, the pure compounds from EExHs could be analyzed to justify the effects. **Conclusions:** This research demonstrates that the secondary metabolites guided cytotoxic and analgesic effects could be extensively studied in multiple models to confirm the effects.

3.11. DISEASE REVIEW

3.11.1. BEDHI – SIDDHA ASPECT

PERUM KAZHICHAL

Syn *: Athisaram*

Migukazhichal

Iyal:

It is the increased frequency & liquidity of the stools, which could either formed or unformed. The food materials are quickly emptied from the intestine resulting in increased frequency. As a result the body is deprived of nourishment causing loss of weight & malnutrition problem.

Noi varum vazhi

1. Increased intake of Mapandam
   - Contaminated food
   - Oil & ghee
   - Decayed food materials
   - Food difficult to digest
   - Improperly cooked food
2. Involving in increased sexual activities when there is Mantham
3. Worm infestation

Murkurikunangal

1. Increased salivation
2. Nausea
3. Hiccup
4. Belching
5. Borborygmi
6. Lower abdominal spasmodic pain
7. Abdominal distension

Noi Enn: 8 types

Kutrangal -4

Extrinsic -4

Kutrangal

1. *Vali*
2. *Azhal*
3. *Iyam*
4. *Mukkutram*

Entrinsic

1. *Suram*
2. *Thodam*
3. *Bhayam*
4. *Kuruthi*

VALI PERUMKAZHICHAL

- Abdominal pricking pain like *Soolai*
- Flatulence
- Unformed stools
- Indigestion
- Sour belching
- Diarrhoea with foul smell
- Incontinence of urine

AZHAL PERUMKAZHICHAL

- Yellowish and frothy stools, dry in nature
- Flatulence
- Odema of limbs, emaciation of body
- Fever
- Pallor
- Headache
- Vomiting
- Pain around umbilical area
- Pain in stomach and anus
- First blackish then frothy stools
- Later bloody diarrhea also occur

IYYA PERUMKAZHICHAL

- Diarrhoea with foul smell and froth
- Diarhoea with pain
- Sometimes with borborygmi and phlegm
- Along with the diarrhea *Suvai* inmai (tastelessness)
- Cough
- Phlegm in chest
- Perumoochu (dyspnea)
- Tenesmus
- Irritation all over the body

MUKKUTRA PERUMKAZHICHAL

- Iraichal
- Diarrhoea with phlegm and blood
- Phlegm in chest
- Cough
- Hiccup
- Vomiting
- Cold extremities
- Noisy abdomen
- Flatulence
- Numbness all over the body

SURA PERUMKAZHICHAL

This perum*kazhichal* is associated with suram

THODA PERUMKAZHICHAL

According to siddha a cause for this disease is said to be birds when pass above babies or is the shadow of birds fall on the baby this disease will occur.

BHAYA PERUMKAZHICHAL

This disease is caused due to increased emotional disturbances. This can be avoided through various ways like meditation.

KURUTHI PERUMKAZHICHAL

This is caused due to increased intake of sour and pungent food materials. Increased dose of mercury containing medicine or defective preparation can also cause this, plants like kundrimani, serankottai, sithiramoolam, moosambiram when given in

higher doses without proper purification and will cause perforation in stomach & hence produce bloody stools.

Pothukurikunangal

- Increased salivation
- Anorexia
- Indigestion
- Sour belching
- Hiccup
- Pain in abdomen
- Bloating abdomen
- Frequency of stool
- Lethargy
- Dryness of skin
- Tiredness
- Depression
- Cold extremities
- Without proper medication it will leads to
    - Cramps
    - Feeble pulse
    - Oliguria
    - Patient remains conscious till death.

Mukkutra Verupadukal

Due to abnormal food habits derangement of *pitham and vatham* occurs. This increases *udanavayu* causing indigestion, salivation, hiccup, belching. Derangement of *abanavayu* causing flatulence, borborygmi, increased evacuation. Emaciation of 7 udal thathus occur resulting in *perumkazhichal.*

Naadi nadai

- *Pithathil ushnam*
- *Vathathil ushnam*
- *Kapha pitham*
- *Kapham*
- *Pitham*

Urine : Decreased, yellowish red.

Fecus : Yellow, black, whitish coloured watery stools.

TREATEMENT

- Normalize the deranged digestive fire
- Regularize the abanavayu
- Appetizers and antidiarrhoeals can be given

Decoctions

1. **Athividayam decoctions**

    Athividayam

    Vilva pazham

    Korai kizhangu

    Iruveli ver

    Add 2 nazhi water prepare as decoction. Then add thippili & chukku – 30-60 ml BD/TDS

2. Mathulam pazhathol & chukku decoction. Add some honey – 30-60 mlBD
3. Thippiliathi kudineer : Azhalperumkazhichal
4. Maramanchal kudineer

Choornam

1. Thayir chundi churnam
2. Chundai vatral podi
3. Annapodi
4. Thaleesapathiri podi

Vadagam

1. Thaleesathi vadagam
2. Pirandai vadagam
3. Vazhai poo vadagam

Pills

1. Kabada pills
2. Kattu vathi pills
3. Oozhi pills
4. Vajrakapada mathirai
5. Jathikkai mathirai

Parpa chendhoorams

1. Ainthuppu parpam
2. Uppu chendhooram
3. Naga parpam

4. Pavazha parpam
5. Nathai parpam
6. Linga thuvar
7. Muthu parpam
8. Annabedi chendhooram

Legiyam

1. Ingi legiyam
2. Jathikkai legiyam
3. Vilvathi legiyam

Manapagu

1. Vilva manapagu
2. Kudasa manapagu
3. Chemparuthi manapagu

Diet

- Watery food substances
- Barley water
- Atthi pinchu
- Two times cooked rice water
- Butter milk
- Avarai pinchu

3.11.2. MODERN ASPECT

DIARRHOEA

INTRODUCTION

Diarrhoea is defined as the passage of more than 200gm of stool daily, and measurement of stool volume is helpful in confirming this. The most severe symptom in many patients is urgency of defecation and faecal incontinence is a common event in acute and chronic diarrhoeal illness.

It may be divided into 2 types

1. Acute diarrhoea
2. Chronic (or) Relapsing diarrhoea.

Acute diarrhoea is extremely common and is usually due to faecal – oral transmission of bacteria or their toxins. Chronic diarrhoea is increased frequency of defecation and loose, watery pellet stools.

Epidemiology:

Death rate of 4.5 million in 1980 for gastroenteritis. Diarrhoea remains the second leading cause of infant mortality (16%) often pneumonia (17%) in this age group. The majority of such cases occur in the developing world with over half of the recorded cases of childhood diarrhoea occurring in Africa and Asia with 96 million and 1.2 billion cases respectively, compared to only 480 million in the rest of the world.

Infectious diarrhoea resulted in about 0.7 million deaths in children under five years old in 2001 and 250 million last school days. In the America's diarrhoeal disease accounts for a total of 10% of death among children aged 1-59 months. While in south east asia, it accounts for 31.3% of deaths. It is estimated that around 21% of child mortalities in developing countries are due to diarrhoeal diseases.

World wide in 2004 approximately 2.5 billion cases of diarrhoea occurred which result in 1.5 million death among children under the age of five. Greater than half of these were in Africa and South Asia.

In India accounts for 13% of all deaths in Indian children younger than 5 years.

There is significant morbidity associated with diarrhoea in children under 5 years of age. Reducing mortality from diarrhoea is clinical to achieving the health care goals.

Causes

- Poor sanitation, open defecation is a leading cause of infectious diarrhoea leading to death.

- Contaminated water
- Poor nutrition
- Malabsorption
- Inflammatory Bowel disease
- Irritable bowel syndrome

Other causes

- Diarrhoea can be caused by chronic ethanol ingestion.
- Ischemic bowel disease
- Bile salt malabsorption
- Harm one secreting tumours Eg : serotonin can cause diarrhoea.
- Viral infection such as rotavirus, enterovirus or a hepatic virus.
- Bacterial infections like E-coli, Salmonella, Shigella can cause the disease.

PATHOPHYSIOLOGY

Diarrhoea is an increase in the volume of stool or frequency of defecation. It is one of the most common clinical signs of gastrointestinal disease, but also can reflect primary disorders outside of the digestive system. Certainly, disorders affecting either the small or large bowel can lead to diarrhoea.

For many people, diarrhoea represents an occasional inconvenience or annoyance, yet atleast 2 million people in the world, mostly children, die from the consequences of diarrhoea each year.

Pathophysiology of diarrhoea have classified into,

1. Secretory diarrhoea
2. Osmotic diarrhoea
3. Inflammatory diarrhoea
4. Iatrogenic or Drug induced diarrhoea.

Prevention:

- By proper sanitation
- Hand washing
- Improved sanitation of drinking water
- Vaccination (Rotavirus)
- Proper nutrition
- Breast feeding exclusively for 6 months after birth.

3.12. PHARMACEUTICAL REVIEW

3.12.1. SIDDHA ASPECT OF THE FORMULATION

PILL (MATHIRAI)

DEFINITION OF PILL

The raw drugs are triturated with the juices of leaves or kudineer. They are rolled into different sizes of pills, dried and stored.

RULES OF TRITURATION

The ingredients should be first purified and powdered into a find powder and then maserated with the prescribed juices or liquid one after the other in their order. Each time it must be grinded till it becomes waxy in consistency does not adhere to the fingers or mortar and pestle, lastly it must be made into pills as prescribed and dried in shade.

ADDITION OF AROMATIC INGREDIENTS

Aromatic ingredients were added just before 24mins (1 naazhigai) of pill rolled from finely grounded paste. The aromatic ingredients added pills should allowed to dry in shade and keep them in a tightly closed container.

SIZE AND SHAPE

Usually round in shape, there fore it also called as 'Urundai'. But it may differ in some preparations. e.g. Urai mathirai. The size, shape and weight of the pills should be made as said in the literature evidence.

SHELF LIFE

1 year

3.12.2. MODERN ASPECT OF THE FORMULATION

DEFINITION OF PILLS (MATHIRAI):

A tablet is a pharmaceutical dosage form it otherwise called as caplet. Medicinal tablets are called as "pills". Originally "pills" referred specifically to a soft mass rolled into a ball shape, rather than a compressed powder.

As per Indian pharmacopeia 2007 defined the tablets are solid dosage forms each containing a unit dose of one or more medicaments. They are anticipated for oral route. A tablet consists an active medicament with excipients which are in powder form are compressed or pressed into a solid dosage form. About two third drugs prescribed are in solid dosage form and tablets include half of them.

Classification

As per IP 2007 tablets are majorly classified into following categories (Indian pharmacopoeia 2007).

1. Uncoated Tablets

This type of tablets contains single layer or more than one layer tablet consisting of active ingredient with the excipients, no additional cover is applied onto it after the compression.

2. Coated tablets

Coated types of tablets have an additional coating layer on it after the tablet was compressed, the coating layer of tablets formed with sugar, gums, resins, inactive or insoluble fillers, plasticisers, polyhydric alcohols, waxes.

3. Dispersible tablets

These are the film coated or uncoated tablets because a uniform dispersion when suspended in water.

4. Effervescent Tablets

These type of tablets which are uncoated and are planned to be dissolved and produce an dispersion before they are administered the dissolution is achieved by the reaction between an organic acid and bicarbonate which produce Co_2, thus produced Co_2 will disintegrate the tablet so which dissolves in the solution to produce an suspension which was rapidly absorbed.

5. Modified-release tablets

These types of tablets are the coated or uncoated tablets which are designed in such a way that the rate or location of the active ingredient released is modified. It includes enteric coated tablets, prolong release tablet or delay release tablet.

A. Enteric- coated tablets

These are also called as gastro resistant tablets as they resistant to the gastric juices; these are formulated by coating the tablet with anionic polymer of methylacrylic acid and their esters or by coating with cellulose acetyl pthylate. Ex. Erythromycin, NSAIDS.

B. Prolonged –release tablets

These types are otherwise called as sustain release tablets or extended release tablets was formulated in such a way that the active ingredient is released for a prolong duration of time and is available in systemic circulation after administration.

C. Delayed –release tablets.

This dosage form was planned to release the drug after some time delay or after the tablet has passed one part of the GIT into another. All enteric coated tablets are type of delayed action tablet but all delayed action of tablets was not enteric or not intended to produce enteric action.

6. Soluble tablets

These are coated or uncoated tablets which are planned to dissolve in water before they are administered.

7. Tablets for use in the mouth:

These are the tablet formulations which are planned to be show local action in the buccal cavity. These include buccal tablet, sublingual tablets and troche or lozenges. Buccal tablets are placed in between the cheek and gingival. Sublingual tablets are placed below the tongue.

Eg : glyceryl trinitrate

8. Tablets for other routes of administration

These include implantable tablets and vaginal tablet. These are inserted into the rectum or vagina for their local or systemic action.

4. MATERIALS AND METHODS

4.1. PREPARATION OF THE DRUG :

Kattuvai Mathirai has been selected from the classical siddha literature Sikitcha Rathna deepam yennum vaidhiya nool, Page No:158

Ingredients of the test drug are Lingam, Ilavampisin, Athividayam, Sathikkai, Sathipathiri, Vilvapazham, Kaichukkatty, Kirambu, Oomathi, Neermulli.

COLLECTION OF THE DRUGS :

The raw drugs lingam, Ilavampisin, Athividayam, Sathikkai, Sathipathiri, Vilvapazham, Kaichukkatty, Kirambu, Oomathai, Neermulli were purchased from authorized drug store in Nagercoil at Kanyakumari district.

IDENTIFICATION AND AUTHENTICATION OF DRUGS :

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

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

INGREDIENTS:

1. *Lingam* (Red sulphide of mercury) - 976mg (2 panavedai)
2. *Ilavampisin* (Gum of Bombaxmalabaricum Linn) 7.65gm (1 1/2 Kalanju)
3. *Athividayam* (Aconitum heterophyllum wall-ex.Royle) - 7.65gm (1 1/2 Kalanju)
4. *Sathikkai* (Mylistica fragrans Houtt) - 7.65 gm (1 1/2 kalanju)
5. *Sathipatthiri* (Myristica fragrans Houtt) - 7.65gm (1 1/2 Kalanju)
6. *Vilva pazham* (Fruit of Aegle marmelos Linn) - 15.3 gm (3 Kalanju)
7. *Kaichukatty* (Extract from Acacia catechu Linn) - 7.65 gm (1 1/2 Kalanju)
8. *Kirambu* (Syzygium aromaticum Linn) - 7.65gm (1 1/2 Kalanju)
9. Leaf juice of Datura metal Linn - Quantity Sufficient
10. Hygrophila auriculata seeds Linn - Quantity Sufficient

PURIFICATION OF INGREDIENTS:

1. *Lingam*: (Red sulphide of mercury)

Impure *Lingam* can be subjected to powdered and saturated with equal parts of lime juice, Cow's Milk, Acalipha indica juice.

2. *Ilavampisin*: (Gum of Bombox malabaricum)

Gum is dissolved in water then filtered and dried in sunlight.

3. *Athividayam*: (Aconitum heterophyllum)

Outer layer is scrapped out and made into pieces & allow to dry in sunlight.

4. *Sathikkai*: (Myristica fragrans)

Scrap the outer skin and roast it in ghee

5. *Sathipathiri* : (Myristica fragrans)

Sathipathiri is allowed to dry in sunlight.

6. *Vilvapazham*: (Fruit of Aegle marmelos)

Remove the outer shell of the fruit.

7. *Kaichukkatty*: (Extract from Acacia catechu)

First Kaichukatty was powdered and it should be dissolved in distilled water, until it became saturated and filtered then allowed to dry completely under sunlight. It takes for complete drying. Then the purified Kaichukatty was made into fine powder.

8. *Kirambu* : (Syzygium aromaticum)

Kirambu is allowed to dry in sunlight.

9. Neermulli : (Hygrophila auriculata)

Clean dust, sand and infected seeds.

10. Oomathai: (Datura metal)

Remove riped and infected leaves.

METHOD OF PREPARATION:

All the above purified ingredients are powdered individually, placed in mortar & pestil and triturated well with the leaf juice of datura metal for 3 hours and then measure the weight of paste. Add 3 fold of Hygrophilla auriculata seed in propation to that paste and again triturated with the above juice for 3 hours and made into pill upto one kundri.

SHELF LIFE :

1 Year

METHODS OF APPLICATIONS:

Adjuvants : Ghee

Dosage : *(Kundrimaniyalavu* – 130mg) One pill morning & evening

INDICATION:

All types of *Bedhi*

Fig : 1 Ingredients of Kattuvai Mathirai

BEFORE PURIFICATION

Lingam

Athividayam

Sathikkai

Sathipathiri

Kirambu

Fig : 1 Ingredients of Kattuvai Mathirai

BEFORE PURIFICATION

Ilavam pisin

Kaichukatti

Vilva pazham

Oomathai ilai

Neermulli vithai

Fig : 1 Ingredients of Kattuvai Mathirai

AFTER PURIFICATION

Lingam

Athividayam

Sathikkai

Sathipathiri

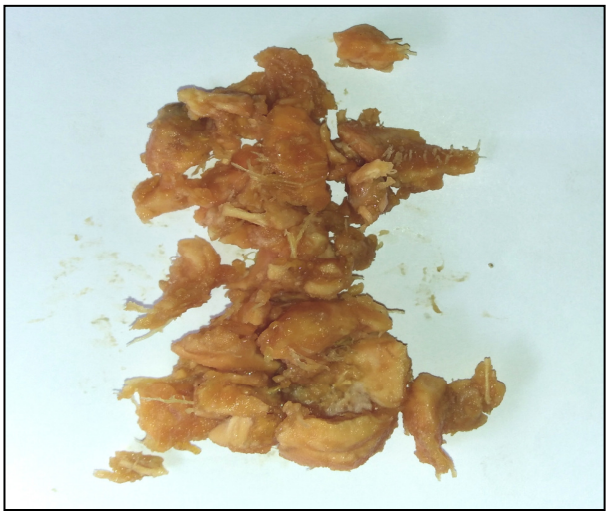
Kirambu

Fig : 1 Ingredients of Kattuvai Mathirai

AFTER PURIFICATION

Ilavampisin

Kaichukatti



Vilva pazham

Oomathai ilai Saru

Neermulli vithai

Fig : 3 KATTUVAI MATHIRAI

On Processing

Prepared Drug - Kattuvai Mathirai

Drug : *KATTUVAI MATHIRAI*

4.2. STANDARDIZATION OF THE DRUG:

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

4.2.1. PHYSICAL ANALYSIS

TESTING PHYSICAL CHARACTERIZATION OF SAMPLE:

Colour Examination:

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

Odour examination:

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

Size examination:

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

Weight Variation Test:

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

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

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

Table. 1 ***Weight Variation limits of Tablets (IP)***

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

Accepted tablet:

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

Suspected tablet:

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

Rejected tablets:

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

Solubility:

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

pH Value:

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

4.2.2. PHYSICO CHEMICAL ANALYSIS

LOSS ON DRYING (INDIAN PHARMOCOEPIA, 1996)

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

TOTAL ASH:

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

a)Acid insoluble ash

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

b)Water soluble extractive

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

c)Alcohol soluble extractive

Macerate 5g of the air dried drug, coarsely powdered, with 100ml of alcohol of the specified strength in a closed flask for 24 hrs, shaking frequently during six hours and allowing to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105^{o}, to constant weight. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

TABLET DISINTEGRATION TEST:

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

MICROBIAL LIMIT TEST OF *KATTUVAI MATHIRAI*

1. Evaluation of Total Aerobic Bacterial Count

1.1. Preparation of Sample for Experimental Work

Weighed 10 gm of the homogenized drug sample aseptically and dissolved in 10 ml of sterile water and made up to 100 ml with the sterile water. The insoluble drug product was suspended in 100 ml of buffered sodium chloride-peptone solution (pH 7.0).

1.2. Serial dilution of Sample

A serial dilution is the dilution of a sample, in 10-fold dilutions. From the sample, 1 ml of the sample was added to 9 ml of sterile distilled water and mixed it well. This dilution was denoted as 10^{-1} dilution. From this dilution, one ml was taken from that mixture is added to 9 ml, and designated as 10^{-2} dilution. The same procedure was repeated up to 10^{-4}.

1.3. Isolation of Total Viable Aerobic Microbial Count

1.3.1. Isolation of Bacteria by Plate Count Method

In this test, the bacteria in sample were made to grow as colonies, by inoculating a known volume of sample into a solidifiable nutrient medium (Casein Soybean Digest agar or Nutrient agar medium) in petridish. The agar plate was prepared by mixing growth medium with agar and then sterilized by autoclaving. Once the agar was cooled to 45^{o}C, approximately 15 to 20 ml of medium was poured into a sterile Petri dish under aseptic condition and left to solidify for 15 minutes. After solidification, each plate was smear with 0.1 ml of sample from the dilution of 10^{-1} and 10^{-2}. After inoculations, all the plates were incubated at 37^{o}C for 24 hours. After incubation, the bacterial colonies were developed as visible to the naked eye and the number of colonies on a plate was counted using Quebec Colony Counter. Plates with an average of from 30 to 300 colonies of the target bacterium were selected for colony count. Because of the statistical problems, plates with lower than 30 colonies greater than 300 colonies were rejected

1.3.1.1. Composition of Nutrient Agar Media

Peptone	: 5.0 gm
Sodium chloride	: 5.0 gm
Beef extract	: 1.5 gm
Yeast extract	: 1.5 gm
Agar	: 15.0 gm
Distilled water	: 1000 ml
pH (at 25°C)	: 7.4±0.2

1.3.2. Isolation of Fungi

From each of the above prepared samples, 0.1 ml of sample was transferred to Sabouraud Dextrose agar (SDA) prepared with Chloramphenicol. The plates were then incubated for 5 days at room temperature (20 to 25°C). After incubation, the fungal colonies were observed and calculated.

1.3.2.1. Composition of SDA

Dextrose	; 40 gm
Peptone	: 10 gm
Agar	: 15 gm
Distilled water	: 1000 ml

1.4. Evaluation of Antimicrobial Activity of Drug

Antimicrobial activity was performed by disc diffusion method on agar.

1.4.1 Preparation of drug extracts solutions for the experiment

The dried drugs were weighed and dissolved in sterile distilled water to prepare appropriate dilution to get required concentrations of about 10, 20 and 30µg/ml. They were kept under refrigerated condition unless they were used for the experiment.

1.4.2. Procedure for the Agar Well Diffusion Test

The antibacterial screening of the drugs were carried out by determining the zone of inhibition using disc diffusion method. All the drug extracts were tested against four pathogenic bacterial strains of gram positive and gram negative organism by disc diffusion method.

1.4.3. Bacterial Inoculums Preparation

Inoculums of *Staphylococcus mutants*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, and *Bacillus substilis* were prepared in nutrient broth medium and kept for incubation at 37°C for 8 hrs.

1.4.4. Agar well-diffusion method

This method was followed to determine the antimicrobial activity. Muller-Hinton Agar media plates were swabbed (sterile cotton swabs) with 8 hour old - broth culture of respective bacteria. After inoculation, wells with the size of 10 mm diameter and about 2 cm a part were made in each of these plates using sterile cork borer. Stock solution of each drug extract was prepared at a concentration of 1 mg/ml in water. About 100 µl of different concentrations of drug solvent extracts were added into the wells and allowed to diffuse at room temperature for 2 hrs. The plates were incubated at 37°C for 24 hrs. After incubation, the diameter of the inhibition zone (mm) was measured and the activity index was also calculated.

1.4.4.1. Composition of Muller Hinton Agar Media

Beef Extract	: 02.00 gm
Acid Hydrolysate of Casein	: 17.50 gm
Starch	: 01.50 gm
Agar	: 17.00 gm

1.5. Evaluation of Specified Microorganisms

1.5.1. Isolation & Identification of *Escherichia coli*

One ml of the prepared sample was added in a sterile screw-capped container containing 50 ml of nutrient broth and mixed well. Then, it was allowed to stand for 1 hour and mixed well again. After one hour, the screw caps of the bottle was loosened and incubated at 37º for 18 to 24 hours.

1.5.1.2. Primary Test

From the above prepared enrichment culture, 1.0 ml was taken and transferred aseptically into a tube containing 5 ml of Mac- Conkey broth. Inoculated tubes were incubated in a water-bath at 36º to 38º for 48 hours.

1.5.1.3. Secondary Test

From the primary test, 1.0 ml of the enrichment culture was taken and transferred aseptically in to 5 ml of peptone water. It was then incubated in a water-bath at 43.5º to 44.5º C for 24 hours and observed the tubes for acid and gas. Then, the culture was subjected to biochemical tests of imvic and the results were observed and correlated.

1.5.1.4. Alternative test

It was done by a loop full of enriched culture in the primary test was streaked on a sterile Mac-Conkey agar medium. Then, the plates were inverted and incubated at 37 º C for 24 hours. After incubation, the pink or brick red color colonies were examined and

transfer them individually into the surface of Eosin Methylene Blue agar medium (EMB), on Petri dishes. Inoculated plates were inverted and incubated at 37 ° C for 24 hours. After incubation, the colonies on medium were checked for their color appearance like green metallic sheen under reflected light. The colonies were subjected to confirmation by further suitable cultural and biochemical tests.

1.5.1.5. Components of Eosin Methylene Blue Agar Media

Pancreatic digest of gelatin	: 10.0 g
Dibasic potassium phosphate	: 2.0 g
Lactose	: 10.0 g
Eosin Y	: 400 mg
Methylene blue	: 65 mg
Agar	: 15.0 g
Distilled water	: 1000 ml

1.5.1.6. Components of Eosin Methylene Blue Agar Media

Pancreatic digest of gelatin	: 10.0 g
Dibasic potassium phosphate	: 2.0 g
Agar	: 15.0 g
Lactose	: 10.0 g
Eosin Y	: 400 mg
Methylene blue	: 65 mg
Distilled water	: 1000 ml

1.5.2. Isolation & Identification of *Salmonella* sp.

One ml of the prepared sample was added in a sterile screw-capped container containing 100 ml of nutrient broth and mixed well. Then, it was allowed to stand for 1 hour and mixed well again. After one hour, the screw caps of the bottle was loosened and incubated at 37º for 18 to 24 hours.

1.5.2.1. Primary Test

From the above prepared enrichment culture, 1.0 ml was taken and transferred aseptically into a tube containing 10 ml of Selenite F broth. Inoculated tubes were incubated in a water-bath at 36º to 38º for 48 hours. After incubation, the culture was subcultured on two of the agar media namely Bismuth sulphate agar and Deoxy cholate citrate agar and incubated the plates at 36º to 38º for 18 to 24 hours. After incubation, colonies were observed on the medium and confirmed the genus *Salmonella* based on guidelines.

1.5.2.2. Secondary test

The suspected colonies of the primary test were subcultured on the slant of triple sugar-iron agar in test tube and in urea broth. Both media were incubated at 37ºC for 24 hours. After incubation, the results were observed according to the development of color change and acid / gas in media. The presence of Salmonella was confirmed by agglutination tests.

1.5.2.3. Composition of *Salmonella Shigella* Agar Media

Beef Extract	: 5.0 gm
Enzymatic Digest of Casein	: 2.5 g
Enzymatic Digest of Animal Tissue	: 2.5 gm
Lactose	: 10 gm
Bile salts	: 8.5 gm
Sodium Citrate	: 8.5 gm
Ferric Citrate	: 1.0 gm
Brilliant Green	: 0.00033 gm
Neutral Red	: 0.025
Agar	: 13.5 gm
Distilled water	: 1000 ml

1.5.3. Isolation and Identification of *Pseudomonas aeruginosa*

From the above prepared enrichment culture, 1.0 ml was taken and transferred aseptically into 100 ml of fluid soyabean-casein digest medium and mixed well. The inoculated tubes were incubated at 37º C for 24 hours. After incubation, the growth of bacteria was checked. From this, a loop full of culture was streaked on the surface of Cetrimide agar medium and Pseudomonas Isolation Agar medium and incubated at incubated at 37º C for 24 hours. After incubation, the colonies from the agar surface of these two media were checked for detection of fluorescein and pyocyanin.

1.5.3.1. Composition of Cetrimide Agar Media

Pancreatic digest of gelatin	: 20.0 g
Magnesium chloride	: 1.4 g
Potassium sulphate	: 10.0 g
Cetrimide	: 0.3 g
Agar	: 13.6 g
Glycerin	: 10.0 g
Distilled Water t	: 1000 ml

1.5.4. Isolation and Identification of *Staphylococcus aureus*

From the above prepared enrichment culture, a loop full of culture was taken and transferred aseptically on Mannitol salt agar and incubated at 37º C for 24 hours.. After incubation, the colonies were subjected to confirmation by hem agglutination test.

1.5.4.1. Composition of Mannitol Salt Agar Media

Pancreatic digest of gelatin	: 5.0 g
Peptic digest of animal tissue	: 5.0 g
Beef extract	: 1.0 g
D-Mannitol	: 10.0 g
Sodium chloride	: 75.0 g
Agar	: 15.0 g
Phenol red	: 25 mg
Distilled Water	: 1000 ml

4.2.3. BIO CHEMICAL ANALYSIS

PROCEDURE:

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

QUALITATIVE ANALYSIS FOR BASIC RADICALS:

Test for Calcium:

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

Test for Iron (Ferric):

The extract is acidified with glacial acetic acid and potassium ferro cyanide.absence of blue colour indicates the absence 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. Gives absence of white precipitate indicates the absence 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 absence of brisk effervescence indicates the absence 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. Gives absence of formation of yellow precipitate indicates the absence of albumin.

Test for tannic acid:

The extract is treated with ferric chloride.Gives absence of formation of bluish black precipitate indicates the absence 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

4.2.4. PHYTOCHEMICAL ANALYSIS OF *KATTUVAI MATHIRAI*

The siddha preparation *KATTUVAI MATHIRAI* was prepared and used for phytochemical analysis. Preliminary test, on the siddha preparation *KATTUVAI MATHIRAI* was carried out for the presence of alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, phenolic compounds, proteins and free amino acids, flavanoids, lignin, fixed oils and fats. The methods adopted for the estimation are as follows:

Test for Alkaloids (Evans, 1997)

A small segment of the siddha preparation *KATTUVAI MATHIRAI* was mixed separately with a few drops of dilute hydrochloric acid and filtered. The filtrates were tested carefully with various alkaloidal reagents as follows:

a) Mayer's test (Evans, 1997):

To a few ml of filtrate, a drop of Mayer's reagent is added by the side of the test tube. A white or creamy precipitate indicates that the test as positive.

b) Hager's test (Wagner et al., 1996):

To a few ml of filtrate, one to 2ml of Hager's reagent is added. A prominent yellow precipitate indicates the test as positive.

c) Dragendorff's test (Waldi, 1965):

To a few ml of filtrate, one to 2ml of Dragendorff's reagent is added. A prominent yellow precipitate indicates the test as positive.

Test for Carbohydrates (Ramakrishnan et al., 1994)

A small quantity of siddha preparation *KATTUVAI MATHIRAI* was dissolved separately in 5ml of distilled water and filtered. The filtrate was subjected to Molisch's test to detect the presence of carbohydrates. Filtrate was treated with 2-3 drops of 1% alcoholic alpha naphthol solution and 2ml of concentrated sulphuric acid was added along the sides of the test tube. Appearance of brown ring at the junction of 2 layers shows the presence of carbohydrates.

Test for Glycosides

The siddha preparation *KATTUVAI MATHIRAI* was hydrolyzed with hydrochloric acid for few h on a water bath and the hydrolysate was subjected to Legal's and Borntrager's test to detect the presence of different glycosides.

(a) Legal's Test:

To the hydrolysate, one ml of pyridine and few drops of sodium nitro prusside solution were added and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red color shows the presence of glycosides and aglycones.

(b) Borntrager's Test:

Hydrolysate was treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Ammoniacal layer acquires pink color, shows the presence of glycosides (Evans, 1997).

Test for Phytosterols (Finar, 1986)

(a) **Liebermann Burchard Test:**

Small amount of the siddha preparation *KATTUVAI MATHIRAI* was dissolved with 3ml of acetic anhydride, a few drops of glacial acetic acid and followed by the addition of few drops of concentrated sulphuric acid. Appearance of bluish green color shows the presence of phytosterols.

(b) **Salkowski Test:**

Small quantities of the siddha preparation *KATTUVAI MATHIRAI* were dissolved in chloroform separately. This chloroform solution was added with few drops of concentrated sulphuric acid. The appearance of bluish green color shows the presence of phytosterols.

Test for Flavanoids

(a) **Shinoda's Test:**

Small quantity of siddha preparation *KATTUVAI MATHIRAI* was treated with alcohol to that a piece of magnesium was added followed by an addition of concentrated hydrochloric acid drop wise and heated. Appearance of magenta color shows the presence of flavanoids (Harborne, 1984).

(b) **Florescence Test:** Small quantity of *KATTUVAI MATHIRAI* was dissolved separately in alcohol and a drop of that extract was placed on Whatman filter paper and observed under UV light. Florescence indicates the presence of flavanoids.

Test for Tannins (Mace, 1963)

Small quantities of siddha preparation *KATTUVAI MATHIRAI* was dissolved separately in water and tested for the presence of phenolic compound and tannins. In the process of testing and treating, the following observations were noted:

a) Dilute ferric chloride solution (5%) gives a dark green color. 38

b) 10% aqueous potassium dichromate solution gives yellowish brown precipitate.

c) 10% lead acetate solution gives a white precipitate.

Test for Proteins and Free Amino Acids (Fisher, 1968; Ruthmann, 1970)

Small quantities of various siddha preparation *KATTUVAI MATHIRAI* was dissolved in few ml of water and the following reaction were carried out

(a) Millon's Test : To 2ml of filtrate, few drops of Millon's reagent were added. A white precipitate indicates the presence of proteins (Rasch and Swift, 1960).

(b) Ninhydrin Test: To 2ml of filtrate 2 drops of ninhydrin solution was added. A characteristic purple color indicates the presence of amino acids (Yasma and Ichikawa, 1953).

(c) Biuret Test: An aliquot of 2ml of filtrate was treated with a drop of 2% copper sulphate solution. To this, 1ml of ethanol (95%) was added, followed by excess of potassium hydroxide pellets, Pink color in the ethanol layer indicates the presence of protein (Gahan, 1984).

Tests for Fixed oils and Fats

(a) **Spot Test:**

A small quantity of siddha preparation *KATTUVAI MATHIRAI* was placed between 2 filter papers. Oil stains produced with any extract shows the presence of fats and fixed oils in the *KATTUVAI MATHIRAI* (Harborne, 1984).

(b) **Saponification Test:**

A small quantity of siddha preparation *KATTUVAI MATHIRAI* was treated with few drops of 0.5N alcoholic potassium hydroxide along with 2 to 3 drops of phenolphthalein. Later the mixture is refluxed for about 2h. Soap formation indicates the presence of fats and fixed oils in the *KATTUVAI MATHIRAI* .

Tests for Lignin

Small quantities of *KATTUVAI MATHIRAI* was dissolved separately in few ml of alcoholic solution of hydrochloric acid and phloroglucinol gives red color, which shows lignin is present.

Test for Saponins (Kokate, 1999)

Frothing Test:

The siddha preparation *KATTUVAI MATHIRAI* was diluted separately with 20ml of distilled water and it was agitated on a graduated cylinder for 15min. Absence of the foam formation shows the devoid of saponins.

4.2.5. INSTRUMENTAL ANALYSIS

Fig. No. 1 SCANNING ELECTRON MICROSCOPE (SEM)

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

Introduction:

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

Principle:

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

SEM MECHANISM

Procedure:

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

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

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

In order to verify the existence of a substance and recognize its shape, the image contrast must be well defined. In other words, even if a system boasts extremely high resolution, if image contrast is poor, it would be extremely difficult to determine the

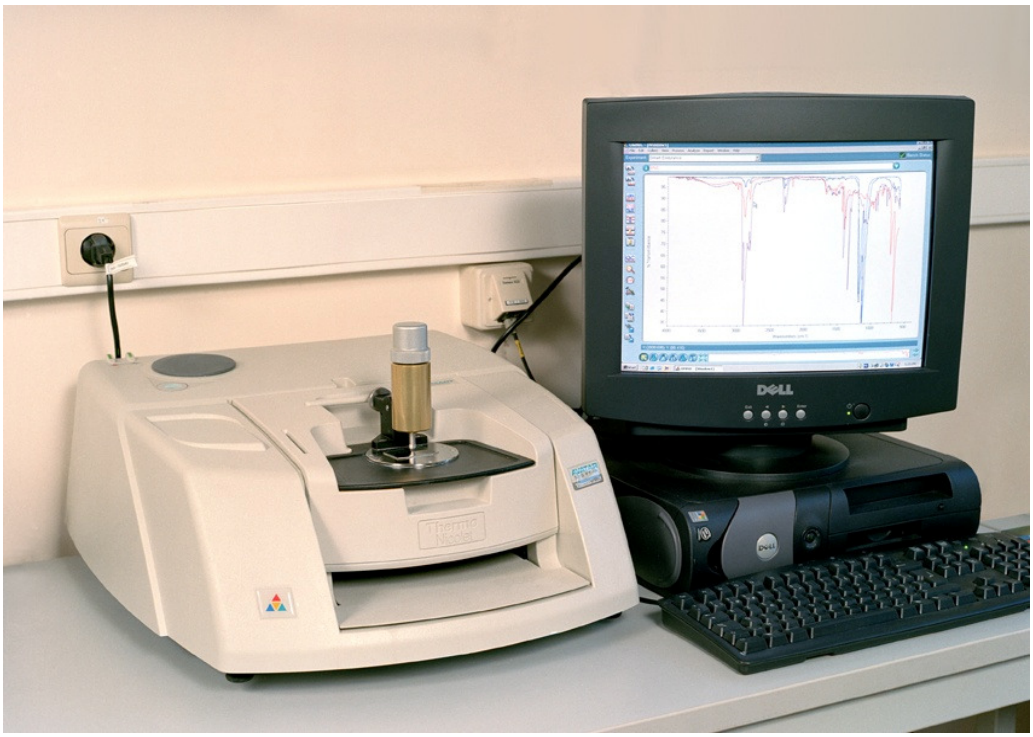
existence of a substance, let alone recognize its shape. Another important feature of the SEM is the three-dimensional appearance of the specimen image, which is a direct result of the large depth of field.

Applications:

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

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

Fig. No. 2 FOURIER TRANSFORM-INFRA RED SPECTROSCOPY (FT-IR)



Introduction:

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

Principle:

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

Source	:	Nernst Glower
Beam splitter	:	It is made up of a transparent material. Thin films of Silicondepositedon Potassium bromide

(KBr) Bromide (KBr) Detectors: DeutratedTriGlycine Sulphate (DTGS).

Scan Range	:	MIR 450to 4000 cm-1
Resolution	:	4.0 cm-1
Sample required	:	50mg, solid or liquid
Sampling Techniques:		There are a variety of techniques for sample preparation physical form of the sample to be analyzed.
Solid	:	KBr or Nujol mull method.
Liquid	:	Csl / TIBr Cells
Gas	:	Gas cells

Measurements Techniques:

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

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

Procedure:

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

1. Then, 300 Al of 20 mM buffer, prepared in H_20 or $2H_20$, pH or p2H 7.2, were added and the sample concentrated again. The p2H value corresponds to the pH meter reading + 0.4. The concentration and dilution procedure was repeated several times in order to completely replace the original buffer with the This buffer.
2. The washings took 24 h, which is the time of contact of the protein with the 2H20medium prior FT-IR analysis. In the last washing, the protein was concentrated to fine a volume of approximately 40 Al and used for the infrared measurements.The concentrated protein sample was placed in CaF2 windows and a 6 Am tin spacer or a 25 Am Teflon spacer for the experiments in H20 or 2H20, respectively. FT-IR spectra were recorded by means of a Perkin-Elmer -Spectrum-1 FT-IR spectrometer using a deuteratedtriglycinesulfate detector.
3. At least 24 h before, and during data acquisition, the spectrometer were continuously purged with dry air at a dew point of 40^oC. Spectra of buffers and samples were acquired at 2 cm_1 resolution under the same scanning and temperature conditions. In the thermal denaturation experiments, the temperature was raised in 5^oC steps from 20 to 95^oC.
4. Before spectrum acquisition, samples were maintained at the desired temperature for the time necessary for the stabilization of temperature inside the cell (6 min).Spectra were collected and processed using the SPECTRUM software from Perkin-Elmer. Correct subtraction of H_20 was judged to yield an approximately flat baseline at 1900-1400 cm_ 1, and subtraction of $2H_20$ was adjusted to the removal of the$2H_20$ bending absorption close to 1220 cm_ 1

KBr Method

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

Nujol Mull Method:

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

Liquids:

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

Applications:

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

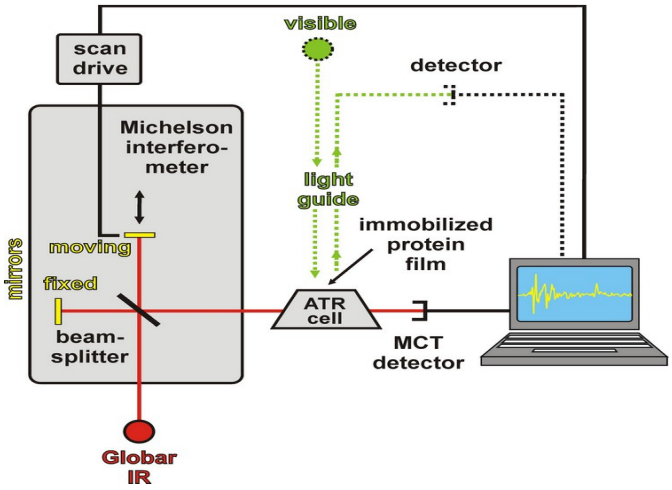


Fig. No. 3 Mechanism of FTIR analyzer

Analytical Capabilities:

1. Identifies chemical bond functional groups by the absorption of infrared radiation which excites vibrational modes in the bond.
2. Especially capable of identifying the chemical bonds of organic materials
3. Detects and identifies organic contaminants.
4. Identifies water, phosphates, sulphates, nitrates, nitrites, and ammonium ions
5. Detection limits vary greatly, but are sometimes $<10^{13}$ bonds/cm^3 or sometimes sub monolayer .Useful with solids, liquids, or gases.

Fig. No. 4 INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETRY(ICP-OES):

Introduction:

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

Mechanism:

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

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

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

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

The intensity of each line is then compared to previously measured intensities ofknown concentrations of the elements and their concentrations are then computed by interpolation along the calibration lines. In addition, special software generally corrects for interferences caused by the presence of different elements within a given sample matrix.

Applications :

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

The author used it for elemental identification and quantitative compositional information of the *KATTUVAI MATHIRAI.*

4.2.5.4 X-RAY POWDER DIFFRACTION (XRD) INSTRUMENTATION

X-ray diffractometers consist of three basic elements: an X-ray tube, a sample holder and an X-ray detector.

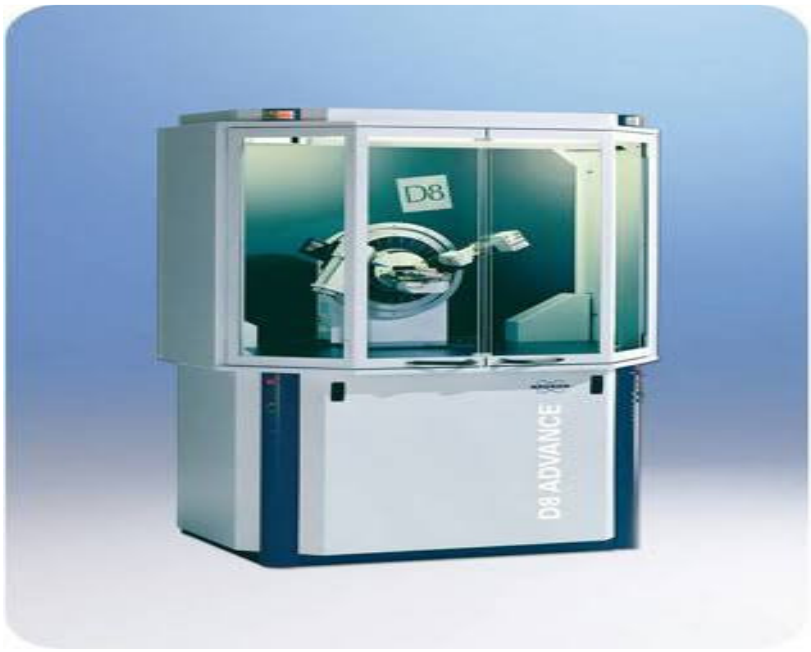


Fig.No. 5 Bruker's X-ray Diffraction D8-Discover instrument.

X-rays are generated in a cathode ray tube by heating a filament to produce electrons, accelerating the electrons towards a target by applying a voltage and bombarding the target material with electrons. When electrons have sufficient energy to dislodge inner shell electrons of the target material, characteristic X-ray spectra are produced. These spectra consist of several components, the most common being K_α and K_β. K_α consists in part of $K_{\alpha1}$ and $K_{\alpha2}$. $K_{\alpha1}$ has a slightly shorter wavelength and twice the intensity of $K_{\alpha2}$. The specific wavelengths are characteristic of the target material (Cu, Fe, Mo, Cr). Filtering, by foils or crystal monochrometers, is required to produce monochromatic X-rays needed for diffraction. $K_{\alpha1}$ and $K_{\alpha2}$ are sufficiently close in wavelength such that a weighted average of the two is used. Copper is the most common target material for single-crystal diffraction, with CuK_α radiation = $1.5148A^{0.}$ These X-rays are collimated and directed onto the sample. As the sample and detector are rotated, the intensity of the reflected X-rays is recorded. When the geometry of the incident X-rays impinging the sample satisfies the Bragg Equation, constructive interference occurs and a peak in intensity occurs. A detector records and processes this X-ray signal and converts the signal to a count rate which is then output to a device such as a printer or computer monitor.

The geometry of an X-ray diffractometer is such that the sample rotates in the path of the collimated X-ray beam at an angle θ while the X-ray detector is mounted on an arm to collect the diffracted X-rays and rotates at an angle of 2θ. The instrument used to maintain the angle and rotate the sample is termed a goniometer. For typical powder patterns, data is collected at 2θ from -5^0 to 70^0, angles that are present in the X-ray scan.

Applications:

X-ray powder diffraction is most widely used for the identification of unknown crystalline materials (e.g. minerals, inorganic compounds). Determination of unknown solids is critical to studies in geology, environmental science, material science, engineering and biology.

Other applications include:

1. Characterization of crystalline materials
2. Identification of the fine-grained minerals such as clays and mixed layer clays that are difficult to determine optically.
3. Determination of unit cell dimensions.
4. Measurement of sample purity.

With specialized techniques, XRD can be used to:

1. Determine crystal structures using Rietveld refinement
2. Determine of modal amounts of minerals (quantitative analysis)
3. Make textural measurements such as the orientation of grains in a polycrystalline sample.

Strengths and Limitations of X-ray Powder Diffraction:

Strengths:

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

Limitations:

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

User's Guide-Sample Collection and Preparation:

Determination of an unknown requires: the material, an instrument for grinding and a sample holder.

1. Obtain a few tenths of a gram (or more) of the material, as pure as possible.
2. Grind the sample to a fine powder, typically in a fluid to minimize inducing extra strain (surface energy) that can offset peak positions, and to randomize orientation.
    a. Powder less than -10 μm (or 200-mesh) in size is preferred.
3. Place into a sample holder or onto the sample surface.
    a. Packing of the fine powder into a sample holder. Smear uniformly onto a glass slide, assuring a flat upper surface.
4. Pack into a sample container
5. Sprinkle on double sticky tape
    a. Typically the substance is amorphous to avoid interference
6. Care must be taken to create a flat upper surface and to achieve a random distribution of lattice orientations unless creating an oriented smear.
7. For unit cell determinations, a small amount of a standard with known peak positions (that do not interfere with the sample) can be added and used to correct peak positions.

Data Collection, Results and Presentation:

Data collection:

The intensity of diffracted X-rays is continuously recorded as the sample and detector rotate through their respective angles. A peak in intensity occurs when the mineral contains lattice planes with d-spacings appropriate to diffract X-rays at that value of θ. Although each peak consists of two separate reflections ($K_{\alpha 1}$ and $K_{\alpha 2}$), at small values of 2θ the peak locations overlap with $K_{\alpha 2}$ appearing as a hump on the side of $K_{\alpha 1}$. Greater separation occurs at higher values of θ. Typically these combined peaks are treated as one. The 2λ position of the diffraction peak is typically measured as the center of the peak at 80% peak height.

Data reduction:

Results are commonly presented as peak positions at 2θ and X-ray counts (intensity) in the form of a table or an *x-y* plot (shown above). Intensity (*I*) is either reported as peak height intensity, that intensity above background, or as integrated intensity, the area under the peak. The relative intensity is recorded as the ratio of the peak intensity to that of the most intense peak (*relative intensity* $= I/I_1 \times 100$).

The d-spacing of each peak is then obtained by solution of the Bragg equation for the appropriate value of λ. Once all d-spacings have been determined, automated search/match routines compare the *ds* of the unknown to those of known materials. Because each mineral has a unique set of d-spacings, matching these d-spacings provides an identification of the unknown sample. A systematic procedure is used by ordering the d-spacings in terms of their intensity beginning with the most intense peak. Files of d-spacings for hundreds of thousands of inorganic compounds are available from the International Centre for Diffraction Data as the Powder Diffraction File (PDF). Many other sites contain d-spacings of minerals such as the American Mineralogist Crystal Structure Database. Commonly this information is an integral portion of the software that comes with the instrumentation.

4.3. TOXICOLOGICAL STUDIES

4.3.1. ACUTE TOXICITY STUDY IN FEMALE WISTER RATS TO EVALUATE TOXICITY PROFILE OF *KATTUVAI MATHIRAI*

OBJECTIVES

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

Guidelines followed:

(a) OECD Guidelines No. 423,

Study Design and Controls:

1) Female Wister Rats in controlled age and body weight were selected.
2) *Kattuvai Mathirai* was administered at 5 mg/kg, 50 mg/kg, 300 mg/kg,1000 mg/kg, 2000 mg/kg, body weight as (Water) as suspension along with blank.
3) The results were recorded on day 0, with single oral dosing period of 14 days.

EXPERIMENTAL PROCEDURE

1. ANIMALS

Supply

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

All animals underwent a period of 20 days of observation and acclimatization between the date of arrival and the start of treatment. During the course of this period, the animals were inspected by a veterinary surgeon to ensure that they fulfilled the health requirements necessary for initiation of the Study.

Housing

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

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.

Table – 2 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 – 2 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

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

Doses

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

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

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

Dose Preparation

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

Administration

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

Observation period

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

Mortality and Morbidity

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

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

Objective

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

Test Guideline Followed

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

Test Item Detail

Kattuvai Mathirai

Test System Detail

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

Acclimatization

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

Randomization & grouping

One day before the initiation of treatment (days 0- last day of acclimatization), the selected animals were randomly grouped into three different groups containing minimum 5 male and 5 female animals per group.

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

Table – 3 Numbering and Identification

Case No	Group No	Animal Marking
1	CONTROL	H,B,T,HB,NM (Male) H,B,T,HB, NM (Female
2	Low dose of *KATTUVAI MATHIRAI* 300mg/kg	H,B,T,HB,NM (Male) H,B,T,HB, NM (Female)
3	Middle dose of *KATTUVAI MATHIRAI* 600mg/kg	H,B,T,HB,NM (Male) H,B,T,HB, NM (Female)
4	High dose of *KATTUVAI MATHIRAI* 900 mg/kg	H,B,T,HB,NM (Male) H,B,T,HB ,NM (Female)

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

Case No	Group No	Animal Marking	Sex
1	CONTROL	H,B,T,HB,NM H,B,T,HB, NM	Male Female
2	Low dose of *KATTUVAI MATHIRAI* 300mg/kg	H,B,T,HB,NM H,B,T,HB, NM	Male Female
3	Middle dose of *KATTUVAI MATHIRAI* 600mg/kg	H,B,T,HB,NM H,B,T,HB, NM	Male Female
4	High dose of *KATTUVAI MATHIRAI* 900 mg/kg	H,B,T,HB,NM H,B,T,HB ,NM	Male Female

Husbandry

Housing

The Wister rats were housed in standard polypropylene cages with stainless steel top grill. Paddy husk was used as bedding. The paddy husk was changed at least twice in a week. From the week before initiation of the treatment, each cage contained a maximum of 10 rats of the same sex and treatment group.

Environmental conditions

The animals were kept in a clean environment with 12 hour light and 12 hour dark cycles. The air was conditioned at 22 ± 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.

Feed & feeding schedule

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

Water

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

Doses

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

Test Group	Dose to Animals (mg/kg body –weight / day	Number of Animals
Group – I	CONTROL	10 (5 Male and 5 Female)
Group – II	Low dose of *KM* 300mg/kg	10 (5 Male and 5 Female)
Group – III	Middle dose of *KM* 600mg/kg	10 (5 Male and 5 Female)
Group - IV	High dose of *KM* 900 mg/kg	10 (5 Male and 5 Female)

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

Dose Preparation

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

Administration

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

OBSERVATIONS

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

Clinical signs of toxicity

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

Food intake

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

Water intake

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

Bodyweight:

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

Blood Collection

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

LABORATORY STUDIES

During the 4th week of treatment, samples of blood were withdrawn from the orbital sinus of 5 males from each group, under light ether anesthesia after fasting for 16 hours. The blood samples are used to evaluate Hematological parameters like RBC, WBC, and PLATELETS etc….. The collected blood samples also centrifuged 10000 rpm in 10 minutes to separate the serum. The separated serum used to evaluate biochemical parameters like SGOT, SGPT, ALP and BILIRUBIN ect……...

Hematology

The following hematological parameters were analysed using Autoanalyser

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

Differential WBC count:

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

Clinical Biochemistry:

The following clinical Bio parameters were analysed using Auto analyser

Total serum protein (g/dl)

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

TERMINAL STUDIES

Sacrifice and macroscopic examination

On completion of the 4 weeks of treatment, 18 Wister rats were sacrificed by ether inhalation. A full autopsy was performed on all animals which included examination of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents both *in situ* and after evisceration. As the number of animals exceeded the number that could be sacrificed in one day, the autopsies were carried out over three consecutive days at the end of the treatment period.

Organ weights:

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

4.4.PHARMACOLOGICAL STUDY

4.4.1. ANTI-DIARRHOEAL ACTIVITY

EFFECT OF KATTUVAI MATHIRAI ON CASTOR OIL – INDUCED SMALL INTESTINAL TRANSIT IN RATS

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

Animal Grouping

Group I	-	Control (Distilled Water)
Group II	-	Castor oil +Charcoal
Group III	-	Castor Oil +Atropine (3mg/kg)
Group IV	-	Castor oil (1ml p.o) + ***Kattuvai Mathirai*** (100 mg/kg)
Group V	-	Castor oil (1ml p.o) + ***Kattuvai Mathirai*** (200 mg/kg)

4.4.2. ANTI-PYRETIC ACTIVITY

EFFECT OF *KATTUVAI MATHIRAI* ON BREWER'S YEAST INDUCED PYREXIA IN RATS

MATERIALS AND METHODS

Experimental Animals

Wistar albino rats of either sex weighing about 150-200g were employed for this study. They were procured from Kalasalingam college of Pharmacy, srivilliputhurand maintained on the suitable nutritional and environmental condition throughout the experiment. They were housed in polypropylene cages with paddy house bedding under standard laboratory condition for an acclimatization periods of 7 days prior to performing the experiment.

Experimental Design .

Protocol

Body weights of the animals were recorded and they were randomly divided into 5 groups of 6 animals each as follows:

Group I : animals served as control

Group II : animals were treated with yeast via subcutaneous injection (10ml/kg).

Group III : animals were administered with yeast (10 ml/kg) and the standard drug paracetamol (150mg/kg b.w.), orally

Group IV : animals were administered with yeast (10ml/kg,) and with KM (100mg/kg b.w.), orally.

Group V : animals were administered with yeast (10ml/kg,) and with KM(200mg/kg b.w.), orally.

Yeast induced pyrexia

Pyrexia was induced by subcutaneous injection of 20 % w/v of brewer's yeast (10ml/kg) in distilled water. Basal rectal temperature was measured before the injection of yeast, by inserting digital clinical thermometer to a depth of 2 cm into the rectum.

The rise in rectal temperature was recorded 19 h after yeast injection. Paracetamol 150mg/kg body weight was used as the standard antipyretic drug. Rectal temperature of animals was noted at regular intervals following the respective treatments.The temperature was measured at 1st, 2nd, and 3rd hour after drug administration.

Experimental Doses calculated as per the standard procedures are:

S.No	Groups	Dose/kg weight	Dose/200 gms weight	Volume of administration
1.	Vehicle Control	-	-	0.5ml
2.	Therapeutic Dose	23mg	4.6mg	0.5ml
3.	Average Dose	115mg	23mg	0.5ml
4.	High Dose	230 mg	46mg	0.5ml

4.4.3.ANTI SPASMODIC ACTIVITY

EFFECT OF KATTUVAI MATHIRAI ON EXCISED GUINEA PIG ILEUM

ISOLATION OF GUINEA PIG ILEUM (IN-VITRO)

Guinea pig were anesthetized and sacrificed by cervical displacement followed by exsanguinations. The ileum was dissected out, immersed in tyrode's solution and cleaned off the mesentery. Respective segments of 2-3 cm long were mounted in a 25ml tissue organ bath, filled with a mixture of 95% O_2 and 5% Co_2 and maintained at 37°C. The composition of Tyrode's solution (in mM for 1 lit) was 9 mg KCl, 0.1mg NaCl, 0.1mg $NaHCo_3$, 0.42mg NaH_2PO_4, 0.6 mg Glucose and pH value was 7.4.

ANTI-SPASMODIC ACTIVITY ASSAY PROCEDURE:

1. Firstly concentration dependent responses of acetylcholine awere 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 level. Contact time of 60 sec, and base line of 30 sec 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+ KM (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 "*Kattuvai Mathirai"* done by **"Agar well diffusion method"** – Kirby – bauyermethod.

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	-	7.6

Procedure:

The method of antibacterial activity study is UPS Diffusion Method. Antibiotic discs are prepared with known concentration of antibiotic are placed on agar plates that has been inoculated with the known pathogenic micro organism. The antibiotic diffuses through the agar producing an antibiotic concentration, gradient antimicrobial susceptibility is proportional to the diameter of the inhibitory zone around the disc. If the microorganism which grows up to the edge of the disc are resistant to the antimicrobial agent. The recommended medium in this method is Muller Hinton Agar, its PH should be between 7.2-7.6 and should be poured to uniform thickness of 4mm in the petri plate (25ml).

Methodology:

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

The prepared disc of *Kattuvai Mathirai* 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 inhibition.

6. RESULTS AND DISCUSSION

STANDARDISATION OF *KATTUVAI MATHIRAI*

The test drug *Kattuvai Mathirai* had been subjected to various studies to establish the works of Siddhar`s to be true. Literary collections, physico-chemical and elemental analysis, pharmacological study, toxicological study and antimicrobial study are done to prove the activity of *Kattuvai Mathirai* as an anti-diarrhoeal, anti-pyretic and anti-spasmodic activities.

Table – 4 Physico Chemical Standardisation.

SL. NO.	PARA METER	RESULTS
1.	Organo leptic characters	
	a. Color	Brown
	b. Odour	Pleasant odour
	c. taste	Astringent, bitter
	d. Sense of touch	Hard
	e. Appearance	Round
1.	Physico chemical standard	
	a. Loss on drying at 70°C	7.30 %
	b. Ash	
	i. Total ash	8.50%
	ii. Acid insoluble ash	0.95 %
	iii. Water soluble	7.75 %
	c. Extractive value	8.20 %
	i. Ethanol soluble extractive	9.20 %
	ii. Water soluble extractive	7.540
	d. pH value (1% solution)	

Interpretation:

The physical parameters like brown in colour, plesant odour, hard to touch, astringent and bitter in taste, round in appearance revealed that *Kattuvai Mathirai* is having the PH 7.540 slightly alkaline.

Determination of loss on drying normal:

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

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

Total Ash:

Ash values are helpful in determining the quality and purity of crude drugs. In this trial drug *KATTUVAI MATHIRAI* (The minerals that present in the trial drug are calcium, chloride, sulphate) the salts, Ca+, Cl-, Sulphate are not harmful one. In this trial drug *KATTUVAI MATHIRAI* is used as a condensation from water extraction . So only water soluble trace elements present here in a very few trace levels. The total ash value of *Kattuvai Mathirai* was 8.50%.

Acid insoluble Ash:

Acid insoluble ash values represents detecting the presence of silica and oxalate in a drugs. In my drug the silica and oxalate that is the acid insoluble ash is very low on 0.95%. So the drug has high quality.

Water soluble ash:

Water soluble ash also indicate the purity of the drug water soluble ash higher than acid insoluble ash represents good quality of the drug which is *KATTUVAI MATHIRAI* is 7.75%. So water soluble ash is higher than acid insoluble ash.

b)Water soluble extractive

Proceed as directed for the determination of Alcohol-soluble extractive , using chloroform water instead of ethanol. Water soluble extractive of Kattuvai Mathirai is 9.20%.

c)Alcohol soluble extractive

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

solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105°,to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug. Alcohol soluble extractive of Kattuvai Mathirai is 8.20%

Microbial Limit Tests

Table 1: Results of Microbial Contamination Test

S.No.	Test Particulars	Colony Counts (CFU/ g)	Limits Value (CFU/g)
1.	Total Viable Aerobic Bacterial Count	13.5×10^2	1×10^5
2.	Total Viable Fungal Count	3.5×10^2	1×10^3

Table 2. Results of Specific Pathogens Test

S.No.	Test for Specified Pathogens	Colony Counts (CFU/ g)	Limits Value (CFU/g)
1.	*Salmonella* sp.	No growth	-
2.	*Staphylococcus aureus*	No growth	-
3.	*Escherichia coli*	No growth	-
4.	*Pseudomonas aeruginosa*	No growth	-

Interpretation

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 contamination. The other pathogens like Escherichia coli, Salmonella sps, Pseudomonas aeruginosa and Staphylococcus aureus were found to be completely absent in the drugs.

Disintegration:

The disintegration of test sample under the specifications not more than 15 minutes. In the present analysis the *KATTUVAI MATHIRAI* disintegration on only 10m 50 sec.

BIO CHEMICAL ANALYSIS

Table – 5 Results of Preliminary test for basic and acidic radicals

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

INTERPRETATION:

The biochemical analysis of *Kattuvai Mathirai* contains the following chemical constituents, Sulphate, Chloride, Starch, Ferrous iron, Unsaturated compound, Reducing sugar, Tannic acid and Amino acid.

1. Sulphate:

Sulphur is a very versatile molecule, because it can exit in several distinct oxidative states, ranging from +6 in sulphate radicle – 2 in Hydrogen sulfide.

Sulfur is a healing mineral and plays a role in metabolism. Sulfur is present in insulin.

2.Chloride :

Chloride regulate the acid base balance of the body fluids, by maintaining the osmotic pressure of the body fluids. In severe *diarrhoea*, vomiting, large amount of water and electrolytes are lost from body. The dehydration has to be treated by administering water and these electrolytes.

3.Starch:

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

4.Ferrous Iron :

Iron is easily soluble and readily absorbed from intestine and involved.

5.Tannic Acid:

Tannins are water soluble polyphenols that are present in many plant foods.Tannins in fruit have natural defence mechanism against microbial infection.Helps in healing of wounds and inflammation of mucous membrane. They restore the Anti-Oxidant status of the organs to almost normal levels. Increases the cellular Anti-Oxidant enzymes.

6. Unsaturated compound:

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

7. Reducing sugar

Reducing sugar is essential for mental function and good physical activity.

8. Amino Acid :

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

PHYTOCHEMICAL STUDY OF *KATTUVAI MATHIRAI*

The *Kattuvai Mathirai* was subjected to qualitative chemical investigation Details of the various tests performed for the presence of phytoconstituents is shown in Table 6.

Table – 6 Phytochemical tests for *KATTUVAI MATHIRAI*

Tests	***KATTUVAI MATHIRAI***
Alkaloids	
Mayer's test	+ve
Dragendroff's test	+ve
Hager's test	-ve
Carbohydrates and glycosides	
Molisch test	+ve
Legal's test	-ve
Borntrager's test for anthraquinones	-ve
Phytosterols	
Liebermann-Burchard test	-ve
Salkowski test	-ve
Flavanoids	
Shinoda test Magnesium turnings and hydrochloric acid (Presence of red color)	-ve
Fluorescence test	-ve
Tannins	
Ferric chloride test	-ve
Potassium dichromate test	-ve
Lead acetate test	+ve
Proteins	
Millon's test	-ve
Biuret test	-ve
Ninhydrin test	+ve
Fixed oils and fats	
Spot test	-ve

Saponification test	+ve
Lignin	
Phloroglucinol test	+ve
Saponins	
Frothing test	-ve

(+ve) indicates the presence of phytochemical, (-ve) indicates the absence of phytochemical.

INTERPRETATION:

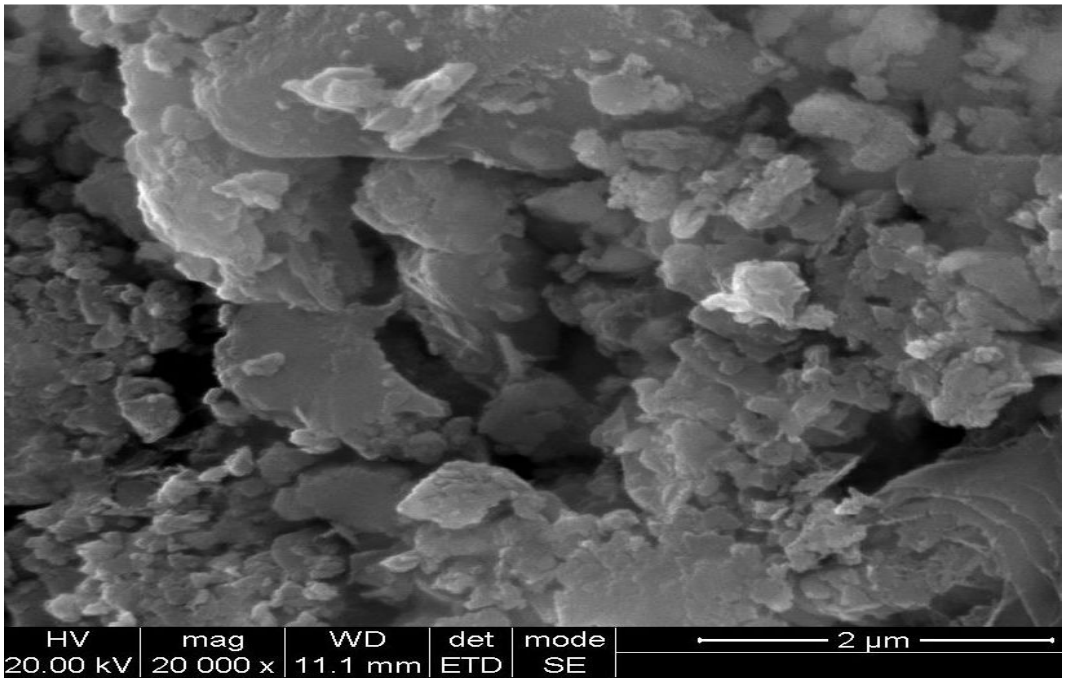
Alkaloids-decreased gastric acid secretion and inhibit the gastric motility

Tannins-Tannins react with tissue promote tissue proteins.

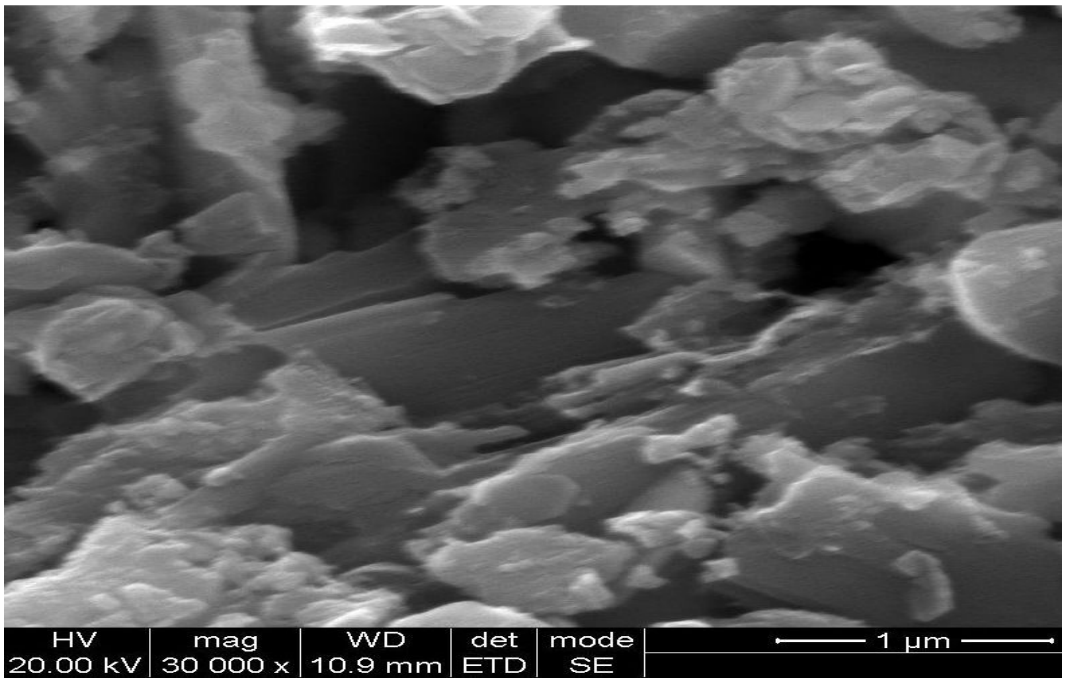
This study revealed the presence of active phytochemicals in Kattuvai Mathirai such as alkaloids, carbohydrates, glycosides, tannins, lignins.

INSTRUMENTAL ANALYSIS

SCANNING ELECTRON MICROSCOPE (SEM)



SEM -20000 Magnification



SEM -30000 Magnification

Figure – 6 Showing SEM Results of Trial Drug *(KATTUVAI MATHIRAI)*

INTERPRETATION :

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

When dispersed in an aqueous medium, these preparations form a negatively charged hydrophobic particle suspension. This hydrophobicity gave these particles a tendency to aggregate together to form larger particles. *Kattuvai Mathirai* exhibited larger sizes and agglomeration of the particles. Therefore, the comparatively larger size may be due to the agglomeration of the particles by repeated cycles of calcinations involved in preparation. SEM analysis of the ***Kattuvai Mathirai*** shows most of the particles present in the sample are nano size, average particle size is **2 - 1µm**.

FOURIER TRANSFORM-INFRARED SPECTROSCOPY(FTIR)

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

FT-IR is a very useful tool in the recognition of the functional groups of bio molecules, thus aiding in their structural elucidation, so confirming the presence of active molecules responsible for the therapeutic activity of Siddha drugs. The results of Table no: 7 and Fig no:7 shows the presence of functional group and inorganic compounds of ***Kattuvai Mathirai***

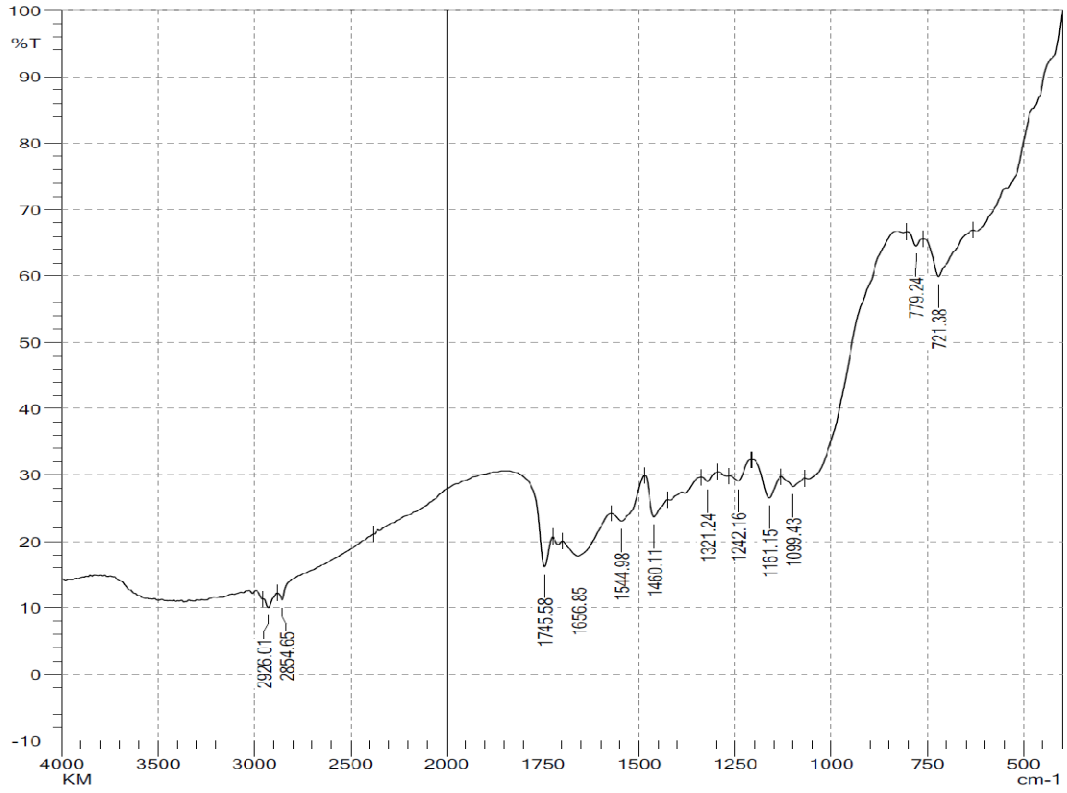


Figure - 7 Showing FTIR Image of *KATTUVAI MATHIRAI*

Table – 7 Interpretation of FTIR Spectrum

S.No	Frequency	Bond	Functional Group
1.	2926.01	C-H Stretch	Alkane
2.	2854.65	C-H Stretch	Alkane
3.	1745.58	C= O	Ester
4.	1656.85	C=N Stretch	Oxime
5.	1544.98	N-O Stretch	Nitro compound
6.	1460.11	CH_3 Bend	Alkane
7.	1321.24	C-N Stretch	Aromatic amine
8.	1242.16	C-N Stretch	Amine
9.	1161.15	C-O Stretch	Tertiary alcohol
10.	1099.43	C-O Stretch	Secondary alcohol
11.	779.24	C-Cl Stretch	Halo - compound
12.	721.38	C=C Bending	Alkane

INTERPRETATION:

1. It confirms that *Kattuvai Mathirai* constitutes Alkanes, Ester, Oxime, Nitro compounds, Amine, Tertiary alcohol, Secondary alcohol, Halo-compound as functional groups.

Alkenes :

When red blood cells reach the end of their lifetime(about 3months)they lise and released red coloured haemoglobin,the molecule that actually transports oxygen in the blood.The heme portion of hemoglobin is metabolized into orange coloured bilirubin.A very small amount of bilirubin dissolves in urine and is responsible for the yellow colour of urine.,but the major route for excretion is more complex.

Amines:

Amines are a class of compounds derived from ammonia by replacement of one or more effective antagonists of SSTR5 (Stomatostatin receptor 5)and are used for treatment, control and prevention of disorders such as Type 2 Diabetes, insulin resistance, lipid disorders and obesity.

Esters:

Esters are organic compound formed when an acid combine with an alcohol and release water. Astringent .

Alcohols:

Has anti microbial action.Acts as a antiseptic agent.

Aromatics:

Aromatics are good pain relievers.Has Anti-pyretic, Anti-inflammatory, Auto-immune activities.

ICP-OES of *KATTUVAI MATHIRAI*

Kattuvai Mathirai (wt:0.41210g)

Elements	Wavelength (nm)	Concentration
Al	396.152	BDL
As	188.979	BDL
Ca	315.807	32.354 mg/l
Cd	228.802	BDL
Cu	327.393	BDL
Fe	238.204	32.354 mg/l
Hg	253.652	BDL
K	766.491	23.171 mg/l
Mg	285.213	01.784 mg/l
Na	589.592	42.280 mg/l
Ni	231.604	BDL
Pb	220.353	BDL
P	213.617	116.227 mg/l
S	180.731	31.324 mg/l
Zn	206.200	01.216 mg/l

BDL: Below Detectable Limit(Normal-1ppm)

1% = 10000ppm,

1ppm = 1/1000000 or 0.0001%

Toxic metals and the permissible limits

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

Result

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

1. ICP-OES reveals high concentration of K in ***Kattuvai Mathirai*** 23.171mg/L.
2. Concentration of Na is 42.280 mg/L, phosphorous is 116.227 mg/L
3. It also has physiologically important minerals like Na, K,P. Ca.

Below detection limit(BDL) of heavy metals As(arsenic), Hg(Mercury), Cd (Cadmium), Pb(Lead), Cu (Copper) and trace elements like Ni(Nikkal), Al(Aluminum) is seen.

INTERPRETATION

The result indicates the presence of **pottassium, sodium, phosphorus, sulphate , zinc, iron, calcium, magnesium, phosporus**

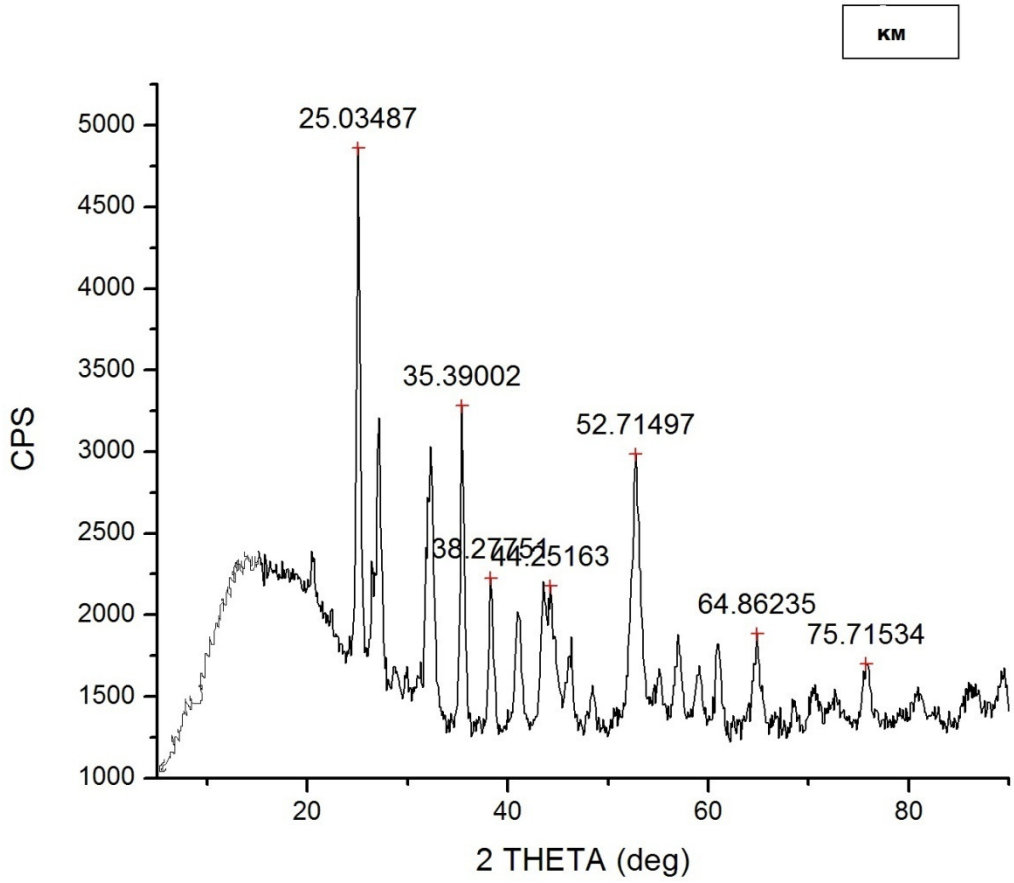
- In the presence of **sodium** and **pottassium**, these two elements are required for the regulation of acid base balance and water balance in the cells.
- **Calcium** participated in several reaction in cascade of blood clotting process.
- **Phosphorus** is important for the maintenance of pH in the blood as well as in the cells.
- **Zinc** is a micro nutrient and is required for wound healing.Zinc has effects against viruses. {Rhino virus}.Zinc may be regarded as an antioxidant, protects the body against free radical damage and cell damage. Zinc is important for a healthy immune system. It enhances absorbtion of iron.It can produce healthy veins and arteries that enhance the blood circulation
- **Magnesium** is a cofactor that regulates diverse biochemical reactions in the body, including protein synthesis, muscle, nerve function, blood glucose control and blood pressure regulation
- **Sulphate** Sulphate may prevent the occurrence of any infection,Sulphate is potent anti oxidant activity in human body

This reveals the safety of the drug.It is evident that the effectiveness of *Siddha* medicine has been proved by the modern scientific way.

XRD (X-Ray Diffraction)

X-Ray powder diffraction is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions.

Fig. No. 8 XRD –Results of *KM*



This XRD finger print shows both the similarities and differences of the sample successfully and is a valuable primary tool for checking the quality control of Herbo-mineral formulations. The different peaks show the presence of minerals in the samples.

TOXICITY STUDIES

8. EVALUATION OF ACUTE TOXICITY STUDY OF *KATTUVAI MATHIRAI*

Effect of Acute Toxicity Study (14 Days) of *kattuvai mathirai*

Table no 8.1 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 8.2. 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 8.3 Hand held observation

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

Table no 8.4 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 *KATTUVAI MATHIRAI*at a dose of 2000 mg/kg to the rats do not produce drug-related toxicity and mortality. So No-Observed-Adverse-Effect- Level (NOAEL) of *KATTUVAI MATHIRAI*is 2000 mg/kg.

DISCUSSION

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

At the 14th day, all animals were observed for functional and behavioral examination. In functional and behavioral examination, home cage activity, hand held activity were observed. Home cage activities like Body position, Respiration, Clonic involuntary movement, Tonic involuntary movement, Palpebral closure, Approach response, Touch response, Pinna reflex, Sound responses, Tail pinch response were observed. Handheld activities like Reactivity, Handling, Palpebral closure, Lacrimation, Salivation, Piloerection, 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 ***kattuvai mathirai*** on the growth rate. Body weight change in drug treated animals was found normal.

INTERPRETATION:

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

KATTUVAI MATHIRAI at the doses of 5mg/kg, 50mg/kg , 300mg/kg, 1000mg/kg and 2000mg/kg to rats.

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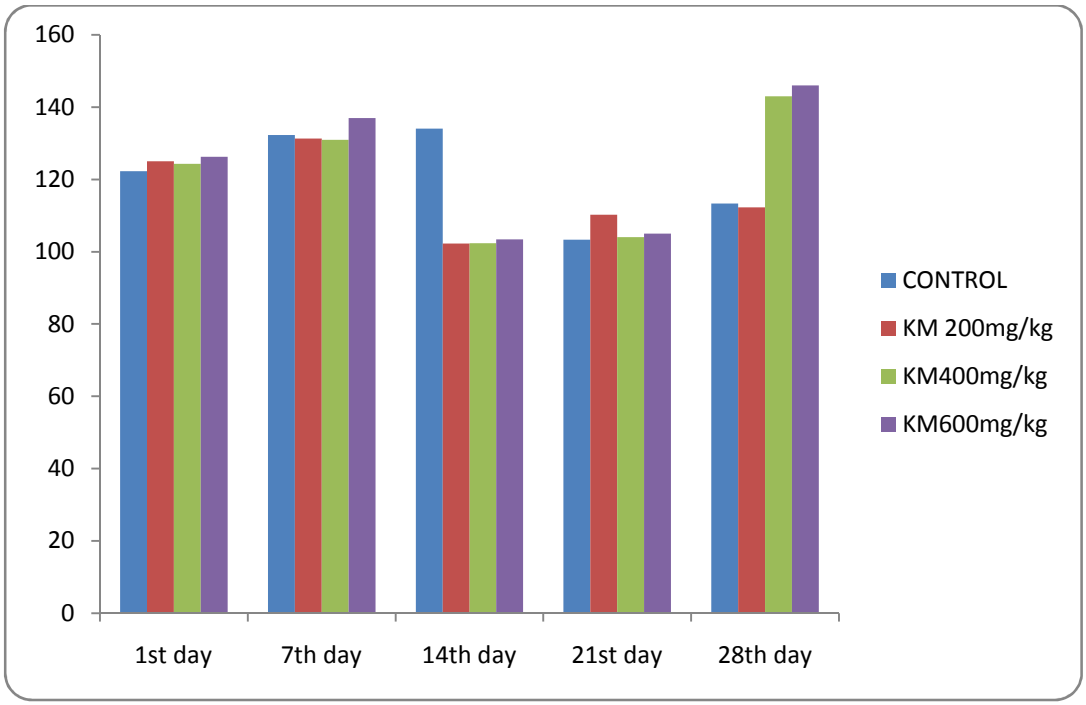
Body weight at weekly interval was measured to find out the effect of ***kattuvai mathirai*** on the growth rate. Body weight change in drug treated animals was found normal.

9. SUB-ACUTE TOXICITY STUDY IN WISTER RATS TO EVALUATE TOXICITY PROFILE OF *KATTUVAI MATHIRAI*

Table :9.1 EFFECT OF SUB- ACUTE DOSE (28 DAYS)OF *KATTUVAI MATHIRAION* BODY WEIGHT IN GRAM

GROUP	CONTROL	LOW	MID	HIGH
1st day	122.3±1.23	125±1.743	124.3±2.431	126.3±2.43
7th day	132.3±1.23	131.3±1.543	131±2.313	137±2.31
14th day	134.1±1.204	102.3±1.32	102.4±2.212	103.4±2.212
21st day	103.3±2.320	110.2±1.701	104±1.331	105±1.33
28th day	113.3±1.241	112.3±1.402	143±2.2405	146±2.240

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

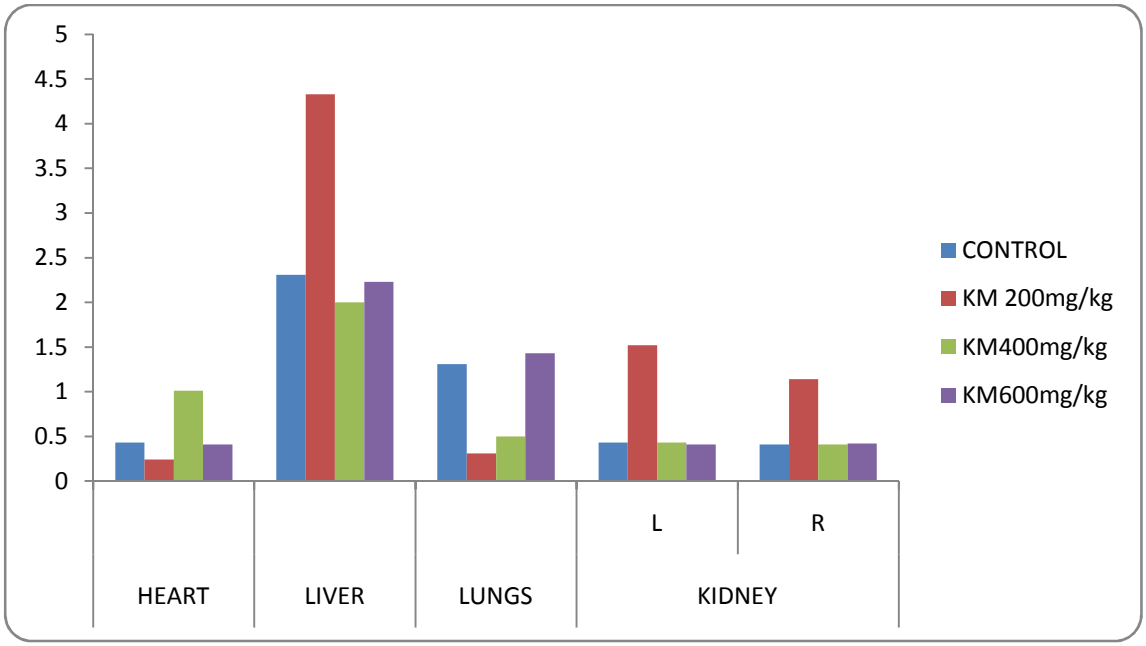


EFFECT OF SUBACUTE DOSE (28 DAYS)OF *kattuvai mathirai*

Table : 9.2. *kattuvai mathirai* ON ORGAN WEIGHT (PHYSICAL PARAMETER) IN GRAM

GROUP		CONTROL	LOW	MID	HIGH
HEART		0.43±0.04	0.24±0.06	1.01±0.31	0.41±0.04
LIVER		2.31± 0.43	4.33±0.43	2.20±0.03	2.23± 0.43
LUNGS		1.31±0.30	0.31±0.34	0.50±0.44	1.43±0.30
KIDNEY	L	0.43±0.04	1.52±0.05	0.43±0.04	0.41±0.04
	R	0.41±0.044	1.14±0.04	0.41±0.044	0.42±0.044

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

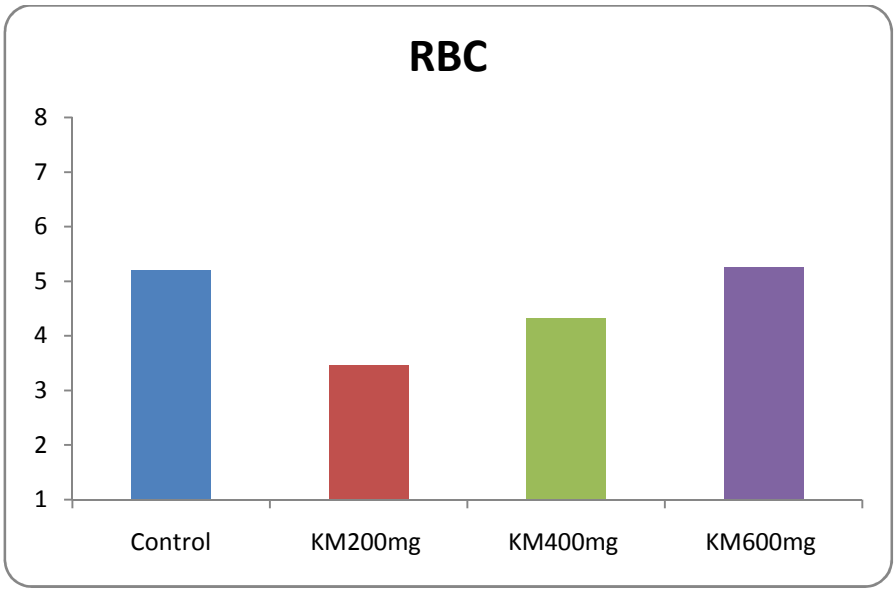


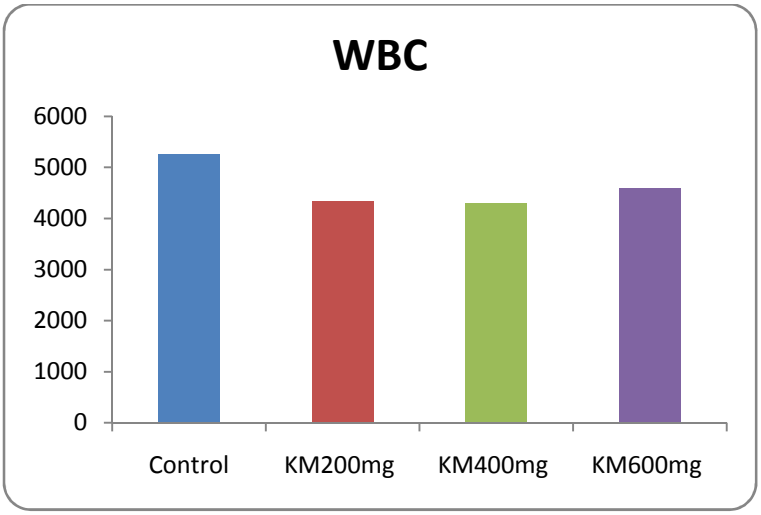
EFFECT OF SUB- ACUTE DOSE (28 DAYS) OF *KATTUVAI MATHIRAI ON* HAEMATOLOGICAL PARAMETERS

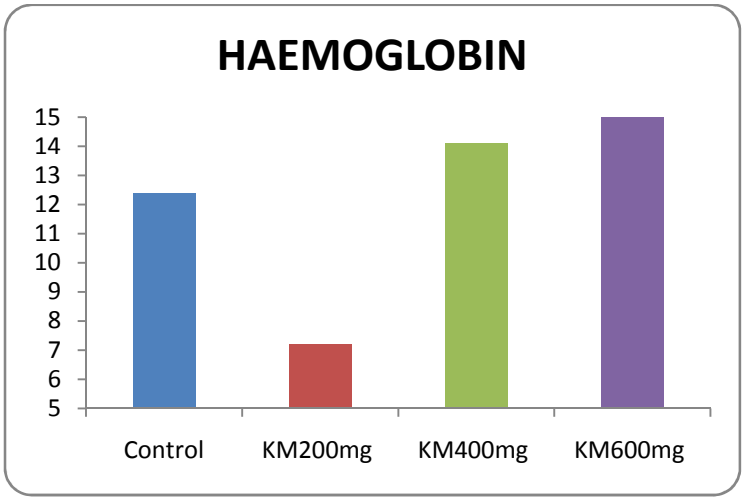
Table no 9.3

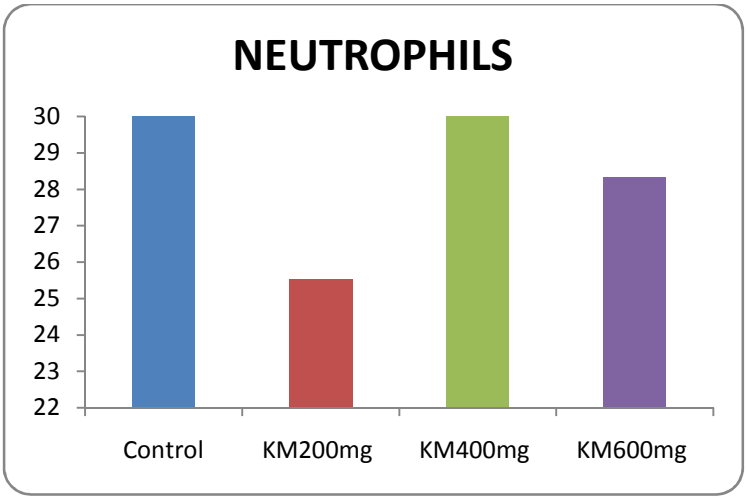
Drug treatment	RBC million cells/cmm	WBC cells/cmm	Haemoglobin gm %	Differential count %			
				Neutrophils	Eosinophils	Monocyte	Lymphocyte
Control	5.21±0.60	5252.41±43.32	12.40±0.65	31.27±1.40	3.53±0.31	0.65±0.35	23.13±3.52
LOW	3.47±0.40	4334.04±43.22	7.20±0.63	25.54±1.61	2.10±0.34	.02±0.50	23.22±3.51
MID	4.33±0.41	4304.25±52.35	14.11±3.03	30.32±2.42	3.44±0.32	0.62±0.60	23.13±3.52
HIGH	5.26±0.41	4588.25±52.35	16.11±3.03	28.32±2.42	3.50±0.32	0.64±0.60	24.13±3.52

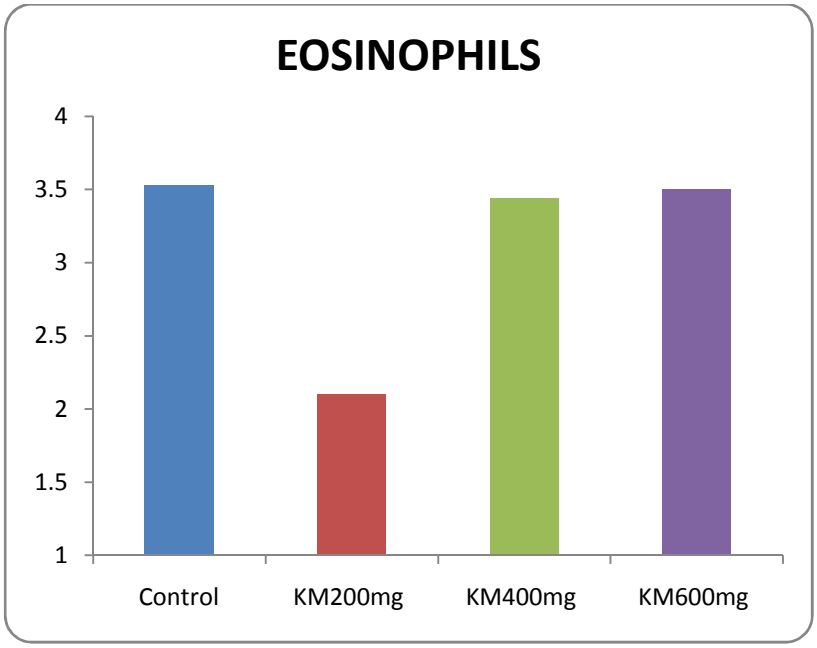
Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by Dunnett's(n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.




WBC
6000
5000
4000
3000
2000
1000
0
Control
KM200mg
KM400mg
KM600mg


HAEMOGLOBIN
15
14
13
12
11
10
9
8
7
6
5
Control
KM200mg
KM400mg
KM600mg


NEUTROPHILS
30
29
28
27
26
25
24
23
22
Control
KM200mg
KM400mg
KM600mg


EOSINOPHILS
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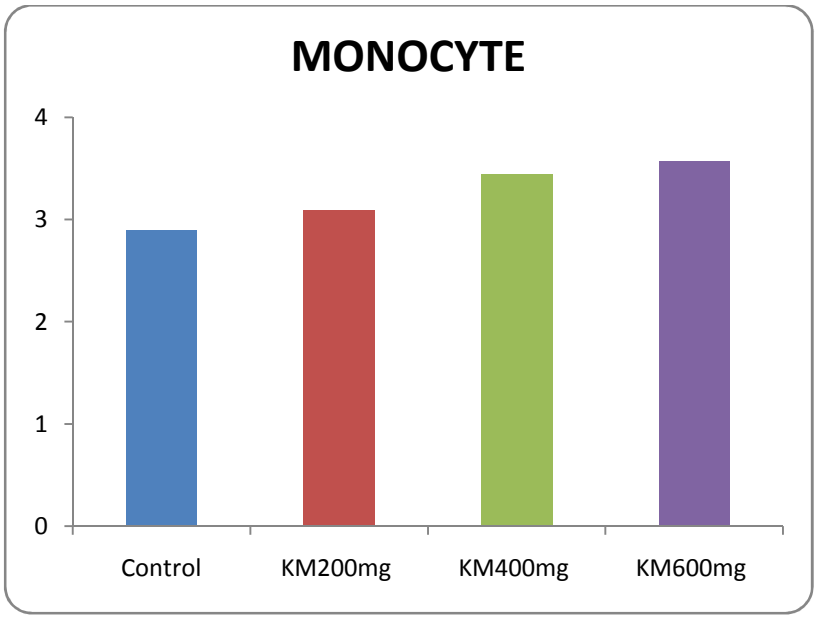
3.5
3
2.5
2
1.5
1
Control
KM200mg
KM400mg
KM600mg


MONOCYTE
4
3
2
1
0
Control
KM200mg
KM400mg
KM600mg

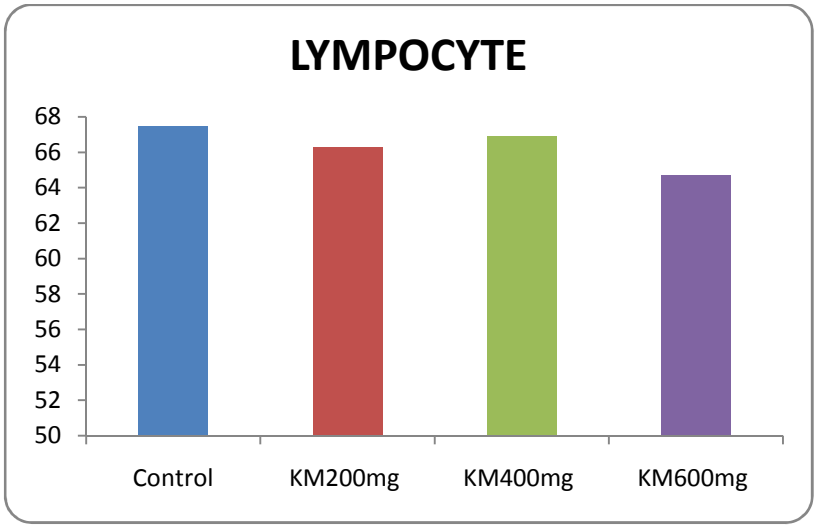
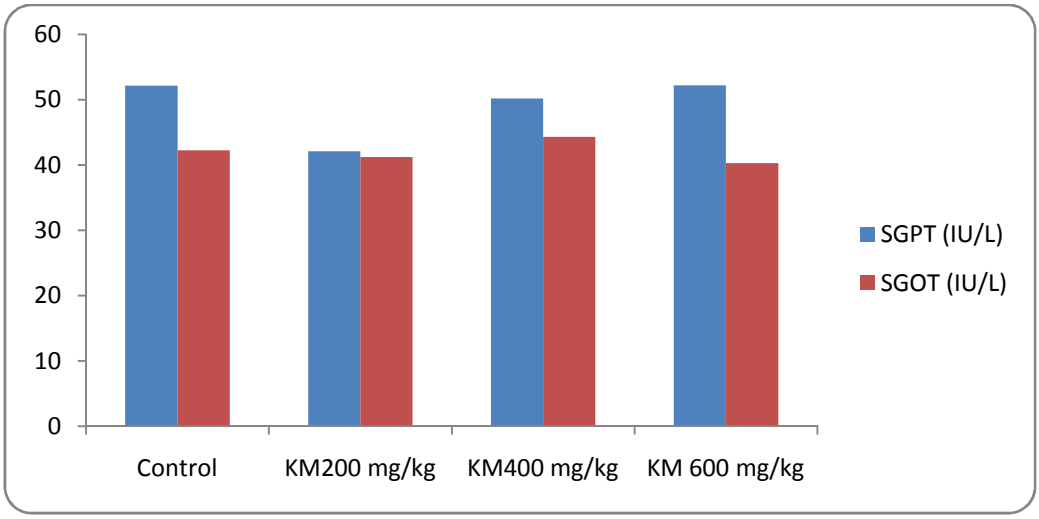
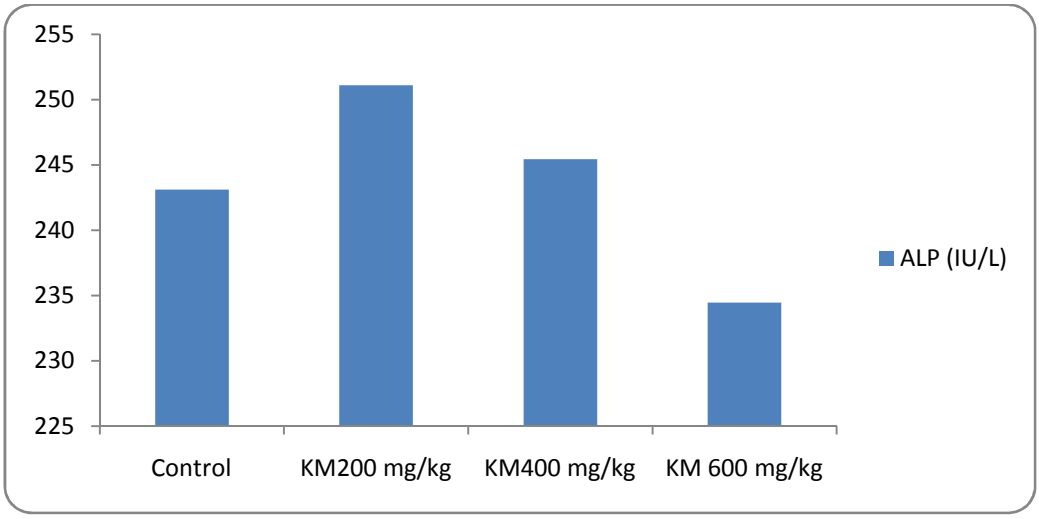
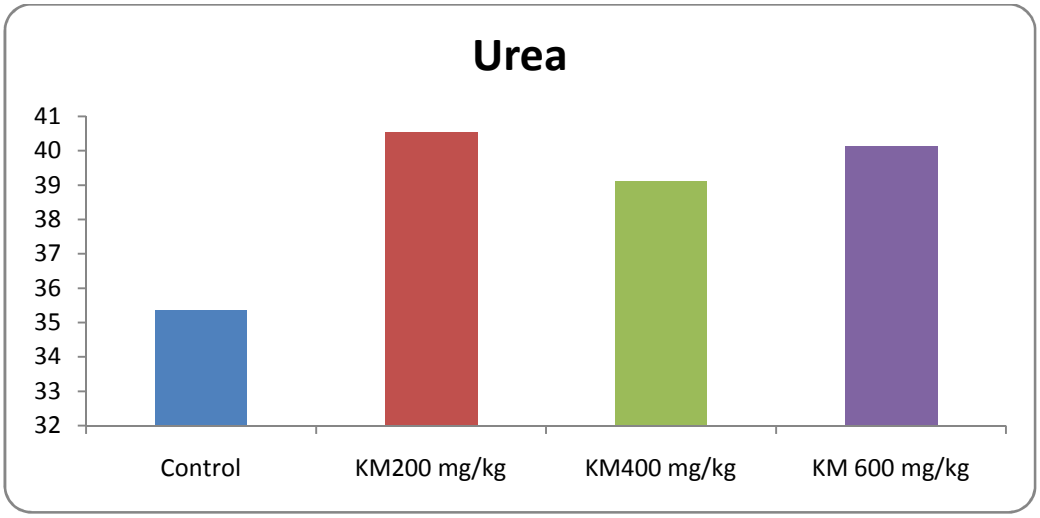

LYMPOCYTE
68
66
64
62
60
58
56
54
52
50
Control
KM200mg
KM400mg
KM600mg

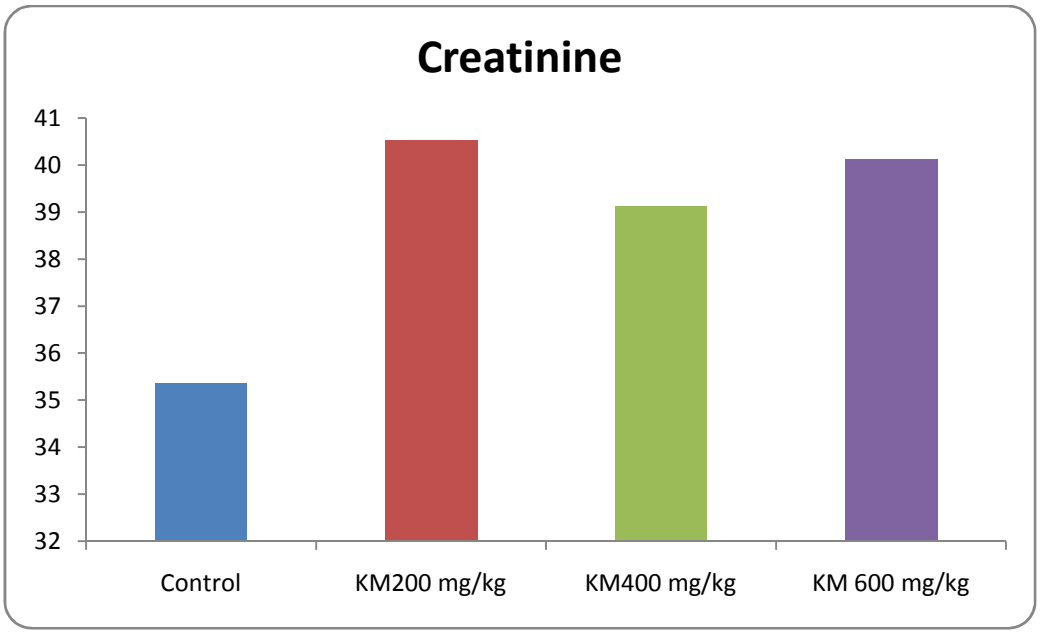
Table :9.4 EFFECT OF SUB- ACUTE DOSE(28 DAYS)OF *KATTUVAI MATHIRAI*ON BIOCHEMICAL PARAMETERS

Drug Treatment	SGPT (IU/L)	SGOT(IU/L)	ALP(IU/L)	Urea (mg/dl)	Creatinine(mg/dl)
Control	52.14±3.22	42.24±4.51	243.12±13.32	35.35±3.20	0.54±0.05
LOW	42.13±3.42	41.23±4.21	251.11±14.42	40.53±2.62	0.50±0.06
MID	50.21±4.64	44.31±2.41	245.45±4.34	39.12±2.42	0.45±0.06
HIGH	52.21±4.64	40.31±2.41	234.45±4.34	40.12±2.42	0.46±0.06






Urea
41
40
39
38
37
36
35
34
33
32
Control
KM200 mg/kg
KM400 mg/kg
KM 600 mg/kg


Creatinine
41
40
39
38
37
36
35
34
33
32
Control
KM200 mg/kg
KM400 mg/kg
KM 600 mg/kg

EFFECT OF SUB- ACUTE DOSE (28 DAYS) OF *KATTUVAI MATHIRAI*

BIOCHEMICAL PARAMETERS

GROUP	**CONTROL**	***kattuvai mathirai* (200mg/kg)**	***kattuvai mathirai* (400mg/kg)**	***kattuvai mathirai* (600mg/kg)**
TOTAL BILIRUBIN (mg/dl)	1.308±0.2457	1.458±0.2827	0.8198±0.3376	0.104±0.199

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

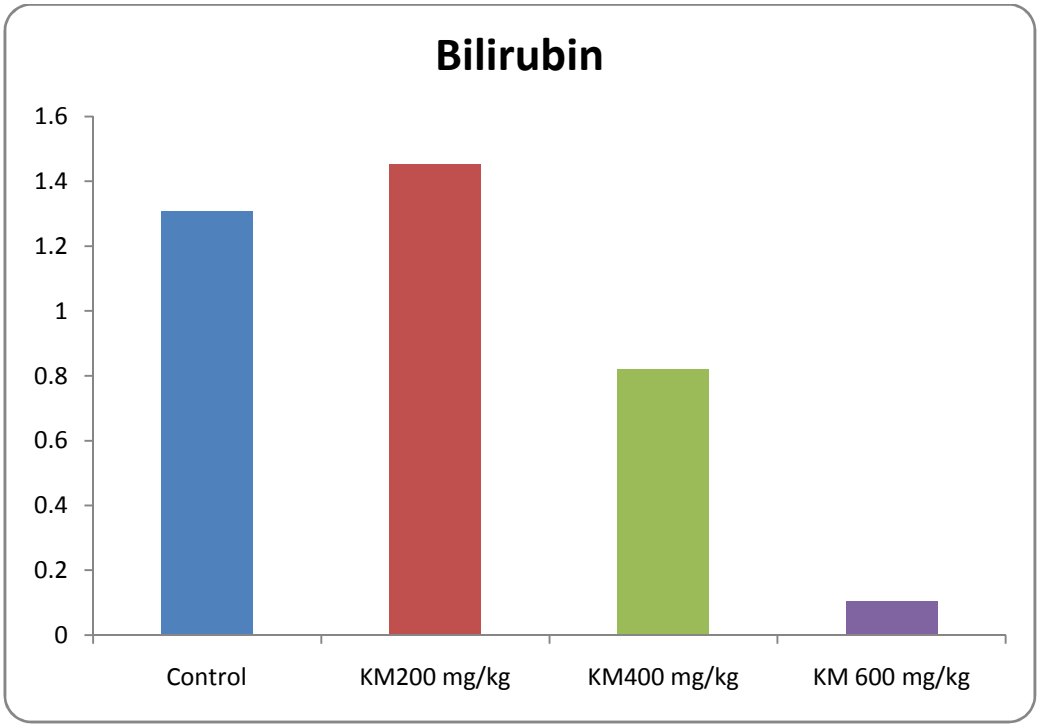


Table: 9.5 EFFECT OF SUB- ACUTE DOSE (28 DAYS) OFON FOOD INTAKE IN GRAM

GROUP	CONTROL	*kattuvai mathirai* (200mg/kg)	*kattuvai mathirai* (400mg/kg)	*kattuvai mathirai* (600mg/kg)
1st DAY	19.33±15.6110	20.1672±17.3	13.10±24.71	18.5±8.82
7th DAY	16.5±14	11.863±15.67	17.73±12.853	12.17±17.41
14th DAY	19.83±8.92	11.83±17.28	11±16.96	20.72±11.981
21st DAY	12.87±15.4	16±8.678	16.88±12.43	20.17±9.22
28th DAY	13.10±13.38	19.38±13.50	11±10.90	11±7.77

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

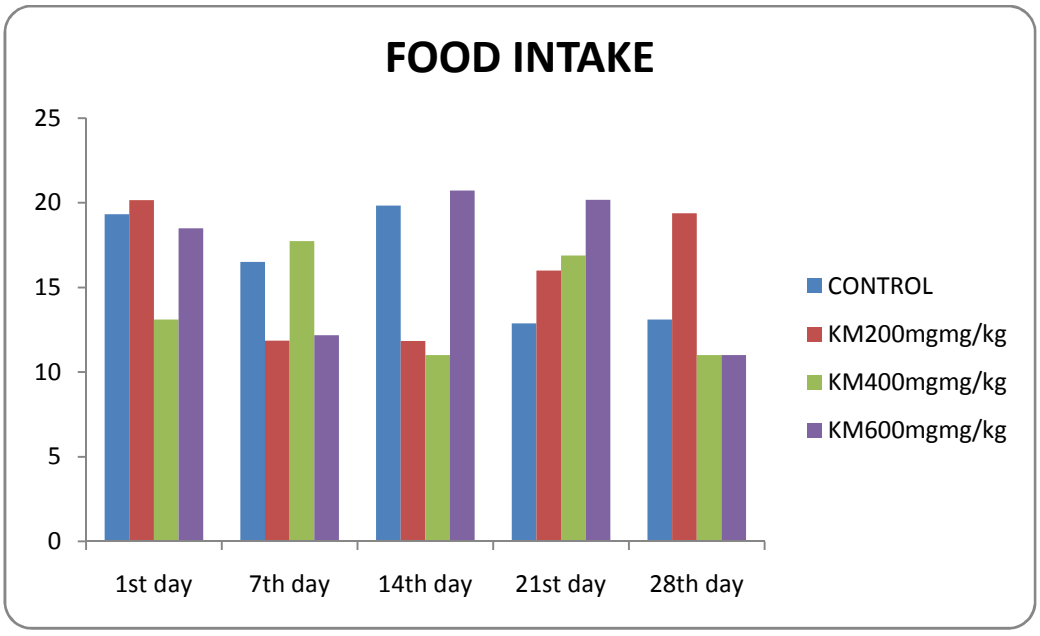


Table: 9.6 Effect of Sub- Acute Dose (28 Days) Of *KATTUVAI MATHIRAI*On Water Intake in ml

GROUP	CONTROL	*kattuvai mathirai* (200mg/kg)	*kattuvai mathirai* (400mg/kg)	*kattuvai mathirai* (600mg/kg)
1st DAY	97.38±13.50	89.12±14.34	102.10±21.719	67.5±7.63
7th DAY	85.5±11.79	100.83±12.60	76.73±9.83	81.17±14.0
14th DAY	58.33±8.717	90.83±14.12	80±13.96	89.162±8.81
21st DAY	91.67±12.49	85±8.42	65.38±9.40	89.17±8.62
28th DAY	82.10±11.40	88.38±11.54	80±8.61	70±7.53

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

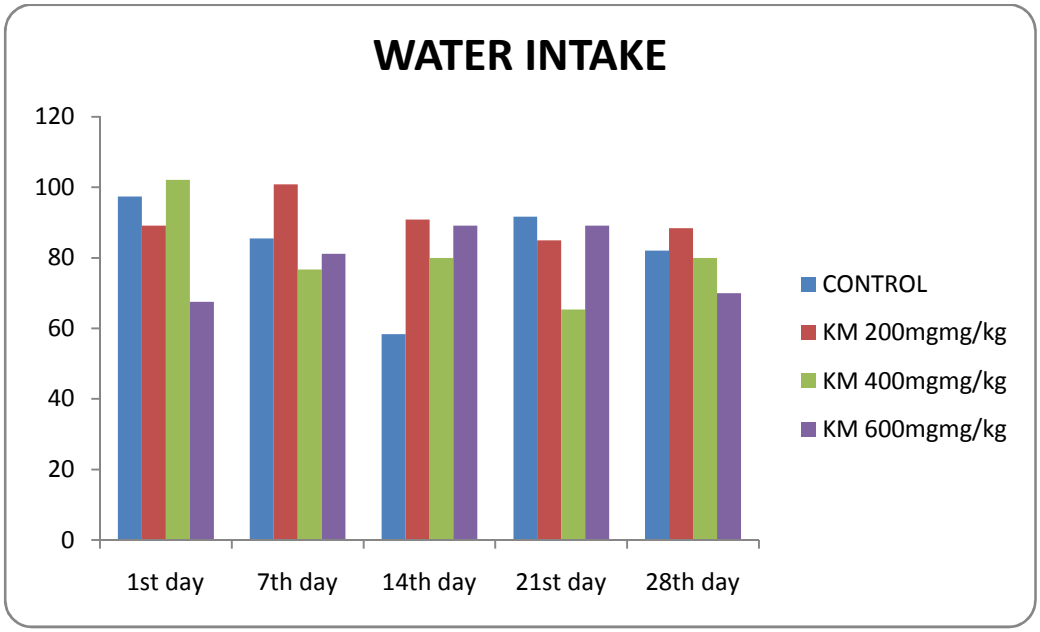
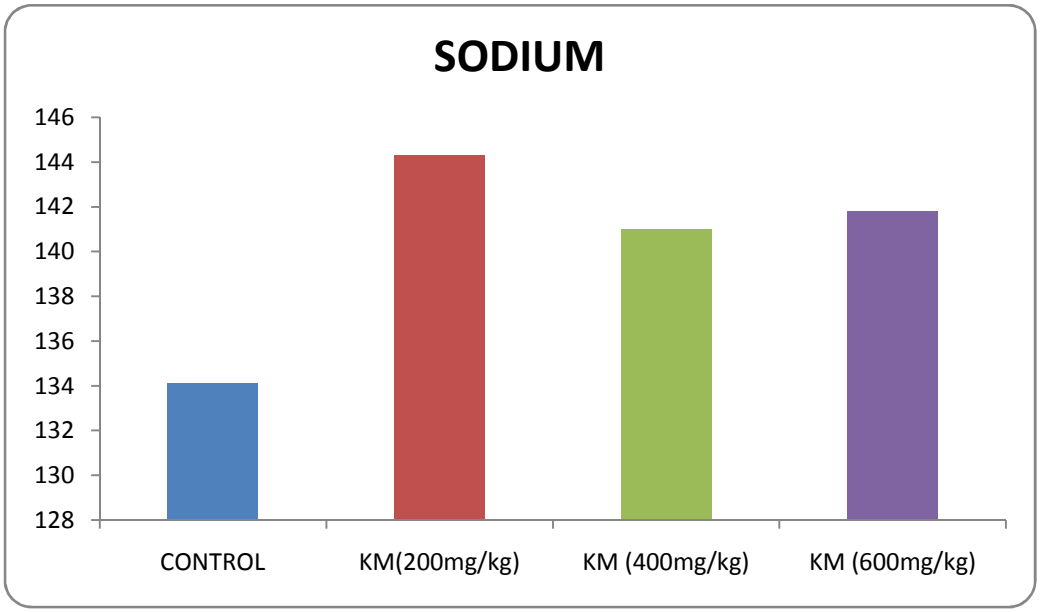
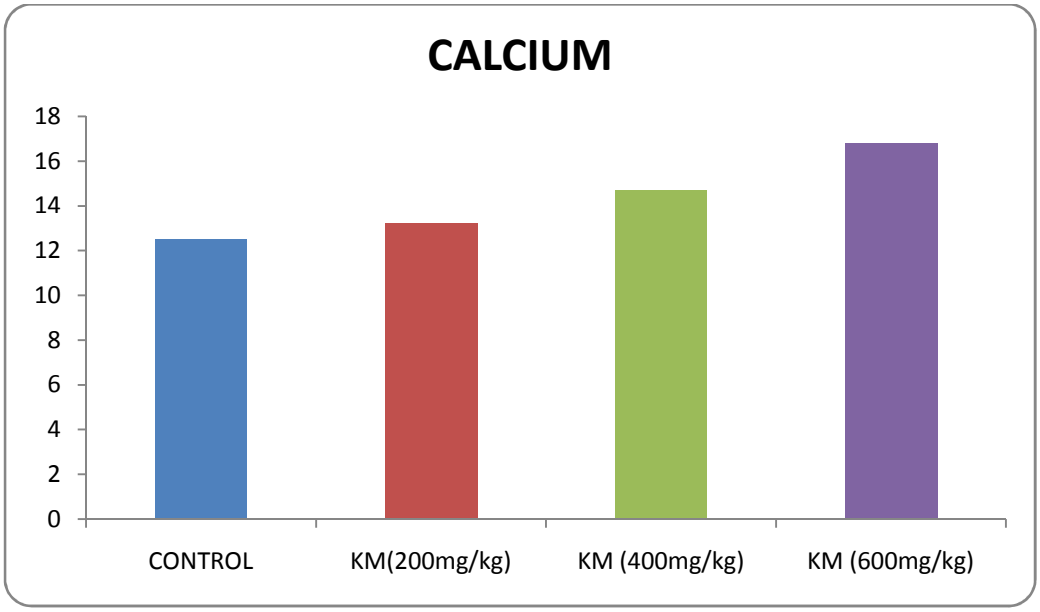


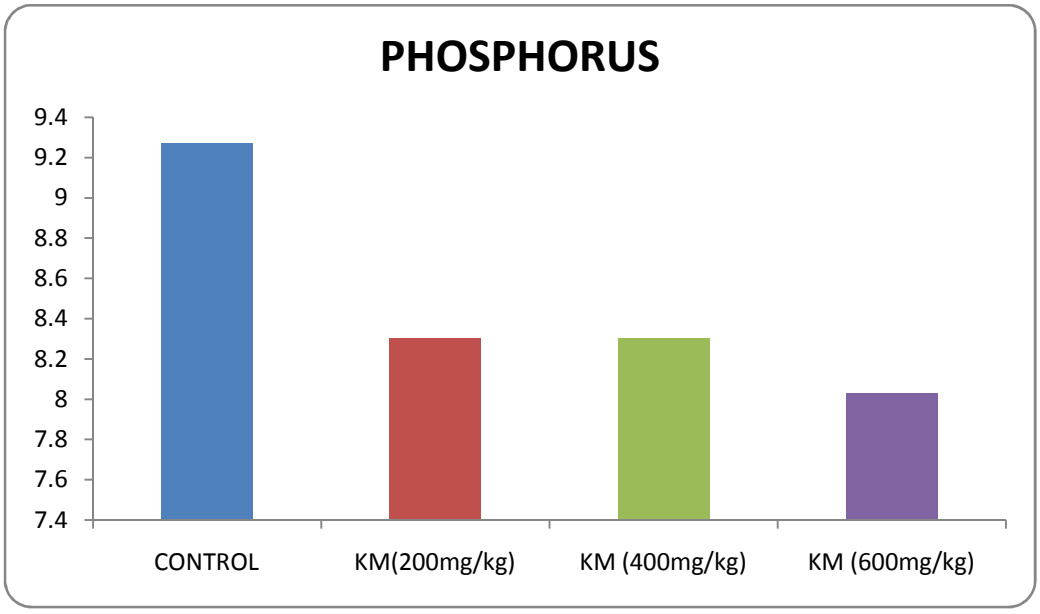
Table: 9.7. EFFECT OF SUB ACUTE DOSES (28 DAY) OF *KATTUVAI MATHIRAI*ON ELECTROLYTES: -

GROUP	CONTROL	*kattuvai mathirai* (200mg/kg)	*kattuvai mathirai* (400mg/kg)	*kattuvai mathirai* (600mg/kg)
Sodium (mg/dl)	134.10±0.55	144.30±0.62	141±0.71	141.80±0.70
Calcium(mg/dl)	12.50±0.19	13.20±0.13	14.7±0.19*	16.80±0.111
Phosphorus (U/L)	9.278±0.7	8.3010±0.0^{ns}	8.30±0.91^{ns}	8.037±0.02*

Values are expressed as mean ± SEM Statistical significance (p) calculated by one-way ANOVA followed by Dunnett's(n=6); NS- non-significant, *p<0.05, **p<0.01, ***p<0.001,







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 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.9.1 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 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 9.4 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.

INTERPRETATION:

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.

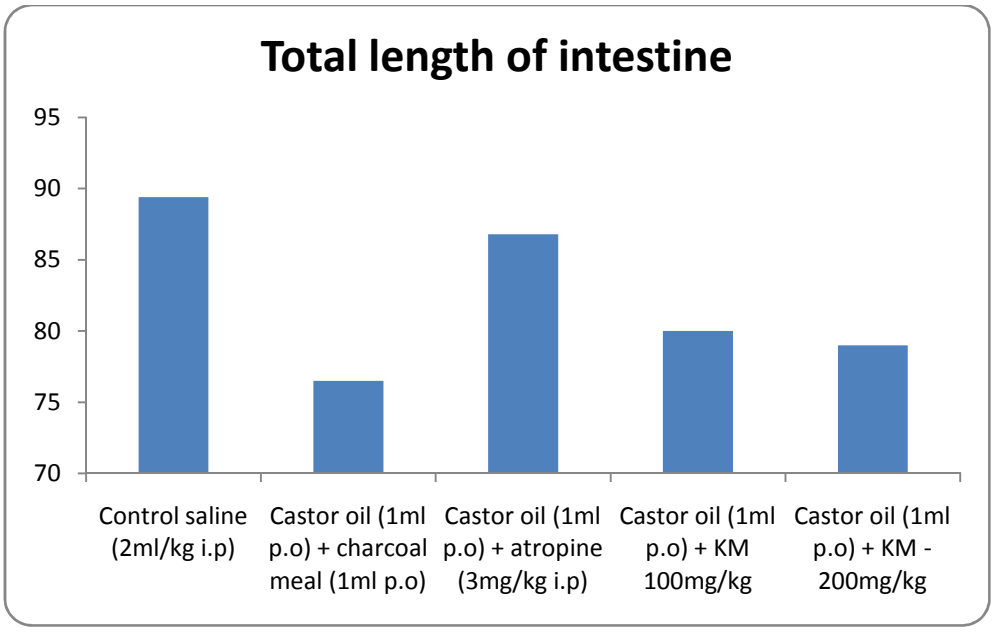
PHARMACOLOGICAL RESULTS

ANTI DIARRHOEAL ACTIVITY

Table : 10 EFFECT OF *KATTUVAI MATHIRAI* ON CASTOR OIL - INDUCED SMALL INTESTINAL TRANSIT IN RATS

GROUP	Total Length of Intestine	Distance Travelled By Marker CHARCOAL	%Intestinal Transit
Control saline (2ml/kg i.p)	90.4±1.3267	0±0	-----
Castor oil (1ml p.o) +charcoal meal (1ml p.o)	78.4±3.9192	65±2.68328	28.09 %
Castor oil (1ml p.o) +Atropine (3mg/kg i.p)	85.4±2.2271	46.6±10.1025	40.56 %
Castor oil (1ml p.o) + KM 100mg/kg	79.2±3.7336	49.8±7.90822	36.31 %
Castor oil (1ml p.o) + KM 200mg/kg	81.8±4.1761	41.6±7.11056	47.47 %

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



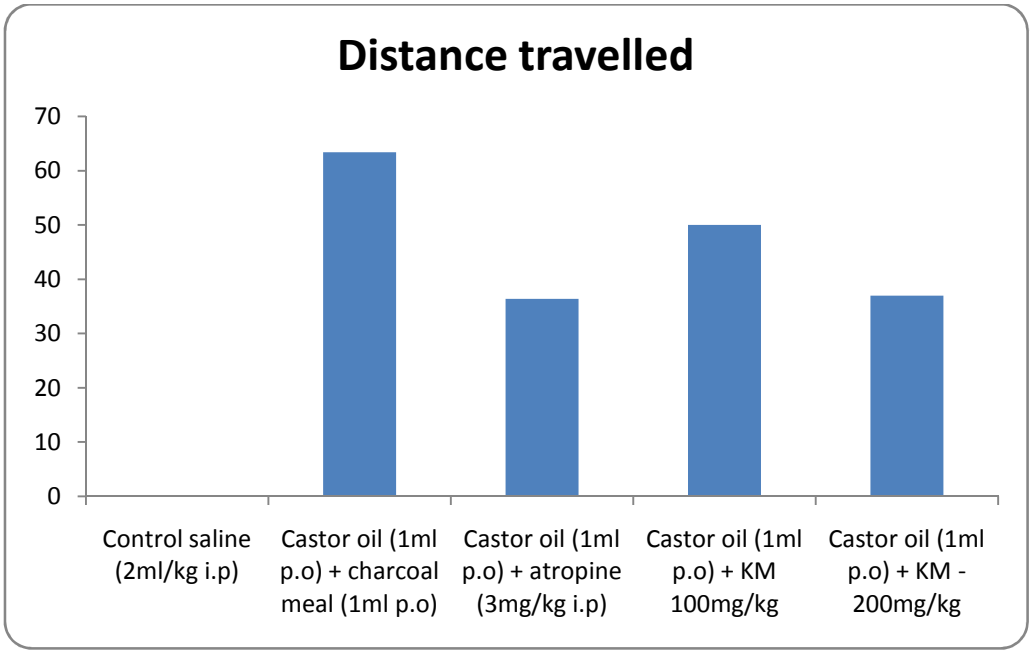


Figure No.9 Effect of Kattuvai Mathirai on castor oil – induced small intestinal transit in rats.

Interpretation

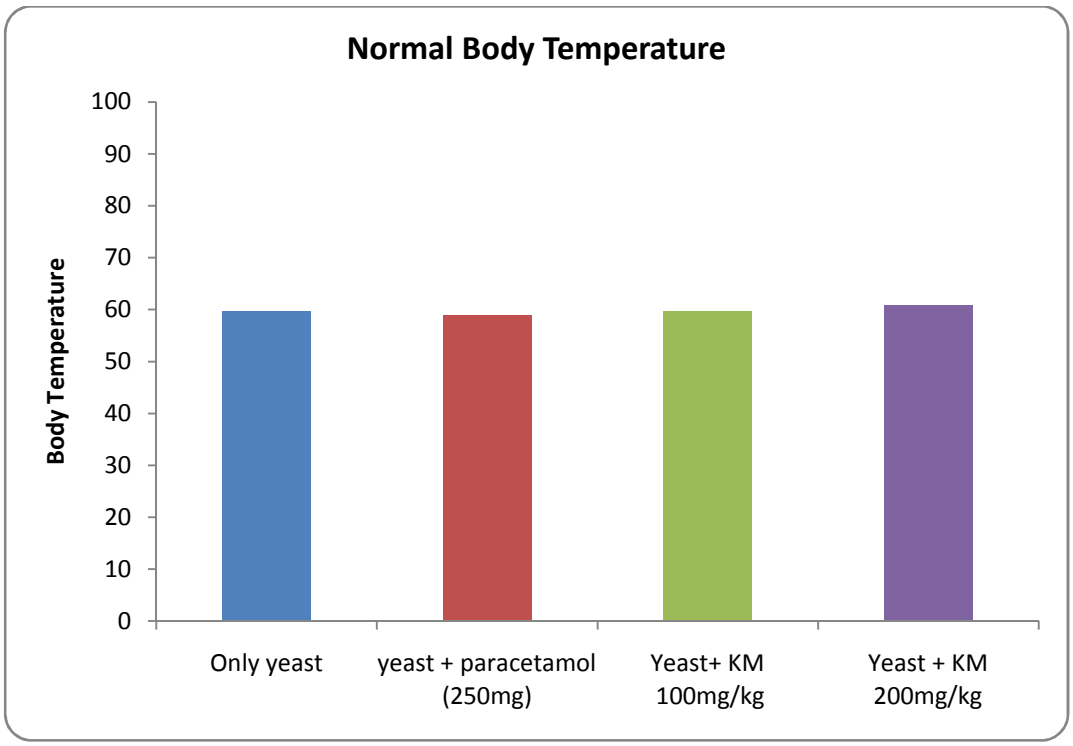
The Anti-diarrhoeal activity of siddha formulation Kattuvai Mathirai at 100mg/kg were tested for their anti-diarrhoeal activity by using caster oil – induced small intestinal transit in rats and the result are tabulated in table no.10. The results reveals that 100mg/kg & 200mg/kg dose of siddha preparation of Kattuvai Mathirai posseses signification anti-diarrhoeal activity when compared to control group.

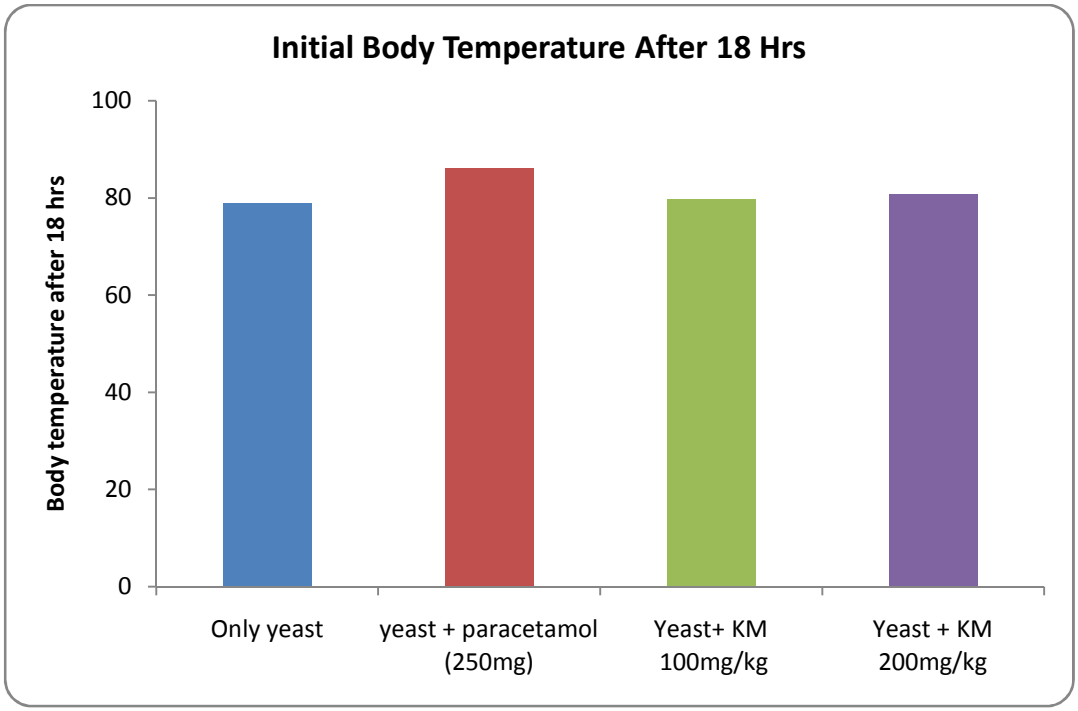
ANTI-PYRETIC ACTIVITY

TABLE NO : 11 EFFECT OF KATTUVAI MATHIRAI ON BEWER'S YEAST INDUCED PYREXIA IN RATS

GPS	Control	Only yeast	yeast + paracetamol (250mg)	Yeast+ KM 100mg/kg	Yeast + KM 200mg/kg
Initial rectal temperature 0hr	58.6±16.720	59.6±18.814	58.8332±18.6342	59.6±18.9276	60.8332±19.3089
Rectal temperature after induction with yeast 18hr	57.5±16.520	78.8664±25.0262	86.1832±27.4032	79.6832±25.2824	80.6665±25.6462
1st hr.	56.5±15.700	84.9666±26.9435	89.1±28.3478	82.7832±26.2956	83.6832±26.6028
2nd hr.	58.3±15.700	81.9832±25.9935	85.8168±27.1768	78.3668±24.8393	76.9832±24.4683
3rd hr.	56.30±32.50	80.2332±25.3776	78.8832±25.007	76.2334±24.1442	74.5±23.6679
4th hr.	56.50±45.92	81.2±25.8892	77.35±24.4649	73.3168±23.235	72.7668±23.1298
5th hr.	57.25±58.30	80.34±25.4612	72.05±22.806	68.6332±21.7312	68.2165±21.6276
6th hr.	52.50±12.20	80.1332±25.3455	62.74±19.8826	65.1±20.5935	63.64±20.1535

I-control (Distilled water-1ml),II-(yeast-10ml), III- Standard (Paracetamol l50mg), IV-yeast10 ml with (KM-100mg),V-Yeast 10ml with KM 200mg.*Significant activity


Normal Body Temperature
Body Temperature
100
90
80
70
60
50
40
30
20
10
0
Only yeast
yeast + paracetamol (250mg)
Yeast+ KM 100mg/kg
Yeast + KM 200mg/kg


Initial Body Temperature After 18 Hrs
Body temperature after 18 hrs
100
80
60
40
20
0
Only yeast
yeast + paracetamol (250mg)
Yeast+ KM 100mg/kg
Yeast + KM 200mg/kg

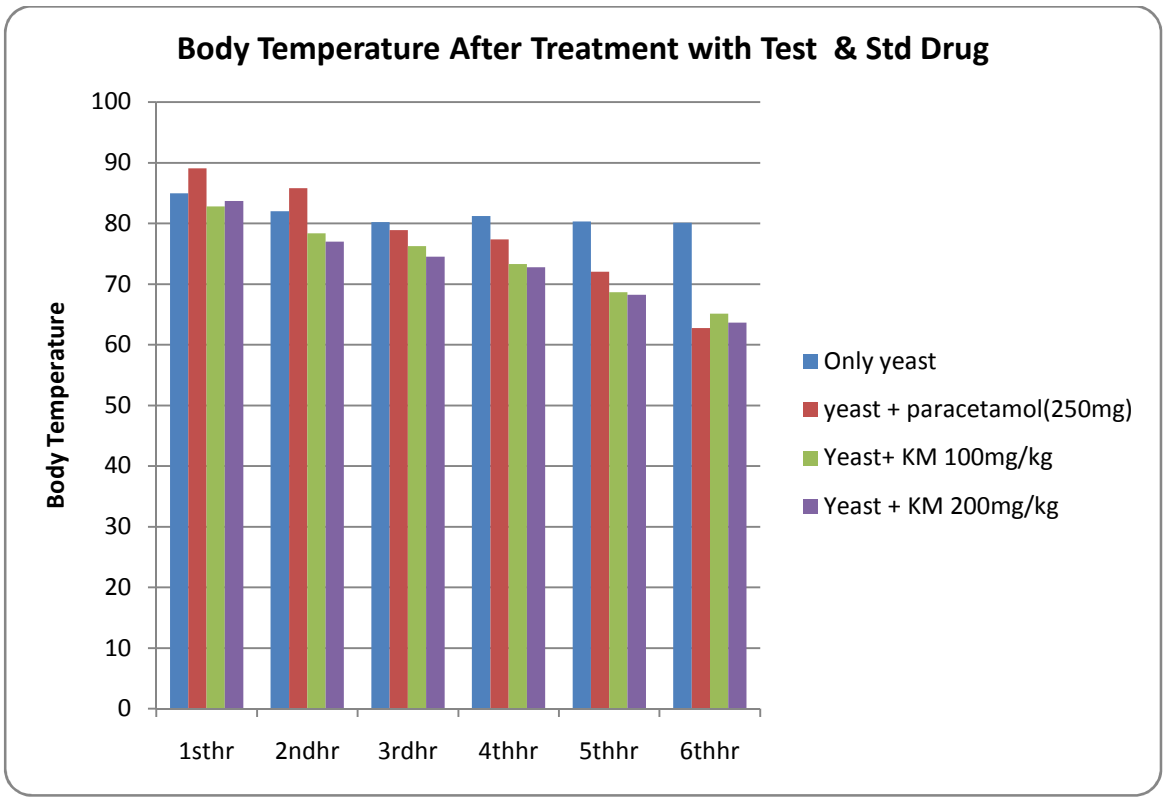


Figure no : 10 Effect of KM on Brewer's Yeast induced pyrexia in rats

Interpretation :

The results are prescribed in table no 11. Group V(KM) showed decrease in rectal temperature after 6 hours when compared to the standard drug. So The test drug "*Kattuvai Mathirai"* has got significant anti-pyretic activity.

ANTI-SPASMODIC ACTIVITY (Smooth muscle relaxant)

EFFECT OF *KATTUVAI MATHIRAI* ON EXCISED

GUINEA PIG ILEUM (IN-VITRO)

Dose Response Relationship Observations of Acetylcholine

Table No: 12

Sl.No	Concentration/dose	Acetylcholine
		Response (cm)
1	0.1 ml	2.8 cm
2	0.2 ml	3.0 cm
3	0.4 ml	3.2 cm
4	0.8 ml	4.0 cm
5	1.6 ml	5.0 cm

Dose Response Relationship Observations of Atropine

Table No: 13

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

Dose Response Relationship Observations of Acetylcholine and *KATTUVAI MATHIRAI*

Table No: 14

Sl.No	Concentration/dose	Acetylcholine + *KATTUVAI MATHIRAI*
		Response (cm)
1	0.1 ml +0.1 ml	3.6 cm
2	0.2 ml +0.2 ml	4.0 cm
3	0.4 ml +0.4 ml	4.6 cm
4	0.8 ml +0.8 ml	5.0 cm
5	1.6 ml + 1.6 ml	6.3 cm

Comparative Dose Response of Acetylcholine followed by *KATTUVAI MATHIRAI*

Table No: 15

Sl. No	Treatment	Dose(ml)	response
1	Acetylcholine	0.1 ml	2.8 cm
2		0.2 ml	3.0 cm
3		0.4 ml	3.2 cm
4		0.8 ml	4.0 cm
5		1.6 ml	5.0 cm
6	Acetylcholine ***+KATTUVAI MATHIRAI***	0.1 ml+0.1 ml	3.6 cm
7		0.2 ml+0.2 ml	4.0 cm
8		0.4 ml+0.4 ml	4.6 cm
9		0.8 ml+0.8 ml	5.0 cm
10		1.6 ml+1.6 ml	6.3 cm

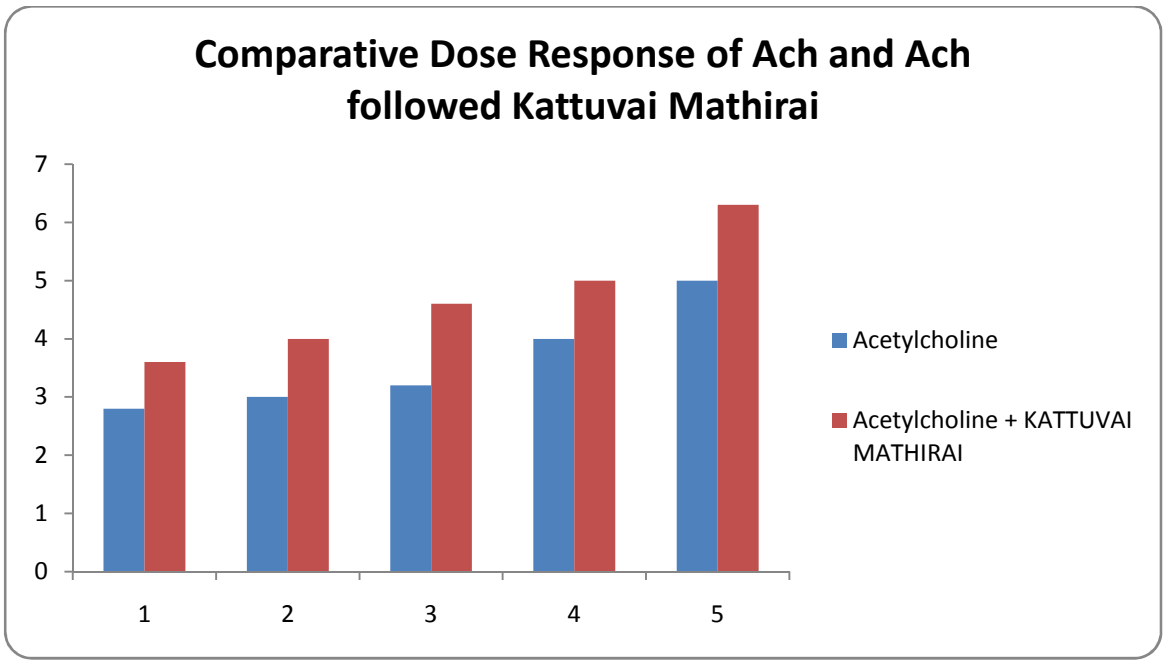


Fig: 11. Comparative dose response relationship of Acetylcholine and*KATTUVAI MATHIRAI*on excised rat ileum.

RESULTS:-

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

INTERPRETATION:

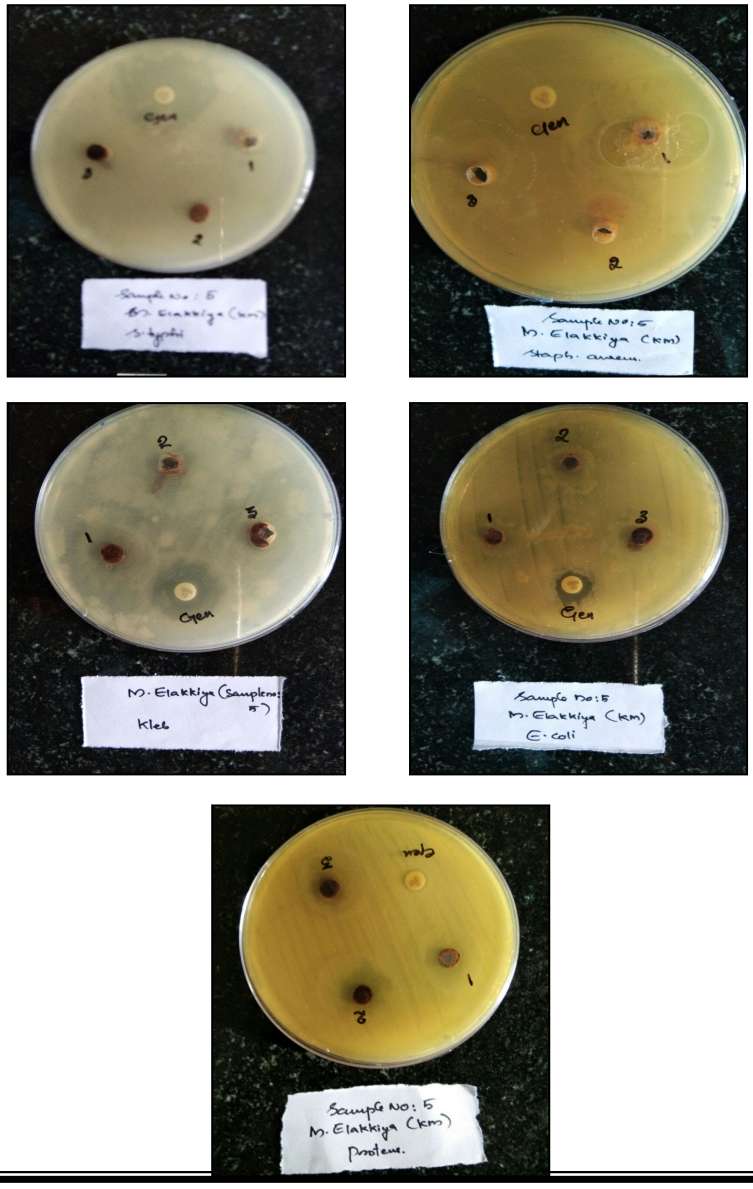
From the present study results it was observed that acetylcholine (Ach) alone causes contraction of excised rat ileum but when acetylcholine was given in presence of ***KATTUVAI MATHIRAI***there was a marked decrease in contraction of ileum was observed. This revealed that ***KATTUVAI MATHIRAI***possess a high degree of anti-spasmodic (smooth muscle relaxant) activity by blocking cholinergic receptors.

ANTIMICROBIAL ACTIVITIES

Table 16. Antimicrobial Activities of Drug by Agar Well Diffusion Method

S.No.	Test Pathogens	Result	Zone of Inhibition (mm)	
			Positive Control (Gentamycin)	Size of Inhibition
1.	*Escherichia coli*	Sensitive	20 mm	11 mm
2	*Klebsiella pneumoniae*	Sensitive	22 mm	13 mm
3	*Staphylococcus aureus*	Sensitive	21 mm	16 mm
4	*Pseudomonas aeruginosa*	Sensitive	22 mm	15 mm
5.	*Salmonella typhi*	Sensitive	19 mm	19 mm

FIGURE 12 : ANTI-MICROBIAL ACTIVITY RESULT



INTERPRETATION

Thus, the present study proves that **Kattuvai Mathirai** is free from microbial contamination and also highlighted the safety of the same. The information obtained from microbial screening tests will be use full in finding out the quality of the drug

The good antibacterial activity of herbal medicines implies that the antimicrobial compounds present in herbal medicines are possibly controlling the microbial activity. Herbal medicines **Kattuvai Mathirai** showed varying degrees of *in vitro* antibacterial activity against test bacteria.

Both Gram positive and Gram negative bacteria *E.coli, Klebsiella pneumoniae Pseudomonas aeruginosa and Salmonella sp* and *Staphylococcus aureus* were found to be sensitive to herbo mineral medicine.

7. SUMMARY

According to siddha aspect, diarrhoea is produced by derangement of pitha and vatha humors. The astringent taste of test drug diminish the deranged pitha humor. Astringent taste which possess the seetha veeriam, very much helpful to diminish the aggravated pitha humor.

According to pancha poothic theory astringent taste is a combination of primitive elements prithivi and vaayu. The prithivi pootha have a character of stability, increasing consistency and vaayu has the character to produce dryness. These three characters very much useful to treat the diarrhoea by making formed stools.

In conclusion, the present study of Kattuvai mathirai possess astringent, pungent, bitter tastes, seetha veeriam which in turn diminish aggravated pitha humor there by treating diarrhoea and pyrexia.

The trial drug of Kattuvai Mathirai was selected from the siddha text Sikitcha Rathna Deepam Ennum Vaithiya Nool, for its anti-diarrhoeal, anti-pyretic and anti-spasmodic activity.

The raw drugs *Sathikkai, Sathipathiri, Kirmbu, Athividayam, Ilavampisin, Kaichukatty, Neermulli vidhai, lingam* were purchased from authorized drug store in Nagercoil at Kanniyakumari district. The fresh *vilva pazham* were collected from Sivankovil at Tirunelveli. The fresh *oomathai ilai* were collected from college campus. All the above ingredients were identified and authenticated by experts of PG gunapadam department, Govt. Siddha Medical College, Palayamkottai, Tamil Nadu.

Collection of relevant literature evidences used in the preparation of Kattuvai mathirai for the ingredients which claims supports area Anti-diarrhoeal, anti-pyretic, Anti-spasmodic activites.

The pharmacological review were done to establish the different methodologies adopted in the preclinical evaluation of the test drug.

Physico chemical and bio-chemical analysis shows the presence of sulphate, phosphate, ferrous iron and amino acids all the above minerals might be responsible for the effectiveness of the drug.

The phytochemical analysis of Kattuvai mathirai shows alkaloids, saponins and tannins.

Regarding instrumental analysis, reveals presence of important minerals like sodium, magnesium, calcium, iron, chloride, phosphorus and zinc these minerals normalize the electrolytes which are cost in diarrhoea.

- FTIR reveals presence of alcohols which has anti-septic activity.
- ICP-OES analysis of these drug shows heavy metals like arsenic, cadmium, nickel, copper and lead are found in below detecting level. The toxic metals are found in BDL. It reveals the drug in safer for long term use. The phosphorus is involved in tissue repair and silicon reduce digestive disorders. Sodium chloride regulates acid-base balance. Zinc has got potent anti-microbial activity.
- SEM photographs revealed that particles are spherical in shapes and sizes are in the range from 1 µm to 5 µm. The size of the particles enables better absorption. Hence the bioavailability is increased and even a minimal dose yields better results.

Acute and sub-acute toxicity were carried out in wister rats according to OECD guidelines (423). This drug has no acute toxicity as there was no mortality seen sub-acute toxicity is carried by repeated dose of test drug for 28 days. Mortality, the functional observations, haematological and bio-chemical investigations were done. There were no significant changes in the bio-chemical and haematological profile. So the toxicological study of these test drug, Kattuvai mathirai establish the safety of the drug for long time administration.

In pharmacological studies, the anti-diarrhoeal activity is carried out in rats by using castor oil-induced method. The test drug Kattuvai mathirai has significant anti-diarrhoeal activity, which reveals the effectiveness of Kattuvai mathirai in treating diarrhoea.

- The anti-pyretic activity of test drug Kattuvai mathirai carried out by using Brewer's yeast induced method. The drug Kattuvai mathirai showed potent anti-pyretic activity.
- Anti-spasmodic activity of test drug Kattuvai mathirai carried out in isolated guinea pig ileum using student's organ bath. This study reveals moderate anti-spasmodic activity of Kattuvai mathirai.

Anti-microbial study of the test drug carried out by Agar well-diffusion method. It is observed that Kattuvai mathirai is sensitive to Escherichia coli, klebsiella

pneumoniae, staphylococcus aureus, pseudomonas aeruginosa, salmonella typhi. These Kattuvai mathirai has significant anti-bacterial activity.

Result and discussion gives the necessary and essential justification to prove the potency of test drug with the scientific validation. Thus the herbo-mineral formulation Kattuvai mathirai is validated for its safety efficiency in the management of diarrhoea and it would be the way for a drug of choice.

8. CONCLUSION

The trial drug of ***Kattuvai Mathirai*** was selected for the elaborate study of its efficacy on bedhi, From the literature review physico-chemical, Biochemical, Phytochemical, pharmacological, microbiological, instrumental analysis, it has been good, Anti-diarrhoeal, Anti-pyretic and Anti-spasmodic activity and hence be effective for bedhi (Diarrhoea).

9. FUTURE SCOPE

The active principle which is responsible for the activity has to be find out through modern scientific analysis having made up of tablet form. ***Kattuvai mathirai*** is extraordinary promise for the prevention and treatment of bedhi. Thus the ancient wisdom siddhars will remains as one important source of future medicine and therapeutics.

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